Harmful Algal Blooms

A Compendium Desk Reference

Editors *Sandra E. Shumway, JoAnn M. Burkholder, and Steve L. Morton*

WILEY Blackwell

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our esteemed colleagues, friends, and mentors.

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Introduction

Toxic microalgae and their associated blooms are regular and natural phenomena and have been recorded throughout history, yet major efforts to study their ecology, physiology, toxins, and impacts have only escalated over the past 4–5 decades as their presence and impacts have expanded globally. Harmful algal blooms (HAB) are caused by a diverse array of microalgal species, and they exert significant negative impacts on human and environmental health, economies, tourism, aquaculture, and fisheries (Figure I.1). The continuing increase in numbers of toxic and harmful algal species worldwide presents a constant threat to these entities, and to the sustainable development of coastal regions. While blooms of toxic algae have been noted in numerous historical documents, dating back centuries, the focus on HAB in North America and their impacts on human health was a relatively new phenomenon in the early 1970s, when the first conference was organized to share information on occurrences predominantly in New England and the Gulf of Mexico (see LoCicero *et al.*, 1975).

As blooms of toxic phytoplankton have continued to increase in their frequency, concentrations, and geographic distribution in marine, estuarine, and fresh waters, the amount of available literature on the topic has also continued to grow. Of the estimated 3400–4000 known species of phytoplankton, only 1–2% (60–80 species) are known to be harmful or toxic, yet their impacts can be devastating. Benthic microalgae and harmful species that do not typically "bloom" are now emerging as vectors of toxins (Chapter 16).

Consumption of contaminated seafood and exposure to contaminated water and aerial-borne toxins lead to seafood safety issues and human health hazards (Chapter 11). These episodes also impact the local economies (Chapter 10) and can cause large-scale ecological disturbances including fish and shellfish die-offs, and mortalities of marine mammals and birds. A conservative, dated estimate of societal costs associated with HAB in the United States is nearly a half-billion U.S. dollars, about half of which is linked to public health effects (Anderson *et al.*, 2000; also see Adams and Larken, 2013; Hamilton *et al.*, 2014; Bingham *et al.*, 2015).

Traditionally, the vectors for toxin transfer were limited to consideration of filter-feeding bivalve molluscs (e.g., oysters, clams, scallops, and mussels), but over time they have grown to include gastropods (snails, limpets, and abalone), cephalopods (squid and octopus), crustaceans (crabs, shrimp, and lobsters), and echinoderms (sea urchins and sea cucumbers) (Chapter 5). Fish and many of these nontraditional food items have been incorporated in routine algal toxin-monitoring programs (Chapter 12) for the most common toxic syndromes such as paralytic shellfish poisoning (PSP), amnesic shellfish poisoning (ASP), neurotoxic shellfish poisoning (NSP), and diarrheic shellfish poisoning (DSP), and emerging toxins such as azaspiracids, palytoxins, yessotoxins, and pectenotoxins.

Aquaculture is the fastest growing component of the food production sector globally, and the possible contamination of aquaculture and fishery products due to microalgal toxins is a major concern for managers charged with guaranteeing safe products for human and animal consumption. This has in turn led to concerted efforts to develop more sensitive, efficient, and affordable tests for algal toxins.

Since the first international conference focused on toxic algae in 1974, there have been 16 international conferences, each of which has produced a volume of contributed papers that provide invaluable information, often at local levels that might not otherwise be made available to the community at large. Bibliographic information

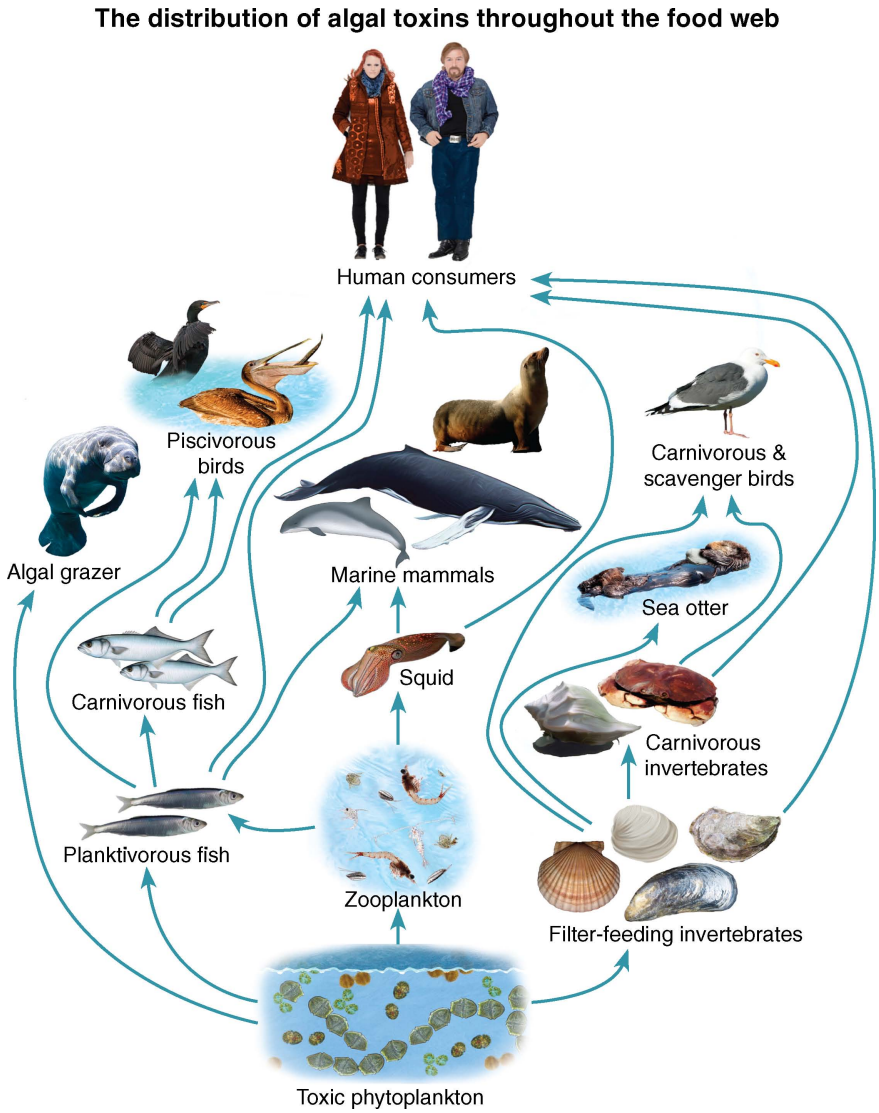

Figure I.1

for these volumes is provided in the "References and Further General Reading" at the end of this Introduction.

The topic is very well studied, and there are numerous comprehensive reviews and volumes available (see "References"). The volume of published material and the exponential growth of the field over the past four decades are the impetus for the current volume – to distill the information into a useable format for managers, newcomers to the field, and those who are not familiar with the scientific literature or do not have easy or affordable access.

The worldwide number of phycotoxin-induced intoxications per year is about 60,000 cases (Gerssen *et al.*, 2010), and, even with the advent of new and improved technologies for detection and monitoring programs, human illnesses still occur on a regular basis. An excellent summary of illnesses and deaths attributed to harmful algae is provided by Picot *et al.* (2011). The greatest threats are with regard to novel species and outbreaks, or areas where monitoring is not routine or does not include all edible species. As new toxins are identified and better technologies developed, monitoring programs continue to evolve. These

monitoring programs are also a valuable source of long-term data sets that are currently being used in modeling efforts to predict the presence and impacts of blooms (see Chapter 3). The high variability in toxin levels between individual animals demands a comprehensive monitoring program (see Chapter 12). The increase in blooms has resulted in development of new and more cost-effective technologies for toxin detection. Among the greatest strides in recent years have been the development of "dipstick tests," which are now routinely used in many areas as preliminary screening tools; the automatized detection of harmful species with specific molecular probes; and the migration from mouse assays to instrumental analyses (see Chapter 2). Successful management and monitoring programs have minimized cases of illnesses associated with toxic algae, and they continue to be refined.

Control, prevention, and mitigation remain topics of considerable interest, and new technologies, especially with regard to manipulated clay, continue to be pursued (Chapter 14), as do efforts to minimize the severity of economic and ecological impacts as well as to reduce threats to human health. The development of educational and outreach materials that promote public understanding and especially those targeted at focused audiences where language may be a barrier (Chapter 13) has been a major factor in engaging the general public and making them more aware of the perils and avoidance means when faced with local harmful and toxic algal blooms.

The current body of knowledge on HAB and their impacts is vast and no longer easily accessible, or understandable, to those not actively engaged in specific research arenas. The present volume is not intended to be a comprehensive review of all topics, but rather to provide basic information to those who are confronted with seemingly boundless sources of information, some conflicting or confusing, or who simply don't know where to begin searching for the information they need. These issues become more urgent when faced with unexpected blooms or known or unknown algal species and the associated risks to human health and trophic consequences in marine and aquatic habitats.

The aim of the current volume is to provide an accessible source of information and references for further investigation for individuals who may not be familiar with the scientific literature, but are in need of technical information when faced with unexpected or unknown harmful algal events.

References and Further General Reading

The available published literature on harmful algal blooms and their impacts is vast and can no longer be covered in any single publication. The goal of this book is to provide an overview for managers and newcomers to the field, and the following list provides an overview of recent publications.

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Causes of Harmful Algal Blooms

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1.1 Introduction

Much has been written about the underlying causes of harmful algal blooms (HAB), the complex interplay of factors that lead to their proliferation, and the unique set(s) of factors contributing to blooms of different species of algae. In general, the overarching causes that have received much attention in the literature include degradation of water quality and increasing eutrophication; increasing aquaculture operations; transport of harmful species via ballast water or shellfish seeding, leading to new introductions; and climate change (e.g., Hallegraeff and Bolch, 1992; Hallegraeff, 1993; Anderson *et al.*, 2002; Glibert *et al.*, 2005, 2014a; Heisler *et al.*, 2008; Wells *et al.*, 2016; and references therein). This chapter reviews these complexities while highlighting the key role of changes in nutrients; estuarine/marine microalgal species are emphasized, and information is also included on some freshwater HAB. While some have suggested that increased monitoring or surveillance has led to a perception of an increase in HAB, there is now compelling evidence from many regions showing conclusively that increases in HAB proliferations are real, not sampling artifacts (Heisler *et al.*, 2008).

What is a HAB? In his seminal paper, Smayda (1997a, p. 1135) stated, "What constitutes a bloom . . . has regional, seasonal, and species-specific aspects; it is not simply a biomass issue. . . . The salient criterion to use in defining whether a 'harmful' species is in bloom and the distinctive feature of such blooms lie not in the level of abundance, but whether its occurrence has harmful consequences." Since the publication of that paper, biomass criteria for a few HAB species have been defined, but more generally HAB continue to be defined in terms of the extent to which they cause harmful events (fish kills), toxic events (shellfish and finfish poisoning), ecosystem disruption (nutritional and/or prey-size mismatches, such as picocyanobacterial blooms), or large biomass events (hypoxia or anoxia). In all cases, for a HAB to occur, the HAB species must be present and its biomass relative to other species in the assemblage changes, although the HAB species does not need to be dominant or in high abundance to elicit some of these effects.

In general, the factors that promote HAB can be reduced to two: changes in the rate of introductions of species to new areas and changes in local conditions leading to conditions more conducive to the growth of individual species. Environmental changes can be subtle and not all factors may change together, leading in some cases to situations where one factor may seem to be favorable, but growth is impaired due to a change in another factor. The success of an introduced species in a new environment is not ensured; instead, there must be a match of environmental factors and the species capable of exploiting the environment. As Smayda (2002) also wrote,

> Anthropogenic seedings are not, in themselves, bloom stimulation events; they are only the first phase of a multi-phase process. A newly vectored, non-indigenous species is initially pioneering: it must either find an open niche or displace a niche occupant as its first step

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> towards successful accommodation within the community. . . . Until colonization is achieved, alien species introduced into water masses that have been modified by cultural nutrient enrichment, water mass conditioning by aquaculture, or climatological disturbances, will not bloom. Successful colonization alone is not decisive, it usually must be accompanied at some point, or coincide with habitat disturbance – a precondition for many HAB occurrences. (p. 292)

Changes in environmental conditions supportive of the increasing global occurrence of HAB are predominantly anthropogenic in nature, such as changes in nutrient loads resulting from expanding human population and associated nutrient pollution from agriculture and animal operations, alterations due to human changes in fishing pressure or aquaculture development, and/or large-scale changes in flow from major water diversion projects. However, changes in environmental conditions may also be due to interactions between trophic and biogeochemical changes that occur once new species become established, or to altered abiotic parameters or physical dynamics, such as temperature and stratification that are caused by climatic changes (e.g., Sunda *et al.*, 2006; Glibert *et al.*, 2011; Glibert, 2015; Wells *et al.*, 2016). The complex set of adaptive strategies associated with different species will lead to some species being more or less successful in contrasting environmental conditions (e.g., Margalef, 1978; Collos, 1986; Glibert and Burkholder, 2011; Glibert, 2015, 2016). The growth of some species can alter the biological and biogeochemical environment, in some cases changing the environment favorably for their own further growth, or for growth of other harmful species. No amount of pressure from an altered rate of species introductions will ensure success of that species in a new environment unless conditions are suitable for its growth (e.g., Smayda, 2002; Glibert, 2015). The success of HAB lies at the intersection of the physiological adaptations of the harmful algal species and/or strain (population), the environmental conditions, interaction with co-occurring organisms (both biogeochemically and trophodynamically), and physical dynamics that alter abiotic conditions and/or aggregate or disperse cells (or can alter abiotic conditions in a favorable or unfavorable manner), in turn promoting or inhibiting their growth. "Strain" is mentioned here because it is well established that there can be high intraspecific variation (strain differences) within a given harmful algal species in a wide array of traits ranging from morphology, reproductive characteristics, and nutritional preferences to toxicity (Burkholder *et al.*, 2005; Burkholder and Glibert 2006, and references therein).

As stated by Wells *et al.* (2016, p. 69) in their review of HAB and climate change, for HAB to be successful, it depends on the "species 'getting there' . . . 'being there' as indigenous species . . . and 'staying there'." The same is true for nutrients and related environmental conditions. They must "get there," often from anthropogenic sources; they must "be there"; and they must "stay there," often through physical dynamics, changes in trophodynamics and biogeochemical processing, or climate-induced changes. Here, using the framework of getting there, being there, and staying there for both cells and nutrients and associated environmental factors, the complexity of factors influencing HAB, emphasizing the intersection of changing habitat, especially nutrient conditions, and adaptive capability of HAB are described. This chapter focuses mainly on microalgae, but also includes several examples of macroalgae. The chapter closes with some suggestions for advancement in the understanding of HAB and nutrients.

1.2 "Getting There": The Classic Perspective on Introduced Species and Links to Cultural Eutrophication

1.2.1 Introduced Species

Transfers of species and their introductions to new areas occur frequently through various pathways. Of particular concern are ballast water introductions (e.g., Hallegraeff, 2010, and references therein; see also Chapter 13, this volume). Many harmful algal species appear to be able to maintain viability during ballast water transport, so the inoculum in the discharge area is often viable (e.g., Burkholder *et al.*, 2007a). Ballast water exchange practices have been linked to the proliferation of previously rare or undetected harmful algae in discharge locations, such as certain toxigenic dinoflagellates in Australian waters (Hallegraeff and Bolch, 1992; Hallegraeff, 1998). Ballast water discharge can alter the abundances of harmful species and set up conditions where previously rare populations proliferate (e.g., Rigby and Hallegraeff, 1996; Forbes and Hallegraeff, 1998; Hallegraeff, 1998). While only a small percentage

of introduced species have become invasive and have caused significant detrimental impact in the receiving environment (Ruiz *et al.*, 1997), in estuaries where the problem has begun to be well studied, it has generally been difficult to separate, with certainty, native from non-native taxa (Ruiz *et al.*, 1997). The fact that many microbial species presently have widespread distributions may reflect a long history of global transport by ships, migratory waterfowl and other animals, winds, water currents, and other mechanisms (Burkholder *et al.*, 2007a, and references therein). The continuing effects of human activities in non-indigenous species introductions and the resulting economic and ecological impacts can be so major that entire ecosystems have been completely changed (Cohen and Carlton, 1995, 1998; Ruiz *et al.*, 1997, 1999).

The expansion of aquaculture worldwide has created another mechanism whereby species can be transported and introduced to new areas (Hégaret *et al.*, 2008 and references therein). Aquaculture products are often shipped worldwide, and harmful species can be carried with these products. Similarly, seed stock and feed are also shipped worldwide, creating opportunities for HAB "hitchhikers." As will be developed in this review, once harmful algal species are introduced, many site-specific factors acting in concert – such as the available suite of nutrient supplies, climatic conditions, season, light regime, the presence of potential predators, mixing characteristics and other physical dynamics, and the presence/abundance of potential competitor microbiota – will control whether a given harmful species can successfully establish and thrive in the new area (e.g., Smith *et al.*, 1999).

1.2.2 Anthropogenically Introduced Nutrients

Over-enrichment of coastal waters by nutrients is a major pollution problem worldwide as the result of human population growth and the production of food (agriculture, animal operations, and aquaculture) and energy (Howarth *et al.*, 2002; Howarth, 2008; Doney, 2010). Population growth and increased food production result in major changes to the landscape, in turn increasing sewage discharges and run-off from farmed and populated lands. A major increase in use of chemical nitrogenous fertilizers began in the 1950s and is projected to continue to escalate in the coming decades (e.g., Smil, 2001; Glibert *et al.*, 2006, 2014a). The global manufacture of nitrogen (N)-based fertilizers has, in fact, increased from < 10 million metric tonnes N per yr in 1950 to >150 million metric tonnes per yr in 2013, with 85% of all chemical fertilizers having been produced since 1985 (Howarth, 2008; Glibert *et al.*, 2014a, and references therein). In contrast to the enormous expansion in the global use of chemical N fertilizers, use of phosphorus (P) fertilizers has shown a much smaller increase, at a rate only about a third that of N (Sutton *et al.*, 2013; Glibert *et al.*, 2014a). Unlike N, there is no anthropogenic synthesis of P, and all P fertilizer comes from mined sources. Of these two major agricultural nutrients, only 10–30% actually reaches human consumers (Galloway *et al.*, 2002; Houlton *et al.*, 2013), and more than half is lost to the environment in direct run-off and atmospheric volatilization/eventual deposition (Galloway *et al.*, 2014).

Nearly 60% of all N fertilizer now used throughout most of the world is in the form of urea ($CO[NH_2]_2$) (Constant and Sheldrick, 1992; Glibert *et al.*, 2006; IFA, 2014). World use of urea as a fertilizer and feed additive has increased more than 100-fold in the past four decades (Glibert *et al.*, 2006). It is projected that from 2012 to 2017, an estimated 55 new urea manufacturing plants will be constructed worldwide, half of them in China (Heffer and Prud'homme, 2013), contributing to a further doubling of global urea use by 2050 (Glibert *et al.*, 2006, 2014a). Urea can be a significant contributor both to total N and to the fraction used by phytoplankton in estuarine and coastal waters (McCarthy, 1972; Harvey and Caperon, 1976; McCarthy *et al.*, 1977; Furnas, 1983; Kaufman *et al.*, 1983; Harrison *et al.*, 1985; Glibert *et al.*, 1991; Kudela and Cochlan, 2000; Switzer, 2008), and the frequency of reports that urea may be used preferentially by many harmful species has increased in recent years (Glibert *et al.*, 2006, and references therein). Urea also rapidly hydrolyzes to NH_4^+ in water, another important N form used by phytoplankton including HAB.

The development of concentrated (confined) animal feed operations (CAFOs) near coastal waters as well as inland is another increasing, major source of nutrient pollution (Mallin, 2000; Burkholder *et al.*, 2007b; United States Environmental Protection Agency, 2013). Animal agriculture is expanding to meet the dietary demands of an increasing population, and increasingly animal production is concentrated in large industrial feeding operations which results in dense animal populations per unit landscape area (Burkholder et al. 1997 and references therein). The high

concentration of wastes per unit area, in comparison to traditional animal production practices, commonly causes contamination of adjacent waters with nutrients and associated pollutants such as suspended solids and pathogenic microorganisms (Burkholder *et al.*, 2007b). To understand the scale of this nutrient source, as an example, in the Cape Fear River basin of North Carolina, it is estimated that there are 5 million hogs, 16 million turkeys, and 300 million chickens produced annually, yielding 82,700 tonnes of N and 26,000 tonnes of P in animal waste (Mallin *et al.*, 2015, and references therein). The estimated "manure footprint" for the United States is about 150,000,000 tonnes (Rumpler, 2016). In China, tens of thousands of CAFOs are estimated to produce more than 40 times as much N pollution as from other types of industries (Ellis, 2008).

Aquaculture can be an important nutrient source and, depending on the size of the operation and concentrations of animals, can be regarded as an aquatic form of CAFO. Nutrient inputs from large-scale culture of finfish, shellfish, macroinvertebrates, and even macroalgae in some areas (Wang *et al.*, 2015) are a growing concern as the importance of aquaculture in providing food supplies continues to escalate. From 1980 to 2012, world aquaculture production volume increased at an average rate of 8.6% per year, and world food fish aquaculture production more than doubled, from 32.4 million metric tonnes to 66.6 million metric tonnes (FAO, 2014). China, in particular, has sustained what has been described as a "dramatic expansion" in cultured fish production; in 2013 alone, it produced 43.5 million tonnes of food fish and 13.5 million tonnes of algae, or about two-thirds of the cultured fish and more than half of the cultured algae worldwide (FAO, 2014).

Localized impacts of "high-input/high-output" finfish and crustacean aquaculture can be severe, such as hypoxia and anoxia, nutrient over-enrichment from discharged waste food and excretory materials, and a shift in sediment biogeochemical processes and benthic communities below fish pens (Carroll *et al.*, 2003; Bissett *et al.*, 2006; Buschmann *et al.*, 2006; Kawahara *et al.*, 2009; Burridge *et al.*, 2010; Keeley *et al.*, 2014). Extreme water quality and habitat degradation have been documented in and around shrimp farms, in particular (Naylor *et al.*, 1998; Páez-Osuna, 2001, and references therein). The cultured species generally has a nutrient retention of 30% or less, the remainder being excreted to the enrichment or lost as undigested feed (e.g., Bouwman *et al.*, 2013a). Global cultured production of finfish and crustacea contributed an estimated 1.7 million tonnes of N and 0.46 million tonnes of P to receiving waters during 2008 (Verdegem, 2013). Within the relatively short period from 2000 to 2006, nutrient release from shellfish cultures increased by 2.5- to 3-fold, and much larger increases are predicted in nutrient contributions from shellfish cultures by 2050 (Bouwman *et al.*, 2011). Aquaculture in many Asian countries is expanding at an apparently unsustainable pace. Asian aquaculture, mostly in China, now contributes nearly 90% of the total global marine aquaculture annually. During 2000–2010, nutrient release from all forms of mariculture in China collectively increased by 44% to 0.20 million tonnes of N, while estimated annual coastal N input from rivers increased by 10% to 2.7 million tonnes of N (Bouwman *et al.*, 2013b). Similar increases were estimated for P. By 2010, Chinese mariculture contributed about 7% of total N and 11% of total P inputs to coastal seas overall, and 4% and 9% of the dissolved N and P, respectively. Various HAB have been associated with estuarine/marine aquaculture, including toxic and fish-killing algae (Wu *et al.*, 1994; Honkanen and Helminen, 2000; Wang *et al.*, 2008; Furuya *et al.*, 2010), and high-biomass HAB (including macroalgae) are often linked to pond production (Alonso-Rodríguez and Páez-Osuna, 2003; Azanza *et al.*, 2005; Wang *et al.*, 2008).

Bivalve culture is generally considered to be less adverse and, in low densities, even benign (Burkholder and Shumway, 2011, and references therein). Nevertheless, when this type of aquaculture becomes so intensive that it exceeds the ecosystem carrying capacity, significant increases in nutrient supplies (especially NH_4^+), noxious phytoplankton blooms, oxygen deficits, and symptoms of cultural eutrophication develop and indeed have been documented in poorly flushed lagoons and embayments (Burkholder and Shumway, 2011). A recent study in Chesapeake Bay, for example, showed an increase of 78% in total NH_4^+ downstream from an oyster aquaculture facility (Ray *et al.*, 2015). Nutrient pollution from finfish and crustacean aquaculture generally is much higher than from molluscan culture, but, as Bouwman *et al.* (2013a) commented, because of relatively "low assimilation efficiency, molluscs can act as pumps in coastal seas transforming the nutrients in algal biomass to dissolved and particulate detrital nutrients; finfish and crustacea similarly act as pumps but with exogenous feed."

In many regions, atmospheric deposition of N contributes significant pollution (e.g., Howarth,

2006; Duce *et al.*, 2008; Galloway *et al.*, 2008). This N is derived because of increasing NOx emission from fossil fuel burning and from volatilization of animal manures and other land-based fertilizer applications. In both European and U.S. coastal waters, anthropogenic atmospheric N deposition contributes from 10 to 40% of new N loading (Jaworski *et al.*, 1997). It has been estimated that N atmospheric deposition reaches >700 mg N m^2 per yr in many regions, particularly the downwind plumes from major cities (e.g., Duce *et al.*, 2008). In eastern North Carolina, atmospheric N deposition (NOx) has more than doubled since the 1970s, a result of urbanization, increased animal operations, and agricultural expansion (Mallin, 2000). Where animal manures dominate, such as in eastern North Carolina (Rothenberger *et al.*, 2009), NH_4^+ emissions account for half of all N deposition (Aneja *et al.*, 2003; Whitall *et al.*, 2003), which has implications for HAB as shown further in this chapter.

Overall, there are many sources of species introductions and diverse routes by which nutrients are contributed to the aquatic environment, across the salinity gradient. That is, there clearly are many paths for both harmful algal cells and nutrients to "get there."

1.3 "Being There": Blooms and Why They Succeed

1.3.1 Nutrient-Related HAB

Increased loading of both N and P has been strongly, positively related to human population density (Caraco, 1995; Smil, 2001). Well-documented examples illustrate an increase of some HAB in relation to increases in N and/or P loading (Lancelot *et al.*, 1987; Anderson *et al.*, 2002; Glibert and Burkholder, 2006; Vahtera *et al.*, 2007; Glibert, 2014a). Among high-biomass bloom formers, pelagic *Prorocentrum* species, especially *Prorocentrum minimum*, has been expanding in global distribution in concert with eutrophication, and in particular with N enrichment (Heil *et al.*, 2005; Glibert *et al.*, 2008, 2012). *Prorocentrum* sp. has been found to be common near sewage outfalls and also near nutrient-rich shrimp ponds (Cannon, 1990; Sierra-Beltrán *et al.*, 2005). In the Baltic Sea, its expansion has been linked to impacts from human activities (Olenina *et al.*, 2010). Worldwide, various species of harmful cyanobacteria have been stimulated to bloom in over-enriched fresh and tidal fresh waters (Burkholder and Glibert, 2013, and references therein), and even in some brackish systems (e.g., McComb and Humphries, 1992; Vahtera *et al.*, 2007; McCulley, 2014). In Northern European waters, blooms of the mucus-forming HAB species *Phaeocystis globosa* have been directly related to the excess NO_3^- content in riverine and coastal waters, that is, the NO_3^- remaining after other species of algae deplete other nutrients (Lancelot *et al.*, 1987; Lancelot, 1995). In the United States, a strong positive relationship has been documented between increased NO_3^- loading from the Mississippi River to the Louisiana shelf and increased abundance of the toxigenic diatom *Pseudo-nitzschia pseudodelicatissima*, based on the geological record of the siliceous cell walls of this species found in sediment cores (Parsons *et al.*, 2002). In Puget Sound, Washington, United States, a striking positive correlation has been found between the growth in documented cases of paralytic shellfish toxins over four decades and the growth in human population, based on United States Census statistics, strongly suggestive of nutrient loading and eutrophication as the causative agent of change (Trainer *et al.*, 2003).

Dinoflagellate blooms off the coast of China have expanded in geographic extent (from km^2 to tens of km^2), duration (days to months), numbers of species, and harmful impacts, and these trends have paralleled an increase in N fertilizer use during the past several decades (Heisler *et al.*, 2008; Li *et al.*, 2009; Glibert *et al.*, 2011, 2014a). Annual N fertilizer use in China has escalated from about ~0.5 million tonnes in the early 1960s to 42 million tonnes around 2010, with the fraction of urea increasing nearly fivefold over just the past two decades (Glibert *et al.*, 2014a, and references therein). River export of N increased from 1980 to 2010 from ~0.5 to >1.2 tonnes km^{-2} per yr in the Changjiang River, from ~0.1 to ~0.2 tonnes per km^2 per yr in the Yellow River, and from ~0.4 to >1.2 tonnes per km^2 per yr in the Pearl River basins (Ti and Yan, 2013). In parallel with these trends in nutrient loading, the number of HAB has increased in virtually all waters of China over the past three decades. In addition to these blooms, "green tides" have increased. These noxious macroalgal blooms (*Ulva prolifera*) received notoriety at the Qingdao Sailing Center during the 2008 Summer Olympics, when the water was blanketed with thick green scum (Hu *et al.*, 2010; Liu and Zhou, 2017). More recently, "brown tides" have become recurrent in the Yellow Sea (Zhang *et al.*, 2012).

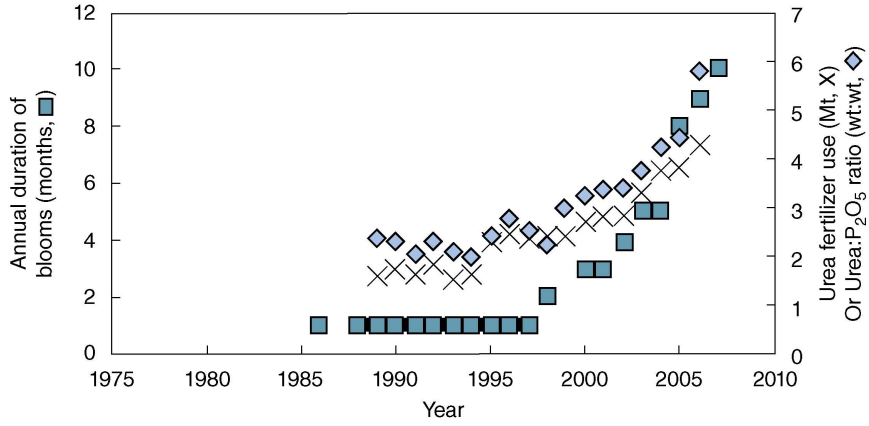

Figure 1.1 Change in annual duration of *Microcystis* blooms in Lake Tai (Taihu) in months, urea fertilizer use (million metric tonnes) scaled to that in the Changjiang watershed and the ratio of use of urea:P_2O_5 fertilizer. *Source*: *Microcystis* data are from Duan *et al.* (2009), reprinted with permission of the American Chemical Society. Data sources for fertilizer use are given in Glibert *et al.* (2014a). http://iopscience.iop.org/article/10.1088/1748-9326/9/10/105001/meta. Licensed under CC-BY 3.0.

Many freshwater HAB that have been described as spectacular or extreme have been documented worldwide in the past two decades. An example of freshwater bloom expansion is in Lake Tai (or Taihu), China, where blooms of the toxigenic cyanobacterium *Microcystis* have increased in duration from ~1 month per yr to nearly 10 months per yr over the past 15 years (Duan *et al.*, 2009), concomitant with increasing fertilizer use in the watershed or other nutrient sources (Glibert *et al.*, 2014a; Figure 1.1). As other examples, over roughly the past decade, toxic *Microcystis aeruginosa* blooms, easily visible from satellite imagery, have expanded to cover the entirety of Lake St. Clair (Michigan and Ontario, Canada; ~1114 km^2 or 430 mi^2) and much of Great Lake Erie (surface area, ~25,745 km^2 or 9940 mi^2) (Michalak *et al.*, 2013; NOAA, 2015; ESA, 2016). In August 2014, the city of Toledo, Ohio, issued a "Do not drink or boil" advisory to about 500,000 people after microcystins in the city's finished drinking water were measured at up to 2.5 µg L^{-1} (Fitzsimmons, 2014). *Microcystis* blooms have become common features in Florida's major river systems, the St. Johns and Caloosahatchee; in Lake Okeechobee (the tenth largest lake in the United States; surface area, 1714 km^2 or 662 mi^2); and in the freshwater tidal St. Lucie Estuary, where huge outbreaks have been visible from satellite imagery and have been sustained seasonally every year over the past decade (Neuhaus, 2016). Although these and other freshwater HAB have been most often been linked to P enrichment (e.g., Schindler *et al.*, 2016, and references therein), it is now recognized that failure to "co-manage" N along with P can be an important factor controlling the magnitude and toxicity of these blooms (Burkholder and Glibert, 2013, and references therein; Monchamp *et al.*, 2014; Glibert *et al.*, 2014a, 2017; Harris *et al.*, 2016).

These examples alone are reason to link the global expansion of some HAB with the expansion of nutrient loads. However, such examples do not fully explain why certain HAB species proliferate and often become the dominant algae, nor do these examples convey the full extent of anthropogenic changes affecting the habitat of these species. What is clear is that the historic view of phytoplankton responses to eutrophication – increased nutrients promote increased chlorophyll and high-biomass blooms, leading to oxygen deduction and losses in habitat (e.g., Cloern, 2001) – is too simplistic for understanding how many harmful algal species respond to changes in nutrients. Anthropogenic activities occurring worldwide are altering landscapes, seascapes, and atmosphere-scapes in complex ways, and the responses by the resident community are equally complex. The complexities of "being there" are next addressed.

1.3.2 Resource Ratios, Nutrient Stoichiometry, and Optimal Nutrient Ratios

Resource ratio theory (Tilman, 1977, 1982, 1985; Smayda, 1990, 1997b) predicts that as the ratios of

different essential elements change, the assemblage structure will change due to competition between algae with different optimal nutrient ratios. The "optimum" N:P is the ratio of the values where the cell maintains the minimum N and P cell quotas (Klausmeier *et al.*, 2004). Changes in this ratio have been compared to shifts in phytoplankton assemblage composition, yielding insights about the dynamics of nutrient regulation (e.g., Tilman, 1977; Smayda, 1990; Hodgkiss and Ho, 1997; Hodgkiss, 2001; Heil *et al.*, 2007; Glibert *et al.*, 2012). Perhaps the clearest demonstration of the effect of altered nutrient supply ratios involves the stimulation of non-diatom species following changes in the availability of N or P relative to silica (Si). Diatom species, which mostly are beneficial, require Si in their cell walls, whereas most other phytoplankton do not. They must sequester major amounts of hydrated Si from the surrounding water for their cell wall formation (Sullivan *et al.*, 1981). As N and P have increased from anthropogenic inputs, the relative proportion of Si has changed. Since Si is not abundant in sewage effluent like N and P, the N:Si or P:Si ratios in many lakes and reservoirs, rivers, estuaries, and coastal waters have increased over the past several decades as human populations have increased (Schelske *et al.*, 1986; Smayda, 1989, 1990; Rabalais *et al.*, 1996). Changes in Si availability have also occurred due to sediment trapping (which would include cell walls of dead diatoms, from which Si is very slowly dissolved) and elemental transformations following construction of dams (e.g., Billen *et al.*, 1999; Vörösmarty *et al.*, 2003; Beusen *et al.*, 2005; Syvitski *et al.*, 2005; see also Section 4.2). Diatom growth declines when hydrated Si availability declines, but other phytoplankton groups that do not need Si can continue to proliferate by using the excess N and P.

Among changes in various nutrient ratios, changing N:P ratios have received considerable attention because of the magnitude of the anthropogenic changes that have occurred due to N and P loading on the one hand, and efforts to reduce nutrient loads on the other. Differences in application rates, together with differences in soil retention of P compared to N, and management efforts that generally have emphasized reductions in P loading relative to N, have led to increasingly skewed N:P ratios in anthropogenic nutrient loads. In many parts of the developed world, reductions mostly in P (e.g., in sewage effluents and laundry detergents; Litke, 1999; Stow *et al.*, 2001; Alexander and Smith, 2006) have been undertaken in attempts to reduce or control algal blooms. The consequence is that many receiving waters are now not only enriched with nutrients, but also these nutrients are in proportions that differ markedly from the proportions of decades past – and also diverge considerably from those that have long been associated with healthy phytoplankton growth, namely, Redfield proportions (Glibert and Burkholder, 2011). It has also been estimated that the atmospheric deposition of nutrients in the ocean is now ~20 times the Redfield ratio for N:P (Jickells, 2006; Peñuelas *et al.*, 2012). The N:P stoichiometry has also markedly shifted in freshwater systems (Elser *et al.*, 2009; Glibert *et al.*, 2014a; Harris *et al.*, 2016). Such changes in the N:P stoichiometry of nutrient supplies have major consequences for HAB.

Various surveys of optimal N:P molar ratios across a broad range of phytoplankton groups have revealed that, while the data tend to cluster around the Redfield ratio (Redfield, 1934), there are numerous examples at both the high and low ends of the spectrum (e.g., Hecky and Kilham, 1988; Geider and La Roche, 2002; Klausmeier *et al.*, 2004). Some analyses even indicate that "Redfield ratios are the exception rather than the rule" in freshwaters (Hecky *et al.*, 1993). Different taxonomic groups (e.g., phyla or classes) of microalgae, and even different species within the same genus, have been shown to have distinct ecophysiological characteristics with respect to nutrient requirements. Given that microalgae span many orders of magnitude in cell volume, from $< 2\,\mu m$ to more than $4000\,\mu m$, it should not be surprising that the elemental demands of different types of microalgae vary (Harris, 1986; Chisholm, 1992; Geider and LaRoche, 2002; Finkel *et al.*, 2010; and references therein). In a meta-analysis of both freshwater and marine studies of phytoplankton stoichiometry, Hillebrand *et al.* (2013) confirmed that phytoplankton N:P ratios become more restricted and lower with increasing growth rate, and that at maximum growth rate N:P converges to an optimal ratio (or a more narrowly defined range) that differs depending on the species and phylogenetic group. The weighted molar averages for optimal N:P ratios appear to be lowest for diatoms (14.9), increase for dinoflagellates (15.1), and increase even more for cyanobacteria (25.8) and chlorophytes (27.0; Hillebrand *et al.*, 2013).

Algal taxa have different optimal nutrient ratios for various reasons. They may have a lower overall requirement for a particular nutrient. Very small cells, such as picocyanobacteria, have a lower requirement for P due to the smaller

need for structural components in the cell (Finkel *et al.*, 2010). Alternatively, or additionally, species that thrive under such conditions may have the ability to "make do with less" by physiological substitution of a P-containing compound(s) with a non-P-containing compound(s), as in the case of substitution of a P-containing lipid with a non-P-containing lipid (sulfolipid). Many cyanobacteria appear to have this capability (Van Mooy, 2009). Thus, the cellular carbon (C):P content of *Synechococcus*, for example, is about 100, whereas that of a typical diatom is about 50 (Finkel *et al.*, 2010). Many HAB species also can upregulate their ability to acquire a particular nutrient if and when it becomes available. As an example, gene expression has been reported for cultured *Microcystis* under conditions of extremely low P; two high-affinity, P-binding proteins and alkaline phosphatase were strongly upregulated by factors of 50- to 400-fold (Harke *et al.*, 2012; Gobler *et al.*, 2016).

Alterations in the composition of nutrient loads have been correlated with shifts from diatom-dominated to flagellated-dominated algal assemblages in many regions. Continuing with the example of China introduced above, in the Huanghai Sea region, inorganic N:P ratios are now about twice Redfield proportions, and about fourfold higher than in the 1990s (Ning *et al.*, 2009; Glibert *et al.*, 2014a). In that region, there has also been nearly a sixfold increase in HAB occurrences and a shift to proportionately more dinoflagellates in comparison to diatoms (Fu *et al.*, 2012a; Glibert *et al.*, 2014a). Similarly, in the South China Sea region, water-column inorganic N:P ratios increased from ~2 in the mid-1980s to >20 in the early 2000s (Ning *et al.*, 2009). In addition to the increase in the number of HAB, a shift in species composition to increasing dominance of genera such as *Chattonella*, *Karenia*, and *Dinophysis* has occurred (Wang *et al.*, 2008).

Nutrient stoichiometry has been shown to be strongly related to blooms of pelagic *Prorocentrum* species (Glibert *et al.*, 2012), but in a manner that changes with the growth state of the bloom. Planktonic *Prorocentrum* blooms are often initiated at N:P levels below Redfield, stimulated by a "flush" of nutrients or organic materials (i.e., by nutrients "getting there" from a run-off or other delivery event). As examples, blooms of *P. minimum* in the Baltic Sea and Chesapeake Bay are characteristically initiated following a flush of organic nutrients (Granéli *et al.*, 1989; Glibert *et al.*, 2001), while blooms of *P. donghaiense* in the East China Sea likely are initiated by an injection of P-rich water from the Taiwan Warm Current and its intersection with N-rich Changjiang River plume water (Tang *et al.*, 2000; Fang, 2004; Zhou *et al.*, 2008; Li *et al.*, 2009). Once the growth rate increases, bloom biomass is able to increase, often reaching nearly monospecific proportions at N:P ratios much higher than Redfield. After the blooms are established, they apparently can be maintained at substantially elevated N:P levels for long periods through mixotrophy or other adaptive strategies that allow balance of cellular nutrients and energy in an environment where nutrients are provided in imbalanced proportions (Glibert *et al.*, 2012, and references therein). Examples of such high-biomass blooms maintained with N:P in excess of Redfield proportions have been reported in the Baltic Sea (Hajdu *et al.*, 2005), the Delaware Inland Bays (Handy *et al.*, 2008), the Neuse River Estuary (Springer *et al.*, 2005), the East China Sea (Li *et al.*, 2009), and Chesapeake Bay (Li *et al.*, 2015). Thus, while high growth rates may enable initiation of blooms, adaptive physiology may allow blooms to be maintained, that is, to "be there" at less than maximal growth rates and at non-optimal N:P ratios. Accoroni *et al.* (2015) applied a similar conceptual model of N:P regulation for blooms of the benthic dinoflagellate *Ostreopsis* cf. *ovata* in the northern Adriatic Sea.

An intriguing curiosity, and one that goes *against* the prevailing notion that HAB occur in response to nutrient enrichment, is the observation that some HAB appear to occur more frequently following reductions, rather than increases, in nutrient pollution. Several specific types of HAB seem to illustrate this phenomenon, such as *Alexandrium* spp. that produce paralytic shellfish toxin. The most commonly cited example of this phenomenon is the Seto Inland Sea, Japan, where nutrient loads were significantly reduced following sewage upgrades. While overall numbers of blooms and their biomass declined, outbreaks of *Alexandrium tamarense* and *Alexandrium catenella* became more prevalent (Anderson *et al.*, 2002). A similar observation was reported from the Thau Lagoon, southern France (Collos *et al.*, 2009). These types of events may be examples of HAB that are promoted not only by nutrient availability but also by changing nutrient proportions. In both cases, P reductions were imposed without concurrent reductions in N, leading to an elevated N:P condition. Overall, it is not necessarily the total nutrient pollutant load that causes HAB, but the change in the composition of those nutrients.

1.3.3 Diversity in Use of Forms of Nitrogen

In addition to nutrient ratios that promote species with a higher or lower requirement for a particular nutrient, the *form* in which the nutrient is supplied may also control whether a specific nutrient load will promote a HAB. Organic nutrients have been shown to be important in the development of blooms of various HAB species, in particular cyanobacteria and dinoflagellates (e.g., Paerl, 1988; Glibert *et al.*, 2001), and the importance of organic nutrient forms in blooms is increasingly recognized worldwide (e.g., Granéli *et al.*, 1985; Berg *et al.*, 1997, 2003; Berman, 1997; Berman and Bronk, 2003; Glibert and Legrand, 2006; Collos *et al.*, 2014). For example, cyanobacterial blooms in Florida Bay and on the southwest Florida shelf have been shown to be positively correlated with the fraction of N taken up as urea, and negatively correlated with the fraction of N taken up as NO_3^- (Glibert *et al.*, 2004). A substantial body of literature suggests that diatoms are NO_3^- specialists (e.g., Lomas and Glibert, 1999a, 1999b; Figueiras *et al.*, 2002; Kudela *et al.*, 2005), while cyanobacteria, and many chlorophytes and dinoflagellates, may be better adapted to use of NH_4^+, urea, or other organic N forms (see reviews by Collos and Harrison, 2014; Glibert *et al.*, 2016). Such differences are consistent with differing evolutionary lineages of these groups, and with increasing insights about the physiology of these different functional groups (Wilhelm *et al.*, 2006; Glibert *et al.*, 2016, and references therein).

The importance of organic N forms is increasingly recognized in algal nutrition, especially in HAB proliferation (Berg *et al.*, 1997; Berman and Bronk, 2003; Bronk *et al.*, 2007; Glibert and Legrand, 2006, and references therein). The pathways by which osmotrophy occurs are numerous, and include direct uptake as well as extracellular oxidation and hydrolysis (Glibert and Legrand, 2006, and references therein). Enzymatic measurements have been used to determine some of the pathways involved in the incorporation and degradation of organic compounds (Chróst, 1991). Urease activity appears to be constitutive for many algal species, but may be higher in many HAB species compared to non-HAB (e.g., Fan *et al.*, 2003; Lomas, 2004; Solomon *et al.*, 2010). For example, urease activity is sufficiently high in *Aureococcus anophagefferens* and *P. minimum* to meet the cellular N demand for growth, but seemingly insufficient to meet the N growth demand for the diatom *Thalassiosira weissflogii* (Fan *et al.*, 2003). In *Alexandrium fundyense*, urease activity was shown to be seasonally variable and positively related to the toxin content of the cells (Dyhrman and Anderson, 2003). Both peptide hydrolysis and amino acid oxidation may be important in some HAB, as shown by Mulholland *et al.* (2002) in studies of *A. anophagefferens* in natural communities. Leucine amino peptidase is another protease that hydrolyses peptide bonds and liberates amino acids (Langheinrich, 1995; Dyhrman, 2005). It is measured by assessing the rate of hydrolysis of an artificial substrate, and has been shown to be of potential significance in dinoflagellates and other HAB classes (Berges and Falkowski, 1996). Stoecker and Gustafson (2003), for example, demonstrated in Chesapeake Bay that leucine aminopeptidase activity was associated with a dinoflagellate bloom, and that in non-axenic cultures of *Akashiwo sanguinea*, *Gonyaulax grindley*, *Gyrodinium uncatenum*, *Karlodinium micrum*, and *P. minimum*, the activity was associated with the dinoflagellates and not the bacteria.

Cyanobacteria, especially picocyanobacteria, have differing abilities to take up and assimilate NO_3^- and NH_4^+ (e.g., Flores and Herrero, 1994; Harris *et al.*, 2016). Even for the picoplankton cyanobacteria that do not include N_2-fixers, there is wide diversity in their ability to use NO_3^- or NH_4^+ (Scanlan and Post, 2008, and references therein). Some picocyanobacteria cannot take up NO_3^- at all (Moore *et al.*, 2002; Rocap *et al.*, 2003), while some can take up both NO_3^- and NO_2^- (Martiny *et al.*, 2009). Many cyanobacteria have constitutive expression of high-affinity NH_4^+ transporters at the cell membrane (Wilhelm *et al.*, 2006, and references therein).

Many experiments have shown that a different algal assemblage can develop when the form of N is altered, even holding the same total N constant and/or at levels that are seemingly saturating for uptake or growth. Early mesocosm experiments by Glibert (1998) showed that different size classes of phytoplankton develop when the proportion of NO_3^- to NH_4^+ varies: a doubling in the ratio of the ambient NO_3^- to NH_4^+ resulted in a nearly 50% increase in the ratio of >10 μm to <10 μm sized algal biomass. In laboratory mesocosm experiments conducted with nutrient-rich water from the Choptank River (a tributary of Chesapeake Bay), Glibert and Berg (2009) showed that NO_3^- uptake was directly related to the fraction of the assemblage as diatoms, while the proportion of NH_4^+ uptake was directly proportional to the fraction of the assemblage as cyanobacteria. Donald *et al.* (2011, 2013) also found, in experiments

conducted in freshwater lakes in Canada, that NO_3^- enrichment led to a proportionately greater increase in chlorophyll-*a* (relative to total wet-weight algal biomass) and a greater initial response by diatoms, while NH_4^+ enrichment led to a proportionately greater increase in cyanobacteria. In experiments conducted in the San Francisco Bay Delta, Glibert *et al.* (2014b) reported proportionately more chlorophyll-*a* and more diatoms in a low-light regime when the N enrichment substrate was NO_3^-, while more cyanobacteria developed in a high-light regime when NH_4^+ was provided as the enrichment substrate in the same concentration. Domingues *et al.* (2011) also showed that enrichment by NH_4^+ in a freshwater tidal estuary favored chlorophytes and cyanobacteria, whereas diatoms were favored under NO_3^- enrichment. The same trends additionally were reported for the phytoplankton assemblage in highly eutrophic Anacostia River water (a tributary of Chesapeake Bay); when experimentally enriched with NH_4^+, a proportionately greater response by cyanobacteria and chlorophytes was observed relative to enrichment of samples with the same amount of NO_3^-, which elicited a greater diatom response (Jackson, 2016).

Toxic cyanobacteria species also appear to be favored over diatoms when N is supplied in chemically reduced forms relative to oxidized forms – as, for example, in the hypereutrophic Lakes Taihu, China, and Okeechobee, Florida, United States (McCarthy *et al.*, 2009). Harris *et al.* (2016) reported that cyanobacterial biomass increased in midwestern lakes when the proportion of NH_4^+ relative to NO_3^- increased. Numerous field studies have shown that dinoflagellates, many of which are HAB formers, are also associated with increased proportion of N in chemically reduced (e.g., NH_4^+ and urea) rather than oxidized forms (e.g., NO_3^- and NO_2; Berg *et al.*, 2003; Glibert *et al.*, 2006; Heil *et al.*, 2007). Thus, as for terrestrial plants, there is a dichotomy in use of oxidized versus reduced N forms, and differential optimal N:P ratios for different phytoplankton functional groups, the end result of which is an altered phytoplankton assemblage composition when nutrient loads, ratios, and forms are altered (Glibert *et al.*, 2016, and references therein; Figure 1.2).

1.3.4 Toxicity

Considerable emerging evidence has shown that nutrient proportions and forms have effects on toxin production as well growth of HAB (e.g., Béchemin *et al.*, 1999; Corcoran *et al.*, 2014). Many toxic algae increase in toxin content under conditions of elevated N:P ratios (e.g., Granéli *et al.*, 1998; Granéli and Johansson, 2003; Granéli and Flynn, 2006; Hardison *et al.*, 2012, 2013). Many toxins are rich in N as well as C, and thus production of toxins might be considered as a dissipatory mechanism whereby cells release the N or C not needed in metabolism (Glibert and Burkholder, 2011; Glibert *et al.*, 2016). Although not conclusively shown for any toxin, toxin production may be part of the complex suite of physiological processes involved in "overflow metabolism" (*sensu* Glibert *et al.*, 2016).

As examples, under conditions of elevated N:P ratios, hemolytic activity per cell has been shown to increase by up to tenfold in the haptophytes *Prymnesium parvum* and *Chrysochromulina* (now *Prymnesium*) *polylepis* (Johansson and Granéli, 1999). Similarly, at higher N:P ratios, neurotoxin production increased in the diatom *Pseudo-nitzschia multiseries.* Excess N and high N:P ratios have also been related to increased microcystin (MC) production under controlled culture conditions (e.g., Lee *et al.*, 2000; Oh *et al.*, 2000; Vézie *et al.*, 2002; Downing *et al.*, 2005; Van de Waal *et al.*, 2009). As recently reviewed by Gobler *et al.* (2016), common cyanotoxins, including MC, nodularins, cylindrospermopsins, and saxitoxins, have amino acid precursors (glutamine, arginine, or leucine), and they, in turn, depend on adequate N supply for their assimilation (Figure 1.3). An adequate N supply is also needed for assimilation of many dinoflagellate toxins (Dagenais-Bellefeuille and Morse, 2013, and references therein), as has been shown for the dinoflagellates *Karlodinium veneficum*, *Alexandrium* sp., and *Karenia brevis* (Granéli and Flynn, 2006; Hardison *et al.*, 2013). In all, the production of metabolites and toxins that contain N tends to be higher when N availability is high, while those that are proportionately more C-rich are more abundant in the cells when N availability is low (Glibert *et al.*, 2016; Harris *et al.*, 2016).

In addition to the proportion of N relative to other elements, the form of N on which cells are growing also affects the synthesis of secondary metabolites, including toxins. Over the growing season of three lakes in Québec known to have toxic cyanobacteria, the cyanobacterial assemblage structure and total MC concentrations of toxins were strongly related to the availability of chemically reduced and organic N forms (DON and

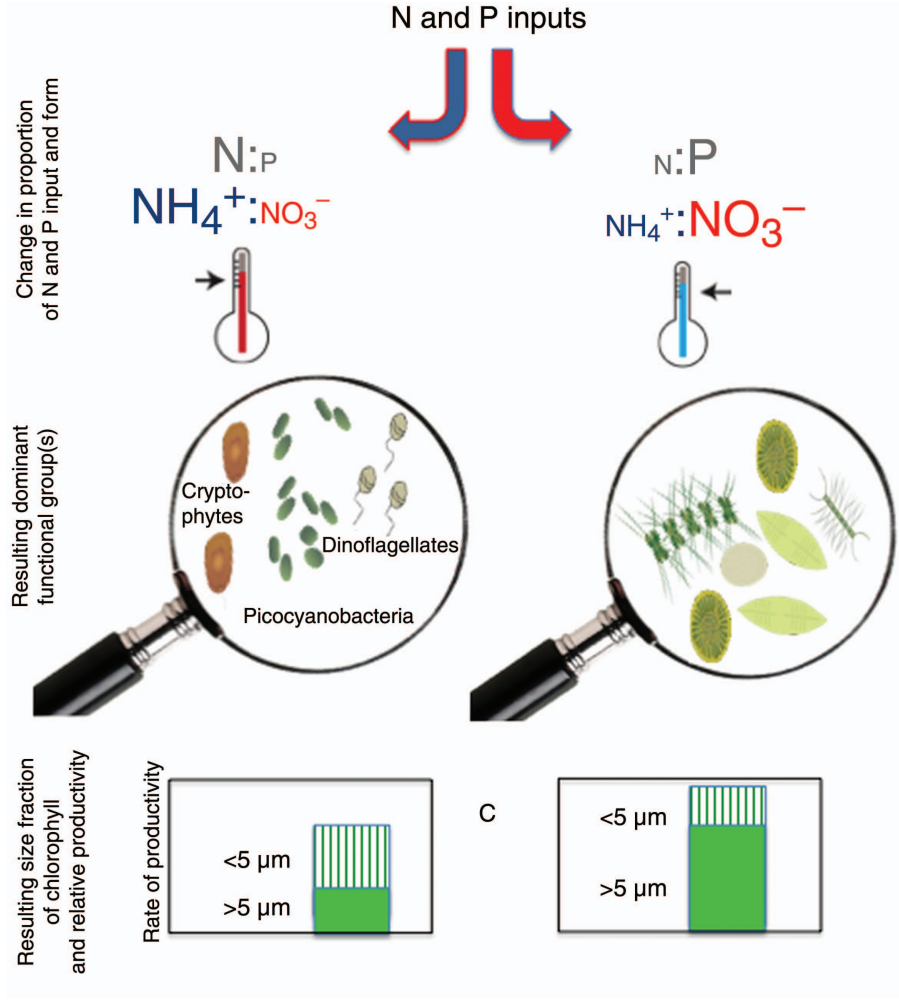

Figure 1.2 Summary conceptual schematic illustrating the effect of changes in the proportion of nitrogen to phosphorus (N:P), the relationship between NH_4^+ and NO_3^- in N loads, and the temperature regime in a natural system. When the N:P ratio is elevated, NH_4^+ is the dominant form, and when waters are warmer, flagellates, cyanobacteria, and chlorophytes commonly proliferate, leading to overall productivity dominated by small-sized algae (here, <5 µm). In contrast, when the N:P ratio is lower, NO_3^- is the dominant form provided, and when conditions are cooler, diatoms are abundant and overall production will likely be dominated by cells of a larger size class (here, >5 µm). Moreover, chlorophyll *a* yield and total production may be higher than under the NH_4^+ enrichment condition. *Source*: Reproduced and modified from Glibert *et al.* (2016). https://creativecommons.org/licenses/by/4.0/. Licensed under CC-BY 4.0.

NH_4^+; Monchamp *et al.*, 2014). Furthermore, in mesocosm studies, additions of NH_4^+ led to higher MC concentrations and cyanobacteria blooms of longer duration compared with experiments in which NO_3^- was the added N substrate (Donald *et al.*, 2011). Recently, Harris *et al.* (2016) reported that in eutrophic midwestern U.S. reservoirs, the concentration of secondary metabolites including toxins increased under elevated NH_4^+:NO_3^- ratios. In work with cultures of the marine dinoflagellate *A. tamarense*, additions of NH_4^+ resulted in higher cell quotas of toxin than did additions of NO_3^- (Leong *et al.*, 2004). Given the extensive research on the differential metabolisms of oxidized versus reduced N in higher plants (e.g., Warncke and Barber, 1973; Johnson *et al.*, 1984; Nakagawa *et al.*, 1984; Fontana *et al.*, 2006; Praveen *et al.*, 2011, among others), it is not surprising that the proportion of nutrients and their forms have direct effects on the metabolites, including toxins, of microalgae (e.g., Van de Waal *et al.*, 2009, 2014; Montchamp *et al.*, 2014; Downing *et al.*, 2015).

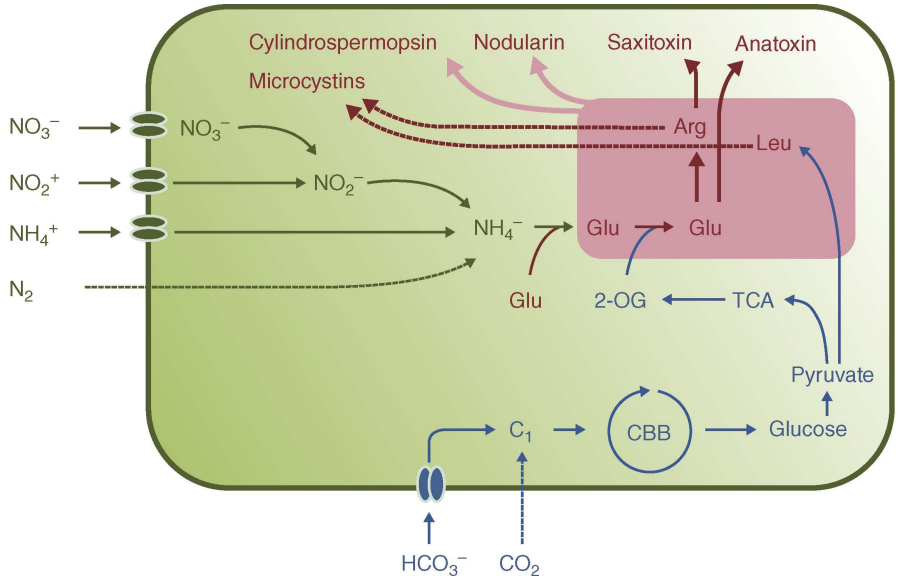

Figure 1.3 Cell schematic illustrating the relationship between nitrogen (N) and carbon (C) uptake and assimilation into amino acids, and synthesis of major cyanobacterial toxins derived from these amino acids. Ci, cellular inorganic carbon; CBB, Calvin–Benson–Bassham cycle; TCA, tricarboxylic acid; 2-OG, 2-oxoglytarate; Gln, glutamine; Glu, glutamate; Arg, arginine; Leu, leucine. *Source*: Modified after Van de Waal (2010) and reproduced from Gobler *et al.* (2016) with permission of Elsevier.

1.3.5 Mixotrophy: Use of "Packaged" and Dissolved Particulate Nutrients

An important advancement in the understanding of HAB and their nutrition over the past decade or so has been the evolving recognition of the importance of mixotrophy in the nutritional ecology of many HAB species, especially those that are prevalent in nutrient-rich environments (Burkholder *et al.*, 2008). Mixotrophy (here, phagotrophy) allows HAB to acquire nutrients in pre-packaged or particulate form; this is recognized as an important strategy when insufficient dissolved nutrients are available in the environment - but it can also be important when the balance of nutrients is unfavorable, regardless of the absolute concentration of the dissolved nutrient (Jeong *et al.*, 2010; Flynn *et al.*, 2013). Essential elements such as N, P, and C are typically rich in microbial prey and, thus, mixotrophy can provide a supplemental supply when there is an elemental imbalance in dissolved (water-column) nutrient substrates (Granéli *et al.*, 1999; Vadstein, 2000; Li *et al.*, 2001; Stibor and Sommer, 2003; Stoecker *et al.*, 2006). In eutrophic environments, although nutrients may be proportionately more available than in oligotrophic environments, nutrients often are out of stoichiometric balance (Burkholder *et al.*, 2008; Burkholder and Glibert, 2013). In the North Sea, for example, as increasing N relative to P proportions developed in response to disproportionate nutrient reductions, mixotrophic dinoflagellates increased, and many of these were harmful species (Burson *et al.*, 2016). Recent laboratory experiments also have shown that, at least for some mixotrophs, grazing is highly dependent on not only their physiological or nutritional state, but also that of their prey (e.g., Lundgren *et al.*, 2016; Lin *et al.*, 2017).

Mixotrophy also may permit growth to be sustained or even accelerated during periods of apparent water-column nutrient deficiency or imbalance (or real nutrient deficiency for non-mixotrophic competitors) if only inorganic forms of dissolved nutrients are considered. The dinoflagellates *Cochlodinium (Margalefidinium) polykrikoides* and *K. brevis* exemplify these benefits of mixotrophy. When growing as a phototroph, *C. polykrikoides* had a growth rate of 0.17 divisions day^{-1} (Jeong *et al.*, 2004). Yet, when grown as a mixotroph with cryptophytes as prey, the division rate of *C. polykrikoides* nearly doubled to 0.34 divisions day^{-1} (Jeong *et al.*, 2004). In the case of toxigenic *K. brevis*, grazing can occur on the cyanobacterium *Synechococcus* sp. (Jeong *et al.*, 2005; Glibert *et al.*, 2009). In laboratory experiments, Jeong *et al.* (2005) estimated that 5 cells $hour^{-1}$ of *Synechococcus* could be grazed by mixotrophic *K. brevis*, while Glibert *et al.* (2009) found that from ~1 to 80 *Synechococcus*

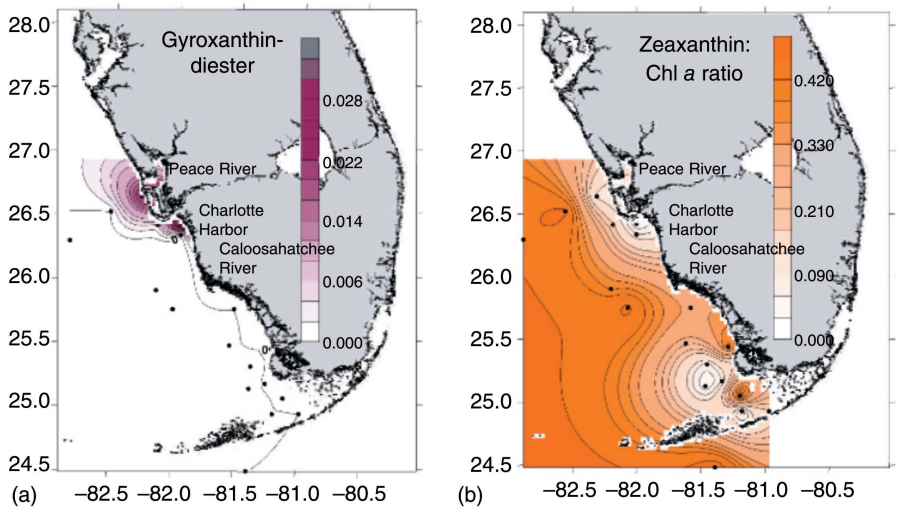

Figure 1.4 Contour maps of (a) abundance of the pigment gyroxanthin-diester (indicative of *Karenia brevis*) and (b) zeaxanthin (indicative of cyanobacteria such as *Synechococcus*) relative to chlorophyll-*a* for the southwestern Florida shelf, sampled in May 2013. *Source*: Reproduced and modified from Heil *et al.* (2007) with permission of Wiley.

cells hour^{-1} were grazed by *K. brevis*, depending on the predator–prey ratio. The growth rate of *K. brevis* increased as the supply of *Synechococcus* increased, indicating that natural variability in this food source may affect growth rates of this HAB species in nature. On the western Florida shelf, an inverse relationship between the spatial extent of *K. brevis* (as measured by the indicator pigment gyroxanthin-diester) and picocyanobacteria (as measured by the indicator pigment zeaxanthin and confirmed microscopically) suggested that mixotrophic grazing contributed to the lower abundance of picocyanobacteria near Charlotte Harbor and the Caloosahatchee River plume (Heil *et al.*, 2007; Figure 1.4). At the very least, availability of a food source may help to sustain the HAB after inorganic nutrient supplies have been diminished.

Jeong *et al.* (2010) described these trophic interactions as serving as "hubs" (in the sense of an airport hub) for energy and nutrient flow through the microbial consortium. Species such as *Prorocentrum* spp. can dominate a hub, serving as both predator and prey of a wide range of species. Such a designation has particular relevance when these species become dominant in a phytoplankton assemblage. The "hub" serves to concentrate nutrients and to transfer them to higher trophic levels. Thus, in addition to providing a growth benefit for HAB species, mixotrophy may provide a physiological mechanism for acquisition of a nutrient that may not be available in dissolved form, and therefore would not be available to competitors without alternate nutrition strategies.

This complexity in response to nutrient proportion and form challenges the commonly held notion that nutrients are only regulating when they are in limiting proportions (e.g., Reynolds, 1999; Davidson *et al.*, 2012, 2014; Wells *et al.*, 2016). Mixotrophy is a common mechanism or "strategy" in waters where nutrients are not stoichiometrically balanced – a condition often enhanced by anthropogenic changes in N relative to P (or Si). Importantly, nutrient limitation does not need to be imposed for stoichiometric imbalanced conditions to develop. Properties of cells such as enzyme activities, gene regulation, cellular pigmentation complement, cell elemental composition, and toxin content all vary across the entire gradient of nutrient supply, not just the limiting range. Thus, as anthropogenic activities change not only the total amount but also the gradient and form of nutrient supply, organisms, including harmful algal species, respond accordingly. The perpetuation of the classic view that nutrient must be in limiting concentration levels to control algal biomass has been a major hindrance in understanding how the composition of algal assemblages changes; nutrient form and proportion have consequences for ecosystem structure and function, whether nutrients are limiting or not.

1.3.6 Other Adaptations

In addition to these strategies for nutrient acquisition, other adaptations can provide benefit to

some HAB species under altered nutrient conditions. Many of these species are flagellates that can swim (while diatoms do not) and exhibit vertical migration behavior. They can move into deeper, more nutrient-rich waters, especially toward the microbially active pycnocline or the sediment–water interface. Under certain environmental conditions, their swimming behavior may result in the formation of high-density patches (e.g., Franks, 1992; Kamykowski *et al.*, 1998). Some cyanobacterial species can similarly regulate their vertical position in the water column by synthesis and collapse of gas vesicles inside their cells (Walsby, 1975). Vertical movement by cells in a stratified environment may help to maximize encounter frequencies for sexual reproduction (for eukaryotic microalgae), minimize grazing losses, and allow cells to obtain nutrients at depth and light at the surface. Some unicellular species form mucilaginous colonies that can impede grazers and/or protect species from viral or bacterial infection (Lancelot *et al.*, 2002).

Furthermore, many harmful algae have benthic cysts or other resting stages that enable cells to withstand hostile or unfavorable environmental conditions. The metabolic switch from resting stages to motile stages often is generally synchronized for an algal population (Kremp, 2001; Vahtera et al., 2014), and the actively dividing cells initiate a bloom. These cysts or spores provide a recurrent seed source or inoculum for planktonic populations, and this characteristic may be a critical factor in determining not only the geographic distribution of species but their eventual abundance as well. All of these behaviors have important implications for harmful algal species success, and serve to underscore the deep complexity of the biology of these seemingly "simple" organisms; there are clearly many factors contributing to harmful species "being there."

1.4 "Staying There": Links to Physical Structure and Climate

1.4.1 Physical Structure: Large-Scale and Small-Scale Natural Hydrological Features

Physical factors influence both nutrient retention and cell retention. Hydrological features such as residence time and turbulence select for various taxa (e.g., Anderson, 1998; Hallegraeff and Fraga, 1998). A major question in HAB biogeography is whether there are biotic provinces that are conductive for specific types of HAB species. Indeed, there are many examples of recurrent HAB in specific biomes, and a few such examples are highlighted here. Highly dynamic estuaries typically have a phytoplankton assemblage distinct from that in quiescent coastal lagoons. The former is more often dominated by diatoms, the latter often by picoplankton such as picocyanobacteria, as in the case of Florida Bay, or pico-pelagophytes, such as "brown tides" *Aureococcus anophagefferens* in Narragansett Bay, Rhode Island; Great South Bay, New York; and the Maryland Coastal Bays, and *Aureoumbra lagunensis* in Laguna Madre, Texas (Buskey *et al.*, 1997; Glibert *et al.*, 2010, and references therein).

It is now well recognized that HAB are a regular and fundamental feature of upwelling marine systems (e.g., Margalef, 1978; Pitcher *et al.*, 2005, 2010; Pitcher and Weeks, 2006). Upwelling may impact coastal phytoplankton blooms and drive new production, but importantly, depending on the coastal configuration, there may be sites where an oceanic supply of nutrients, coupled with terrestrial sources of nutrients and retention zones, may concentrate blooms. Generally, the seasonal succession of microalgae in upwelling systems mirrors that of most temperate systems, with spring diatoms giving way to increasing contribution of dinoflagellates by late summer/early fall (Pitcher *et al.*, 2005, 2010). As an example, the regularity of seasonal transitions between upwelling and downwelling favorable conditions, and the seasonal succession of HAB, has been shown for the Galician coast. There, chain-forming diatoms, including toxic *Pseudo-nitzschia* spp., dominate spring and summer upwelling events when N species are dominated by NO_3^-, but as summer progresses during stratified conditions, dinoflagellates, and particularly the genera *Ceratium*, *Dinophysis*, *Protoperidinium*, *Gymnodinium*, *Gyrodinium*, and *Prorocentrum*, increase in abundance as the N species shift to more regenerated forms (Figueiras and Rios, 1993; Moita, 2001). By the end of summer, heterotrophic dinoflagellates (e.g., *Noctiluca scintillans* and *Mesodinium rubrum*) are increasingly present (Cabeçadas *et al.*, 1983). Finally, blooms of efficient swimmers, including the toxigenic chain-forming dinoflagellate *Gymnodinium catenatum*, occur during the upwelling–downwelling transition (Moita *et al.*, 1998).

As summarized by Pitcher *et al.* (2010), coastal upwelling contributes to the enrichment of surface waters and high productivity, and also contributes

to the transport or export of production from the shelf and coastal regions (Largier *et al.*, 2006). Coastal features such as banks, canyons, or islands have influences on upwelling and its local spatial effects. This is exemplified in the effects of local coastline on blooms in the Santa Barbara Channel. In this region, there are various oscillations of upwelling, relaxation, and convergence. At times these features can form eddies that concentrate plankton (Nishimoto and Washburn, 2002), including HAB. The formation of such eddies, following local upwelling in the Santa Barbara Channel, strongly influences the distribution and toxicity of blooms of *Pseudo-nitzschia* spp., moving the most toxic cells to the center of the eddy (Anderson *et al.*, 2006). Similarly, in the northern California Current system, the Juan de Fuca eddy, a cold, cyclonic gyre located over the continental shelf near the mouth of the Juan de Fuca Strait is associated with advection of nutrient-rich water to the surface by estuarine circulation. The eddy, which increases over the course of the summer as the California undercurrent water is upwelled, is characterized by enhanced phytoplankton biomass and has been implicated as a site for the initiation of toxic *Pseudo-nitzschia* affecting the Washington coast, particularly during summer and autumn (Trainer *et al.*, 2002, 2009). These toxic blooms only impact coastal environments when subsequent downwelling favorable conditions exist, allowing the toxic cells to exit the eddy and to be advected onshore (MacFayden *et al.*, 2005).

Small-scale turbulence and stratification also can play important roles in bloom aggregation, especially in regions offshore, away from shallow waters where tidal mixing and wind mixing dominate. HAB often occur when such systems are highly stratified, in some cases at the very smallest of scales. Subsurface layers of HAB have often been reported in highly stratified waters, wherein the HAB cells accumulate at the intersection of light and nutrients. With a source of light from above and nutrients from below, the subsurface layer provides a habitat where both resources are low but neither inhibits growth. Thin layers, in some cases only centimeters to meters thick in the ocean water column, can be sites of dense HAB aggregations. Bjørsen and Nielsen (1991) described a "magic carpet" of *Gyrodinium aureolum*, while Gentien *et al.* (1995) noted that some thin layers have up to 100% dominance of dinoflagellates.

In addition to the physical aggregation of cells in stratified systems, and especially within thin layers, such layers also provide unique microhabitats for HAB. These microhabitats are often reducing environments (Wang *et al.*, 2015); thus, chemically reduced forms of N are more prevalent than oxidized forms of N. Heterotrophic nutrition is also common in species that dominate thin layers. *Dinophysis*, for example, depends on mixotrophy. There can also be microstructure in the layers. In the thin-layer structure of the Bay of Biscay, for example, it was shown that one layer consisted of *Chaetoceros sociale* and a second layer consisted of *Dinophysis acuminata* (Lunven *et al.*, 2005). Thus, HAB species are capable of finding habitats in which their adaptations are advantageous for acquiring the specific nutrients they need and prefer.

1.4.2 Physical Dynamics: Anthropogenic Hydrological Changes

In addition to natural hydrological features such as upwelling, anthropogenic activities are altering hydrology in many regions. River discharge is changing throughout the world due to dam construction and other in-river consumptive uses. Dams capable of generating more than 1 megawatt electricity that have been constructed, or are under construction, number in the thousands (Zarfl *et al.*, 2015), and extremely large dams (capable of generating >1 gigawatt) are primarily located in Asia along the Changjiang River Basin (e.g., the Three Gorges Dam), and along the Amazon Basin. These dams have significant and complex effects on river flow and accordingly on environmental conditions conducive to HAB. In addition to the reductions in overall river flow that occur from dam construction (and their associated reservoirs), large river systems with dams can become fragmented, preventing free movement of organisms, and severe modification of river flow alters temperature regimes, dramatically reduces sediment transport (Vörösmarty *et al.*, 2010; Lehner *et al.*, 2011; Liermann *et al.*, 2012), and alters nutrient loads and proportions in downstream waters. For example, retention of Si upstream following construction of the Three Gorges Dam in China has been considered an important factor leading to altered nutrient proportions favoring HAB in the East China Sea (e.g., Zhang *et al.*, 2015).

In the United States, where dam construction is no longer accelerating and in fact is declining, critical issues of upstream consumption versus downstream flow and effects on microbial biodiversity are increasingly argued in court (e.g., Pillion, 2014). Reductions in flow due to upstream

consumption in that case have been related to reductions in overall nutrient loads to the downstream estuary, leading to altered phytoplankton assemblage composition including increased abundance of picocyanobacteria and some HAB at the expense of diatoms (Viveros Bedoya, 2014). Estuarine flow is also highly regulated in the San Francisco Bay Delta (Kimmerer, 2002; Winder *et al.*, 2011; Glibert *et al.*, 2011), and strong, bivariate correlations have been reported between flow and organisms of all levels in the food web. In that estuary, sufficient flow is rigorously maintained through strictly established flow criteria in order to increase the low-salinity habitat for endangered fish. Of particular significance is that over the past several decades (prior to the drought beginning in 2013), the phytoplankton assemblage of the Bay Delta shifted from large diatoms, which were common up to the mid-1970s, to smaller flagellates and cyanobacteria since the mid-1980s (Lehman *et al.*, 2005, 2008; Brown, 2010; Glibert *et al.*, 2011), as a consequence of managed flow, increased pollution by N, and altered grazing communities. Important lessons emerge from both examples: altered flow can change the export of nutrients in terms of total loads and proportions, and these changes can create "windows of opportunity" for HAB to develop or at least create conditions where phytoplankton assemblages shift in composition.

1.4.3 Reinforcing Feedbacks

1.4.3.1 Trophic Disruptions

Harmful algal species can also proliferate because of grazing failure, assuming that physicochemical factors (such as nutrients, salinity, light, and temperature) are favorable (e.g., Buskey *et al.*, 1997; Hart, 2006; Kang *et al.*, 2015; Smayda, 2008). Recent work by Harvey and Menden-Deuer (2012), for example, documented fleeing behavior by the toxigenic raphidophyte *Heterosigma akashiwo* in response to a ciliate predator and predation-derived cues, wherein predator-induced changes in the HAB species movements led to a reduction in encounter rate and a threefold increase in net algal population growth rate. Such behaviors that significantly reduce predation pressure were suggested as a new mechanism for HAB formation.

Bakun and Broad (2003) suggested that "loopholes" in the fields of biological control organisms can result from disruptive environmental perturbations, and this may lead to highly successful recruitment of different species. Building on this idea, Irigoien *et al.* (2005, p. 313) proposed that perturbations in factors such as nutrients and light alter the microbial loop, whereby the harmful algal species can escape predation pressure and form blooms. Using data on phytoplankton and microzooplankton (ciliates and heterotrophic dinoflagellates) biomass from 12 geographic regions, they proposed that HAB can escape microzooplankton control through predation avoidance mechanisms (e.g., larger size, colonies, spines, and toxins) at bloom initiation. Total exclusion from grazing is not required; only a disparity of grazing rates is needed, so that the harmful algal species attains positive net growth while other phytoplankton remain under grazing control. In support of this notion, Mitra and Flynn (2006) reported that HAB can develop through a "self-propagating failure of normal predator-prey activity, resulting in the transfer of nutrients into HAB growth at the expense of competing algal species." The rate limitation of this nutrient transfer provides continual nutrient stress that results in various grazing-deterrent behaviors by the harmful species, protecting them from grazing control. This process can be self-stabilizing as long as nutrient demand exceeds supply, which would be most likely under eutrophic conditions with skewed nutrient ratios.

Many HAB also cause subtle, indirect, or complex changes to food webs and trophic interactions. For example, toxicity or allelopathy may serve as an inhibitor of competitors and prey, allowing organisms that would not otherwise have a growth benefit to thrive without competition. Of course, the most notable effect on food webs is fish kills that many harmful algal species can cause through toxicity, gill clogging, and/or oxygen depletion. Direct toxic effects on grazers of a wide range of HAB species have been documented. As examples, toxins from *Karenia mikimotoi* can inhibit zooplankton grazing and growth of competing algae (e.g., Hansen, 1995; Gentien, 1998). In the toxigenic haptophyte *Prymnesium parvum*, the cellular toxins also poison or deter grazers (Granéli and Johansson, 2003), thereby decreasing grazing rates and, in turn, positively influencing growth of the toxic algae. Chapter 7 of this volume describes the food web effects of HAB in detail; here, the point is emphasized that as a HAB becomes established, changes in the food web may contribute to conditions promotive of further HAB maintenance.

Various disruptions of trophic interactions caused by HAB are well documented (e.g., Sunda *et al.*, 2006, and references therein). If such

disruptions lead to reductions in grazing, the bloom taxa can be maintained at a lower growth rate since compensation for grazing loss is not needed. As examples, blooms of the picoplankter *Aureococcus anophagefferens*, common for many years in the coastal lagoons of Long Island and Maryland, had severe negative effects on bay scallops, causing mass mortality and recruitment failure (Bricelj *et al.*, 1989), and also negatively affected growth of juvenile and adult hard clams (Greenfield and Lonsdale, 2002; Wazniak and Glibert, 2004). The small size, unpalatability, and/or nutritional quality of the brown tide species were thought to cause these adverse effects, rather than direct toxicity (but note that toxic activity of this species from a dopamine-like substance that inhibits feeding may also have been a factor; see Bricelj and Kuenstner, 1989; Draper *et al.*, 1990; Gainey and Shumway, 1991). Microzooplankton grazing was also reduced (Gobler *et al.*, 2002), and may have contributed to the prolonged maintenance of these blooms. Blooms of another picoplankter, *Synechococcus*, common in subtropical coastal lagoons like Florida Bay (Glibert *et al.*, 2004, 2010), also have been shown to negatively affect grazers. In this case, losses of filter feeders such as sponges may help prolong these blooms (e.g., Hall *et al.*, 1999; Phlips *et al.*, 1999). The release of polysaccharides by *Synechococcus*, another physiological "overflow" metabolic pathway, is believed to obstruct the canal system of the sponges and thereby disrupt normal feeding (Sunda *et al.*, 2006, and references therein). Therefore, in a natural algal assemblage, some HAB species may be outcompeted by more rapidly growing species, but when grazers are disrupted and nutrient supply favors the HAB species, the probability of their success increases greatly. Thus, growth rate alone need not be the best "strategy" for bloom success; slowly growing HAB can become established and even dominant if their competitors or grazers are inhibited.

1.4.3.2 Biogeochemical Alterations

Many HAB species, if able to establish and grow, can alter the chemical or biogeochemical environment in ways that may further sustain them. In highly productive, high-biomass systems, pH values vary substantially over diel periods or over the life span of a bloom. In highly productive freshwaters and estuaries, the pH trajectory is often one of increase, rather than the decrease that is the dominant direction of change occurring due to increased CO_2 and climate change; pH values > 9 commonly occur during dense blooms (Shapiro, 1997; Jacoby *et al.*, 2000; López-Archilla *et al.*, 2004; Glibert *et al.*, 2011; Gao *et al.*, 2012). Many HAB species are superior competitors under elevated pH, as many have highly effective C-concentrating mechanisms that allow them to sustain photosynthesis when other algae become C-limited or otherwise stressed from exposure to elevated pH (Jähnichen *et al.*, 2007; Glibert *et al.*, 2011, and references therein).

These dynamic pH swings create different challenges for organisms than do the acidification effects of acid deposition and climate change that affect oligotrophic waters (e.g., large oligotrophic lakes and pelagic ocean waters). Such elevated pH conditions may, in turn, alter bacterial metabolism and may also affect the biogeochemical cycling of N, including the chemistry of NH_4^+ and NH_3 and processes such as nitrification, denitrification, and dissimilatory NO_3^- reduction to NH_4^+ (DNRA), as well as the efflux of P from the sediment (Seitzinger, 1991; Kemp *et al.*, 2005; Tank *et al.*, 2009; Gao *et al.*, 2012; Figure 1.5). As the pH increases, fundamental physical-chemical relationships related to P adsorption and desorption change, leading to enhanced release of this important nutrient (Jordan *et al.*, 2008; Glibert *et al.*, 2011, 5). Enhancement of sediment P release under elevated water-column pH conditions has been observed in eutrophic lakes and estuaries (Andersen, 1974; Drake and Heaney, 1987; Jensen and Andersen, 1992; Xie *et al.*, 2003) and tidal freshwater/oligohaline estuaries, and the success of the cyanobacterium *Microcystis* in high-biomass blooms has been attributed to that effect (Seitzinger, 1991; Glibert *et al.*, 2011; Gao *et al.*, 2012). While P may not be available in the water column in concentrations that would be considered sufficient to sustain these blooms, the rate of recycling from the benthos can supply the required P and/or N. Efflux of NH_4^+ also increases under elevated pH. The elevated pH conditions that are created under high-biomass blooms may be unfavorable for many algal species, but not necessarily for the HAB species.

MacIntyre *et al.* (2004) suggested another biogeochemical feedback pathway that may contribute to some HAB, wherein the accumulation of biomass and the concomitant reduction in light attention lead to loss of productivity at the benthos due to light shading. If benthic microalgae decrease, competition for nutrients at the sediment surface is reduced, favoring the water-column species. Loss of benthic primary producers may also increase the rate of flux of nutrients from the sediments because the benthic microalgae are

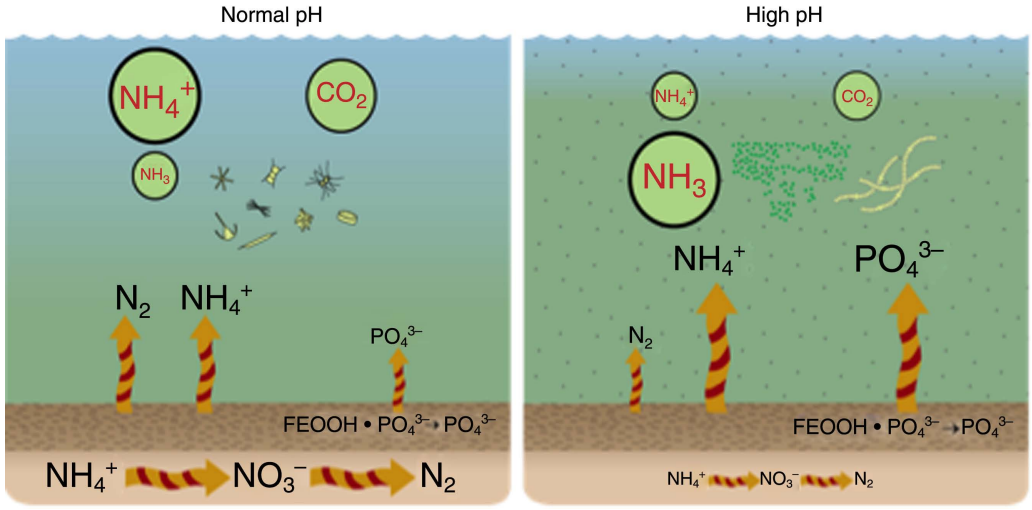

Figure 1.5 Conceptual diagram of the effect of altered pH and altered salinity on the processes of exchange of PO_4^{3-} and NH_4^+ from the sediment to the water column. With a rise in pH, or a shift to higher salinity, the sediment flux of NH_4^+ and PO_4^{3-} increases via the mechanisms described in text. The pH also alters the equilibrium between NH_4^+ and NH_3, leading to higher NH_3 at high pH. *Source*: Reproduced from Glibert *et al.* (2011) with permission of Taylor & Francis.

no longer present to "intercept" these nutrients at the sediment–water interface (*sensu* Carlton and Wetzel, 1988). Also, loss of benthic algae can destabilize the cohesion of sediments, allowing for increased nutrient flux and resuspension under wind events (MacIntyre *et al.*, 2004; Sunda *et al.*, 2006). These examples are just a few of the types of feedbacks that can help to sustain a system in a condition suitable for HAB species once they have become established.

1.4.4 Climate Change

Climate controls many of the fundamental parameters regulating algal growth, including water temperature, nutrients, light, and grazers, and thus can be expected to influence changes in the species composition, trophic structure, and function of aquatic ecosystems. Global temperatures are on the rise, a fact now well accepted to be related to anthropogenic activities (Intergovernmental Panel on Climate Change [IPCC], 2007, 2014). Average sea surface temperatures are expected to rise as much as 5 °C over the coming century, leading to a freshening of many oceanic regions due to ice melt and altered precipitation (e.g., Moore *et al.*, 2008; Doney, 2010; Fu *et al.*, 2012b, and references therein). These changes, in turn, will alter stratification, availability of nutrients and their forms and ratios, pCO_2, and light regimes, and grazing activity among other factors (e.g., Boyd and Doney, 2003), all of which control the extent to which HAB become established, recurrent features.

Temperature affects growth rate, motility, germination, pigment content, enzyme reactions, photosynthesis, and various other processes, affecting the ability of cells to thrive in a particular area (e.g., Wells *et al.*, 2016, and references therein). Increasing temperatures positively affect taxa with higher temperature optima for growth, and negatively influence taxa that have lower temperature optima. Negative influences of increasing temperature disproportionately affect diatoms, as they generally thrive in colder seasons, are more abundant in polar and temperate regions, and tend to have colder temperature optima than other microalgal groups (Harris, 1986; Graham *et al.*, 2016, and references therein). Moreover, rates of uptake of NO_3^- and of its reduction to NH_3 generally decrease at higher temperatures, especially above 15–18 °C (e.g., Lomas and Glibert, 1999a; Glibert *et al.*, 2016), further suggesting that diatoms are negatively affected as temperatures rise. In contrast, many cyanobacteria and dinoflagellate species, including HAB species, prefer warmer temperatures (e.g., Paerl and Huisman, 2008; Paerl and Scott, 2010). Temperatures also affect the consortium of organisms that co-occur with the harmful algal species, including bacteria, viruses, competing phytoplankton taxa, and grazers (Wells *et al.*, 2016, and references therein). Toxicity of many harmful algal species also increases with warming (Davis *et al.*, 2009; Fu

et al., 2012, and references therein). The combination of elevated pCO_2 together with nutrient limitation and altered nutrient ratios appears to be especially potent in affecting the toxicity of some harmful algal species. On the other hand, for some species, higher toxicity associated with warming may promote slower growth rates (e.g., Ogata *et al.*, 1989; Lewis *et al.*, 1993), but, as noted in this chapter, slower growth rates need not be detrimental to HAB formation.

Examples can be found through the United States and European coasts where long-term data are available, showing positive correlations between increasing mean water temperature and shifts in HAB species and the timing of their outbreaks. Warmer temperatures can contribute to range expansion of particular species. Higher temperatures promote increased water-column stability and increased thermal stratification which, in turn, favor known bloom-forming, toxigenic cyanobacterial species that control their vertical position through internal buoyancy regulation (e.g., Walsby, 1975; Visser *et al.*, 2016, and references therein). Under higher vertical mixing, diatoms are superior competitors; thus, as stratification increases, diatoms are more apt to sink out of the water column (Visser *et al.*, 2016, and references therein). Moreover, at higher temperatures water can become less viscous, buoyant cyanobacteria can change their vertical position more rapidly, and sinking diatoms sink faster (e.g., O'Neil *et al.*, 2012, and references therein). Thus, with climate change, diatoms may be negatively affected in several ways.

As mentioned, warming trends are causing changes in the C cycle, resulting in acidification of the oceans and some estuaries, an effect on pH opposite that due to high biomass blooms and their drawdown of CO_2. As pH declines, there is some evidence that some cyanobacteria can increase growth rates and therefore outcompete eukaryotic algae under such conditions (O'Neil *et al.*, 2012, and references therein). Flynn *et al.* (2015), in a series of experiments coupled with modeling approaches, illustrated the complexity of effects of ocean acidification coupled with eutrophication (and oligotrophication) on phytoplankton species succession. They reported a potential for altered primary production, depending on local conditions and bloom composition. Their work also suggested that, when coupled with effects on grazers, ocean acidification and eutrophication will increase the frequency of HAB, including blooms of mixotrophic species.

The interacting effects of pCO_2, temperature, and nutrient supply complicate the interpretation of effects of pCO_2 on HAB toxicity (e.g., Boyd and Hutchins, 2012; Gobler *et al.*, 2016). High CO_2 may also affect toxicity of HAB through various routes. An overall trend of increasing toxicity with increasing pCO_2 has been reported for numerous strains of cultured *Alexandrium ostenfeldii* (Kremp *et al.*, 2012), but culturing approaches can affect the extent to which relationships are observed between elevated pCO_2 and toxicity in some harmful algal species (Van de Waal *et al.*, 2011). The synthesis of at least some toxins is light dependent, as for karlotoxin production by *Karlodinium veneficum* and saxitoxin production by *Alexandrium catenalla* (Proctor *et al.*, 1975; Adolf *et al.*, 2008), suggesting that as photosynthesis is affected by changing pCO_2, toxin synthesis is also altered. Species that produce copious amounts of reactive oxygen species (ROS), such as raphidophytes (Raphidophyceae), also produce more ROS under high light conditions (Fu *et al.*, 2012b, and references therein). In the toxigenic diatoms *Pseudo-nitzschia multiseries* and *P. fraudulenta*, concentrations of their toxin, domoic acid, have been shown to increase at high CO_2/low pH (e.g., Sun *et al.*, 2011; Tatters *et al.*, 2012). This effect has been more pronounced when cells were nutrient-limited, or when forms of N shifted from oxidized to reduced forms (Glibert *et al.*, 2016, and references therein).

Climate change may further influence harmful algal species expansions due to altered precipitation patterns, including increases in droughts in some regions and increased frequency or intensity of storm events in others. Episodic storm events and climate variability affect the timing of freshwater flow, water residence times, the magnitude and timing of nutrient pulses, and resulting biotic responses; in other words, these factors affect the magnitude and timing of how nutrients "get there" (e.g., Miller *et al.*, 2006; Burkholder *et al.*, 2006; Mallin and Corbett, 2006; Paerl *et al.*, 2006; Heisler *et al.*, 2008). As examples, within days after Hurricane Isabel in 2003, a large phytoplankton bloom developed in Chesapeake Bay, linked to increased nutrient loads (Miller *et al.*, 2005). Algal blooms and extensive hypoxia/anoxia occurred shortly after several hurricanes affected the lagoonal Neuse River Estuary in the 1990s (Burkholder *et al.*, 2004, 2006). A bloom of the picocyanobacterium *Synechococcus* in eastern Florida Bay, lasting more than 18 months, followed an injection of nutrients from the high freshwater discharge caused by Hurricanes Katrina, Rita, and Wilma in 2005 (Glibert *et al.*, 2009). Also in Florida, releases of Lake Okeechobee water to the lagoons

along the northeast were accelerated in 2016 due to heavy rains, and the nutrient pollution carried with these flows resulted in what was termed a "guacamole-thick" bloom of cyanobacteria, mostly *Microcystis* (Mettler, 2016).

In coastal lagoons, where riverine input is not the dominant source of nutrient delivery, climate variability can alter the input of groundwater nutrients (e.g., LaRoche *et al.*, 1997). Long-term changes in, or intensification of, climate forces such as monsoons or interannual oscillations, such as those related to the El Niño Southern Oscillation (ENSO), or longer term cycles, such as the North Atlantic Oscillation (NAO) and Pacific Decadal Oscillation (PDO), can also alter conditions for HAB species. In the northern Iberian Peninsula, for example, the harmful dinoflagellate *G. catenatum* was abundant during the mid-1980s when there was a transition from downwelling-favorable conditions to upwelling-favorable conditions, following a shift in the NAO index (Alvarez-Salgado *et al.*, 2003). In late 2013, and continuing through much of 2016, anomalously warm water developed in the northeastern Pacific Ocean (e.g., Bond *et al.*, 2015; Freeland and Whitney, 2015), a feature associated with the unusually strong El Niño event and the PDO. The warm water moved over the continental margin, eventually extending from southern California to Alaska by spring 2015. Coupled with seasonal upwelling, conditions were ideal for *Pseudo-nitzschia* that had sufficient nutrients, and the right nutrient forms, to proliferate and suitable temperatures for rapid growth. Regulatory limits of domoic acid were exceeded along the entire coast for months, and toxin impacts were sustained at many levels of the food web, from razor clams and Dungeness crabs to sea lions and whales and porpoises (McCabe *et al.*, 2016). This was the largest toxic *Pseudo-nitzschia* bloom on the West Coast thus far, and portends of future outbreaks with conditions of increasing temperature and nutrient supply. Collectively, the intersection of nutrient loading with changes in precipitation patterns, temperature, and CO_2 patterns alters the trajectory of both cells and nutrients to "get there," and the resulting environmental conditions make it more conducive for the harmful algae to "be there."

1.5 Conclusions

This review has emphasized that the success of harmful algal species, and therefore the cause of blooms, lies at the intersection of environmental conditions, particularly nutrient conditions, physiological adaptations of the harmful species (or strain), interactions with co-occurring organisms (trophodynamically and biogeochemically), and physical dynamics that can serve to aggregate or disperse cells or their nutrients. There is ample evidence to conclude that the global expansion of HAB, both marine and freshwater, is due to anthropogenic changes. The anthropogenic footprint is not, however, limited to nutrient pollution. Anthropogenic activities have affected trophodynamics from both overfishing and intensive aquaculture, hydrodynamics of major river systems and flow due to dam construction and increasing consumptive water demands, and climate.

In all, there is overwhelming evidence that both species introductions and nutrient loads are changing regionally and globally; they "get there" and are "getting there" more often, through many sources and pathways. Much has been learned about the physiological adaptations of many phytoplankton, both HAB and non-HAB species, including their differing optimal N:P ratios; their preferences for, or tolerance of, different nutrient forms; and pathways of "overflow metabolism" under conditions of excess nutrients. They have good physiological reasons for "being there." Physical dynamics that lead to retentive zones or favorable hydrographic conditions, including anthropogenically altered flow dynamics, help cells "stay there." Changes in climate and other reinforcing feedbacks, through trophodynamic and biogeochemical changes, also contribute to HAB "staying there" when they occur. Warming temperatures, precipitation changes, changes in water-column acidification due to changes in atmospheric CO_2, and alteration of the C system due to excessive productivity (basification from dense bloom formation), coupled with changes in water-column stability, are creating many "windows of opportunity" for HAB to thrive. More blooms are occurring in more places, more often, and lasting longer. There are, of course, various examples where blooms have occurred uncoupled to anthropogenic changes. Nevertheless, production of new biomass requires nutrients. Therefore, for HAB blooms that develop high biomass above what is naturally supported, an exogenous nutrient supply must have been provided. Anthropogenic nutrient pollution is a common source of these exogenous nutrients. Warmer, more stable water columns help to accelerate the change in habitat suitability.

There are many opportunities to advance understanding of HAB and environmental changes. Such

advances need to be achieved across the full spectrum of scales, from the dynamic regulation of genes and physiology of different function groups and species, to the land-, sea-, and air-scape changes that are occurring due to the vast array of global changes, and the feedbacks between biogeochemistry and trophodynamics (Glibert *et al.*, 2013; Kana and Glibert, 2016). There is an urgent need to advance our conceptual understanding of nutrient regulation of algal assemblages beyond the classic focus on nutrient limitation. Compositional changes in algal assemblages occur when nutrients change in proportion or form, at all concentration levels, from limiting to super-saturating. Recognition of the vast anthropogenic effects that nutrient pollution, harvesting and production of food (including associated fertilizer use), and altered hydrodynamics for water consumption or electricity generation are having on the globe is fundamental to understanding how these changes affect ecological function and biodiversity, including microbial biodiversity.

The many challenges of an increasingly nutrient-enriched, HAB-impacted globe should be cause to motivate the development of a new suite of dynamically and stoichiometrically complex models, and new experimental investigations to provide the requisite data to parameterize them so that they are reliably predictive. The major management implication is clear: the most effective actions to reduce HAB and their impacts will be continued efforts to reduce nutrients. New management approaches that focus on dual nutrient (N and P) control and regulation of nutrient forms, beyond simply TN and TP, will be required for effective management of HAB. It must be emphasized that singular nutrient reductions, which characterized many management efforts in the past, can exacerbate the widespread imbalances in nutrient ratios. The challenges of controlling nutrients and managing HAB (getting there, being there, and staying there) will continue to be great.

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2

Detection and Surveillance of Harmful Algal Bloom Species and Toxins

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2.1 Introduction

The ability to mitigate the adverse impacts of harmful algal blooms (HAB) on humans, wildlife, fisheries, and ecosystems, as well as to identify the environmental factors driving HAB population growth and toxicity, is based largely on early detection of causative organisms and their toxins. Indeed, a recently published scientific summary of HAB aimed specifically at policy makers identified sustained monitoring activities as crucial to providing short-term warnings for health threats and long-term assessment of changes in HAB frequency and abundance (Kudela *et al*., 2015). Whereas not all potentially harmful species are capable of producing toxins, there is wide-ranging variation in the toxicity of those that can synthesize these compounds (Cembella and John, 2006; Granéli and Flynn, 2006). This variation reflects intrinsic genetic differences and/or external environmental factors that influence the expression of toxin genes. Thus, the integrated detection of organisms *and* toxins is essential to assess accurately the risk associated with an impending bloom event and to make well-informed management decisions that will adequately protect public, animal, and ecosystem health. Moreover, delivering these data in real or near-real time is critical not only for timely decision making but also for supporting predictive models used in the forecasting of HAB development and trajectories. There now exists a diverse collection of methods, tools, and advanced technologies for bloom and toxin detection and surveillance, including those suitable for *in situ* deployment or field-portable application as well as more conventional laboratory use. Many of these uniquely powerful tools are available commercially as instruments or kits that are providing the management and research communities with the ability to rapidly respond to and more thoroughly investigate HAB events, respectively.

Regardless of the specific approach or technology, all organism detection methods target one or more components within a suite of morphological, molecular, or biochemical constituents/processes that are generally common to all HAB species. These characteristics can be interrogated using a biological probe or physical principle applied in the context of a given detection technology, as summarized in Table 2.1. For example, morphological traits can be interrogated using photons or electrons as applied using light or electron microscopy, respectively, whereas signature DNA or RNA sequences can be interrogated with oligonucleotide probes or primers as applied using sandwich hybridization or quantitative polymerase chain reaction (qPCR) assays, respectively. By comparison, all toxin detection methods target either structural or functional attributes of a compound or suite of closely related compounds. Analytical and certain *in vitro* (e.g., through the use of binders) approaches rely on recognition of physicochemical characteristics of molecules, whereas other *in vitro*, as well as *in vivo*, methods take advantage of the biological activity or function of a given toxin or toxin class to generate a response in an assay or test (Table 2.2). As an example, an *in vitro* method can

Harmful Algal Blooms: A Compendium Desk Reference, First Edition. Edited by Sandra E. Shumway, JoAnn M. Burkholder, and Steve L. Morton.

Table 2.1 Summary of cellular targets that may be employed either alone or in combination for detection, discrimination, and/or identification of harmful algal bloom organisms.

Class of cellular target	Cell constituent or process	Biophysical probe/ principle	Detection technology
Cell morphology	Cell wall (e.g., frustule, thecal plates), flagella	Photons, electrons	Light microscopy, epifluorescence microscopy, electron microscopy
Cell surface moieties	Cell membrane, glycoproteins, exopolysaccharides, peptidoglycan layer, mucoid sheath	Antibodies, lectins	Epifluorescence/immuno-fluorescence microscopy, flow cytometry
Intracellular proteins	Enzymes (e.g., RUBISCO, alkaline phosphatase, nitrogenase, catalase); cellular compartment- or organelle-specific proteins (e.g., phycobilins, histones)	Antibodies	Epifluorescence/immuno-fluorescence microscopy, flow cytometry
Nucleic acids	Genes, rRNA, mRNA	Oligonucleotide probes, peptide nucleic acid probes, (q)PCR primers	Sandwich hybridization assay, fluorescence *in situ* hybridization assay, qPCR, ARISA, microarray, flow cytometry
Optical properties	Photopigments (e.g., chlorophyll, phycocyanin, phycoerythrin, gyroxanthin-diester), photochemistry (e.g., photosystem II cross-section and efficiency)	Fluorometry, radiometry, laser excitation	*In vivo* fluorescence, satellite and airborne remote sensing; *in situ* spectroradiometry, flow cytometry

Note: The various target classes are described according to their corresponding cell constituents or processes. The primary technologies used for detection of these constituents or processes are also identified, along with the biophysical probe and/or principle on which these technologies are based. See text for additional details.

Table 2.2 Summary of toxin properties that may be employed either alone or in combination for detection, discrimination, and/or identification of harmful algal bloom toxins.

Toxin property	Method type	Biophysical probe/principle	Detection technology
Physicochemical structure	Analytical	UV spectroscopy	HPLC-UV, CE-UV
		Fluorescence spectroscopy	HPLC-FLD, CE-FLD, laser-induced fluorescence
		Mass spectroscopy	Mass spectrometry (LC-MS, CE-MS, tandem, MALDI-TOF), Raman spectrometry
		Fluorometry, radiometry, laser excitation	*In vivo* fluorescence, satellite and airborne remote sensing; *in situ* spectroradiometry and flow cytometry
	In vitro	Antibodies	ELISA, lateral flow assay, Luminex® (bead-based array), surface plasmon resonance biosensor, electrochemical biosensor
		Aptamers	Surface plasmon resonance biosensor, electrochemical biosensor
Biological activity/function	*In vivo*	Whole organism toxicity	Rodent (mouse, rat) bioassay, insect/invertebrate bioassay
	In vitro	Pharmacological receptor	Receptor binding assay
		Enzymatic activity	Enzyme inhibition assay
		Cellular toxicity	Cytotoxicity assay
		Cellular morphology	Light or electron microscopy

Note: The primary technologies used for detection of toxins are identified along with the biophysical probe and/or principle on which these technologies are based. See text for additional details.

employ an antibody in an immunoassay format (e.g., enzyme-linked immunosorbent assay [ELISA]) to recognize a physical site or epitope on a toxin molecule(s), yet can also adopt a pharmacological receptor as a recognition element in a receptor binding assay to detect a toxin or toxin class based on its biological function.

In this chapter, a wide range of organism and toxin detection methods and technologies are identified and their fundamental principles of operation/conduct described. A section dedicated specifically to coverage of emerging technologies that allow autonomous, *in situ* application of certain methods is also presented. References providing the reader with detailed protocols for specific applications are included where available. When appropriate, consideration of the potential for adopting a given approach to address a specific management or research need are presented, as well as several examples where certain technologies have been deployed as components of observing systems or networks. Finally, the conclusions and prospects for future advances in bloom and toxin detection/surveillance capabilities are outlined, along with identification of outstanding gaps and priorities that need to be addressed.

2.2 Organism Detection

2.2.1 Visual/Optical

2.2.1.1 Light Microscopy (LM)/Utermöhl's

Conventional LM, long considered the workhorse of phytoplankton identification because of its broad accessibility, has been widely adopted for the detection of HAB species in field samples. Moreover, taxonomic descriptions of HAB species have traditionally relied exclusively on LM observations, until the now-routine practice of incorporating DNA sequences to define and delineate taxa more clearly was adopted (Rausch *et al.*, 1989; Medlin *et al.*, 1991). Use of LM techniques can still provide very useful qualitative, semiquantitative, and quantitative data on presence, relative abundance, and concentration of HAB taxa, respectively. Moreover, many monitoring programs, especially those in Europe where LM is the EU-required regulatory method, routinely employ such analyses to either provide early warning of bloom events or track the development and movement of a bloom population. In the case of the former application, LM can yield a very quick determination of whether a HAB organism is present and its relative abundance in a sample, which is very effective as a rapid screening for potential bloom events. By comparison, accurate quantitative information on the concentration of individual HAB species involves more tedious and time-consuming techniques (see below). Regardless of the type of data desired, a level of technical expertise is essential for the accurate identification of HAB taxa, which increases considerably depending on the taxonomic detail required (e.g., genus, species, etc.). A number of general as well as geographically specific phytoplankton identification (e.g., Tomas, 1997; Horner, 2004; Throndsen *et al.*, 2007) and microscopy methods (Sournia, 1978; Hallegraeff *et al.*, 2003; Karlson *et al.*, 2010) manuals are available, as are courses offering training in phytoplankton taxonomy and identification (e.g., the IOC-UNESCO HAB Programme Training Courses and UNESCO Advanced International Course for Phytoplankton Identification).

Sampling for LM involves either hauling a fine-mesh phytoplankton net (mesh size of 10 µm or 20 µm) through the water column, normally in a vertical direction, or obtaining a "whole water sample" (i.e., unconcentrated) in a bucket or using a standard sampling bottle (e.g., a Niskin bottle) from the surface or at selected depths. Net tows are generally considered nonquantitative, because the volume sampled is unknown, although a flow meter can be positioned at the mouth of the net to provide an estimate of the volume passing through the net. Whole water samples are the standard for generating quantitative LM cell count data (e.g., cells L^{-1}) when processed using a settling technique such, as the Utermöhl method (Utermöhl, 1958). Whole water samples can be taken from a fixed depth or by pumping water either from a fixed depth or over an integrated column of water. Cell concentration information can be used in conjunction with corresponding measurements of HAB toxin concentration to estimate bloom toxicity (based on cellular toxin quota) and thus the potential for adverse impacts to human health and local economies.

2.2.1.2 Light Microscopy/Flow Cytometry

The powerful integration of visual imaging technology with particle analyzers based on flow cytometry (FCM) or fluid flow has been realized in the form of several commercially available instruments. These instruments include the FlowCAM® (Fluid Imaging Technologies, Inc., Scarborough, Maine, United States), the Imaging Flow Cytobot (IFCB; McLane Research Laboratories, Inc., East

Falmouth, Massachusetts, United States), and the CytoSense/CytoBuoy (CytoBuoy b.v., Woerden, the Netherlands). The latter two instruments are suitable for autonomous, *in situ* deployment and will be considered separately in Section 2.4. The FlowCAM is a benchtop instrument that has some degree of portability (e.g., dockside, shipboard, etc.), and can accept either discrete or continuous flow-through sample introduction. The instrument uses a computer-controlled syringe pump to pull fluid through a flow cell perpendicular to the optical path where particles are illuminated, interrogated using optics similar to a microscope, and the resulting images captured and stored for processing and analysis based on morphological features, light scattering, and numerous morphometric and grayscale/color measurements. Laser excitation of particles (488 or 532 nm) with variable emission filters provides for fluorescence-based detection and characterization of particles. Evaluation of the FlowCAM for quantitative phytoplankton analyses, including several pros and cons, was included as part of an IOC-UNESCO manual (Karlson *et al.*, 2010). The primary advantages of this technology as compared to traditional microscopy include reducing manual labor required for sample processing and handling, acquiring nonbiased digital records and image analysis data for all particles, the potential to analyze a wide particle size range, and instrument portability; whereas disadvantages include the need for critical focus to correctly identify cells (but see Camoying and Yñiguez, 2016), multiple "runs" required to analyze a wide size range of target taxa, limited options for sample preservation when using fluorescence-based triggering, and susceptibility to heavy particulate loads in samples. Several investigators have used the FlowCAM to monitor and/or quantify certain HAB species in marine (Buskey and Hyatt, 2006) and freshwater (Tarrant *et al.*, 2009; Lehman *et al.*, 2013) systems. Acceptable correlations between FlowCAM and microscope data were generally reported, and although image recognition software was useful, accurate interpretation of images required an operator with taxonomic expertise.

2.2.1.3 *In Vivo* Fluorometry

The concentration of photosynthetic phytoplankton biomass in the water can be estimated using the technique of *in vivo* fluorometry, which has been incorporated into a wide range of laboratory, field-portable, and *in situ* instrumentation that measures artificially stimulated fluorescence. Field-portable devices can be used to obtain measurements from either discrete or flow-through, pumped samples, whereas *in situ* fluorometric sensors can acquire and transmit continuous, instantaneous measurements while submerged. "Natural fluorescence" signals from phytoplankton exposed to sunlight at the surface of the ocean can be measured using satellite-based spectroradiometric sensors (Behrenfeld *et al.*, 2009). *In vivo* fluorometry is based on the principle of fluorescence excitation/emission and the fact that when light energy (either artificial or natural sunlight) absorbed by photosynthetic pigments (primarily chlorophyll-*a*) in a phytoplankton cell is channeled to the photosynthetic reaction centers, that energy is either dissipated as heat or re-emitted as fluorescence. The intensity of the fluorescence signal emitted by a sample containing phytoplankton cells is a function of the physiological status, light history, and number of cells present, and is generally proportional to the concentration of cells over a linear range. A detailed review of *in vivo* chlorophyll-*a* fluorescence and its use in estimating phytoplankton biomass concentrations was provided by Falkowski *et al.* (2004).

Measurements of *in vivo* chlorophyll-*a* fluorescence as a proxy for phytoplankton biomass have been used extensively by research scientists and resource managers to study phytoplankton dynamics and monitor algal growth in the context of bloom development, respectively. The benefits as well as constraints of employing *in vivo* fluorescence to estimate algal biomass have been thoroughly documented (e.g., Falkowski *et al.*, 2004; Huot and Babin, 2010; Lawrenz and Richardson, 2011). The primary advantages of this approach are that a wide array of user-friendly, commercial instruments is available for various deployment scenarios (i.e., laboratory, field, and *in situ*); raw data are generated instantaneously; and a diverse global database extending over several decades is available throughout the published literature. A major limitation associated with employing *in vivo* fluorescence for HAB detection is the inability to identify or distinguish between species or genera of phytoplankton. As a result, even though bloom development may be detected rapidly and effectively, no detailed information on the presence of HAB

species or their potential toxicity is acquired, and thus *in vivo* fluorescence measurements must be accompanied by more directed analyses (e.g., microscopy, molecular methods) for targeted management applications.

2.2.1.4 Spectral Absorbance/ Spectroradiometry

The suite of photosynthetic pigments contained in a phytoplankton cell, although variable in terms of percent composition as a function of physiological status, exhibits a characteristic absorbance spectrum that can be measured when illuminated with a light source. Moreover, certain HAB species possess unique pigments that can be used to discriminate between their absorbance spectrum and those of other phytoplankton. For example, the HAB species *Karenia brevis* contains a relatively unique carotenoid pigment called gyroxanthin-diester, described by Bjørnland *et al.* (2000), that provides a biomarker for this taxon and distinguishes it from most other phytoplankton taxa (Millie *et al.*, 1995, 1997); however, several other dinoflagellates (e.g., *Karlodinium veneficum* [= *Gymnodinium galatheanum*]; Bjørnland *et al.*, 2000) as well as two pelagophytes and four haptophytes species have also been reported to contain this or a similar pigment (Zapata, 2005). Gyroxanthin-diester is targeted by the Optical Phytoplankton Discriminator (OPD; Kirkpatrick *et al.*, 2008), which uses *in vivo* spectral absorbance measurements to estimate the percent abundance of *K. brevis* based on the contribution of this carotenoid to the total absorbance spectrum. Nonetheless, knowledge of regional phytoplankton assemblages and the possible occurrence of other species containing this or other potentially diagnostic pigments is required in order to determine if such an approach is valid for a given location. The OPD will be considered further under "Autonomous, *In Situ* Technologies" (Section 2.4). Other investigators have used high-performance liquid chromatography (HPLC) to analyze extracted pigments as a means of quantifying gyroxanthin-diester concentration and extrapolating to *K. brevis* cell density (Richardson and Pinckney, 2004); yet, the high variability of gyroxanthin-diester cell quotas coupled with the high sensitivity of the HPLC method suggest that this approach is better suited for early detection and tracking of bloom populations than generating quantitative cell concentrations.

In addition to their intrinsic absorbance spectra, phytoplankton and their pigments also affect the color of surface waters in marine and freshwater systems which can be observed from both airborne and satellite-based spectroradiometers. The topic of detecting, mapping, and analysis of phytoplankton blooms using remote sensing was reviewed by Blondeau-Patissier *et al.* (2014), and an article focused specifically on developing a synthesized framework for remote sensing of HAB has also been published recently (Shen *et al.*, 2012).

2.2.2 Molecular

Molecular methods used to detect organisms are potentially faster and more accurate than traditional LM methods because of the abundance of cryptic species that cannot be differentiated by any other means and because of the extensive training required to distinguish morphologically similar species. Molecular techniques have been used for identification of phytoplankton in a wide variety of applications (Ayers *et al.*, 2005; O'Halloran *et al.*, 2006; Diercks *et al.*, 2008a, 2008b, 2008c; Gescher *et al.*, 2008a; Greenfield *et al.*, 2008). The small-subunit (SSU) and large subunit (LSU) ribosomal RNA (rRNA) genes have been established as efficient and effective targets used to characterize complex microbial samples (Amann *et al.*, 1990). Direct cloning and sequencing of the SSU and LSU rRNA genes from natural samples provide a broad view of community structure and composition (López-Garcia *et al.*, 2001), and have led to the discovery of an enormous amount of hidden biodiversity (Sogin *et al.*, 2006). Because the rRNA database continues to increase in size and scope, it is possible to design probes from higher taxonomic groups down to the species level (Guillou *et al.*, 1999; Groben *et al.*, 2004; Kumar *et al.*, 2005). Species-specific probes can be applied for the analysis of phytoplankton communities with detection by whole-cell methods in which the cell remains intact (e.g., fluorescence *in situ* hybridization [FISH]) and thus also the morphology, or by cell-free methods in which total nucleic acids are extracted and probes applied directly to the nucleic acid target (e.g., sandwich hybridization assay [SHA], microarrays, or biosensors). Detailed step-by-step protocols for nearly all of the methods described here can be found in the UNESCO manual for quantitative phytoplankton analysis edited by Karlson *et al.* (2010) and for microarrays in Lewis *et al.* (2012).

2.2.2.1 Whole Cell Format

2.2.2.1.1 Antibodies

Monoclonal antibodies (MAbs) and polyclonal antibodies have been used to detect cultured and field-collected cells of a wide variety of harmful algae (see Anderson *et al*., 1999). MAbs, which typically require development of hybridoma cell lines produced by fusing myeloma cells with spleen cells of mice immunized with the target antigen, are considerably more difficult and technically demanding to produce than polyclonal antibodies; nonetheless, immortal hybridomas yield an unlimited supply of MAbs. Anderson *et al*. (1999) have shown that immunofluorescence techniques using MAbs targeted to cell surface antigens can be advantageous because no cell permeabilization is required as in FISH, and the fluorescence intensity is usually far greater than that of DNA probes and is less affected by the physiological state of the cell; however, both empty and whole thecae can be recognized by the antibodies, so visual or flow cytometric counting is still required. Both MAbs and polyclonal antibodies can be coupled to immunomagnetic beads to achieve separation of the target cells from a mixed phytoplankton assemblage (Aguilera *et al*., 1996).

2.2.2.1.2 FISH

When probes are coupled with a fluorescent marker, the target organism can be easily identified at the LM level by a technique known as *fluorescence in situ hybridization*. The detection of different species and even the separation of closely related, morphologically similar species or strains (Amann, 1995) can be achieved. FISH has been successfully applied for the detection of harmful algae as well as other algal groups (Miller and Scholin, 1996, 2000; Simon *et al*., 1997, 2000; John *et al*., 2003; Groben *et al*., 2004; Anderson *et al*., 2005; Groben and Medlin, 2005; Mikulski *et al*., 2005; Mikulski *et al*., 2008). Probes for algal cells are usually labeled with fluorescein isothiocyanate (FITC) and fluoresce green, which can be easily distinguished from the orange autofluorescence of the cell's chlorophyll. FISH with phytoplankton for epifluorescence microscopy is normally performed on polycarbonate filters of different pore sizes with one or two fluorescently labeled oligonucleotide probes targeting the 18S or 28S ribosomal gene of the target species. The filter can be cut in several pieces for the detection of different algal species from one sample. The probes bind to their target rRNA located in the ribosomes in the cytoplasm (Figure 2.1). This results in a bright labeling of the entire algal cell because of the high number of targeted ribosomes in the cells of interest.

The broad diversity in different types of cell walls and membranes in the marine phytoplankton creates a challenge to develop a FISH protocol capable of fixing all kinds of algal cells. For example, some naked cells rupture with some fixatives (Medlin and Strieben, 2010). The saline ethanol method originally developed by Scholin and coworkers (1999, 1997, 2003) used probes with more

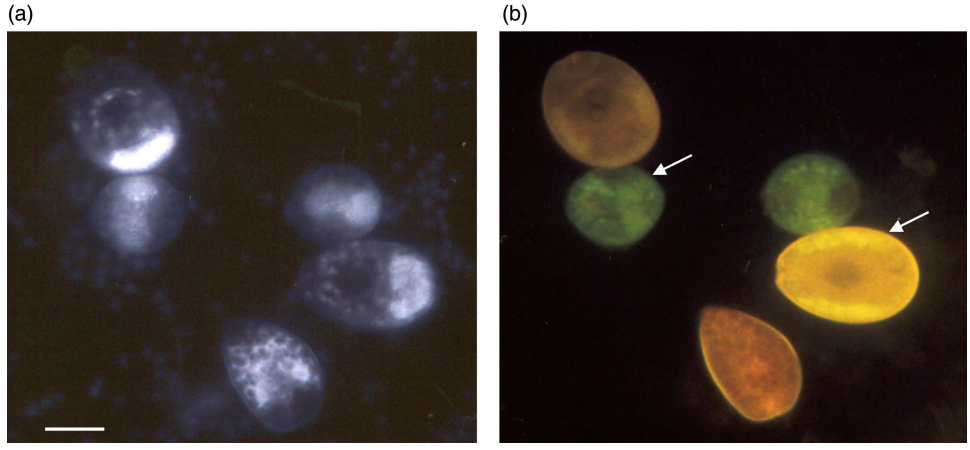

Figure 2.1 (a) DAPI (40,60-diamidino-2-phenylindole) counterstain of *Alexandrium tamarense* (SZN 01), *Prorocentrum micans* (BAHME 04), and *Prorocentrum lima* (CCMP 1743). (b) *In situ* hybridization of the same cells with probe ATAM01. Arrows indicate the positive green signal of the *A. tamarense* cell and the yellow autofluorescence of the other species. Scale bar represents 20 μm. *Source*: Redrawn from John *et al*. (2005) with permission of Oxford University Press.

than two mismatches between target and nontarget sequence. His conditions were found by Groben and Medlin (2005) not to be sufficiently stringent for a wide range of species and insufficient to distinguish single base mismatches between target and nontarget. The Scholin protocol was modified to achieve a protocol that could be used with the widest range of phytoplankton cells from the most delicate to the most rigid while maintaining the stringency needed to discriminate single base mismatches. Formamide was added to the hybridization buffer, and the salt concentration in the last washing step was reduced to make the hybridization more stringent. Formamide concentrations, which range normally between 0 and 50%, must be empirically established for each probe. It became clear during the testing of different fixation/hybridization protocols that the type of detergent used to perforate the cell membrane to allow probe entry into the cell was one of the most important components to be altered. Sodium dodecylsulfate (SDS), which is often used in hybridization buffers, destroys the more fragile cells (e.g., unarmored dinoflagellates). IGEPAL-CA630 (or the chemically identical NONIDET-P40) maintains cell stability, permitting efficient probe penetration. The latter detergent in the hybridization buffer enabled the investigation of certain delicate dinoflagellates (e.g., *Gymnodinium mikimotoi*).

The saline ethanol fixative also extracts the chlorophyll from the cells and bleaches them, thus permitting better visualization of probe signals. This is an advantage for FISH experiments with phytoplankton exhibiting strong autofluorescence; however, if autofluorescence is strong and persistent, or if even prolonged ethanol treatment is insufficient to remove all of it, 50% dimethylformamide (DMF) can be added to remove the chlorophyll from the cells more effectively than ethanol alone (Groben and Medlin, 2005). Note that DMF is toxic and should be added only if autofluorescence is persistent (Medlin and Strieben, 2010).

Although the use of rRNA probes in FISH reactions is not regularly included in monitoring programs for toxic algae, it has often been used for identification of harmful microalgal species in field samples (Scholin *et al*., 1997; Anderson *et al*., 1999; Groben and Medlin, 2005; Mikulski *et al.*, 2005). Target cells labeled by FISH can be visualized by epifluorescence microscopy or by automated cytometric techniques. The whole cell stays intact, and co-occurring phytoplankton species can be discriminated when counterstained with an overall DNA stain (Figure 2.1).

2.2.2.1.3 Flow Cytometry with FISH, CARD FISH, and Solid-Phase Cytometry

Cell counting with epifluorescence microscopy after FISH can be time-consuming and susceptible to human error. Therefore, automated counting is recommended for analyzing many samples. FCM is a suitable tool to detect and count cells after FISH. Both liquid– and solid-phase cytometers (LFCs and SPCs) are available for this operation.

LFCs measure the size of and count microalgal cells in suspension by their optical characteristics as they pass through a narrow laser (Veldhuis and Kraay, 2000). This technique can be enhanced with the addition of FISH probes for greater differentiation of phytoplankton populations. Fluorescein isothiocyanate is normally used as the fluorescent label in FISH and LFC applications, but Cy5 has also become popular as a fluorescent label in LFC protocols (Shapiro, 2003). For phytoplankton, the red labeling of the cells can be difficult to separate from the orange autofluorescence of the chlorophyll. Thus, dual labeling in one sample of phytoplankton is possible. The presence of additional lasers allows the discrimination of different species in one phytoplankton sample. The specialized analytical software supplied by the Cytosense Cytometer (see below) enables the laser to pass over the entire surface of the cell to produce enhanced detail of the cell surface and can even permit the counting of cells in a chain in contrast to the single emission data gathered by all other cytometers.

FISH for LFC has to be performed in suspension. The cells are fixed in a tube, then centrifuged, then resuspended, repeatedly, for the various stages of the FISH protocol. The biggest disadvantage of this method is the risk of losing cells during these stages. Treating the tubes with surfactants, and adding surfactants to the cells, can remedy this impediment (Biegala *et al*., 2003).

2.2.2.1.4 CARD FISH on a Slide or in Suspension for Liquid Flow Cytometry

In certain epifluorescence and FCM applications, the fluorescence signal of the FITC-labeled bound probe can be too low for detection. High autofluorescence of the microalgae and low target number from a small-sized cell or from a senescent cell, and therefore a lower cellular ribosome content, can cause low fluorescence signals. An additional reason for a low fluorescence yield can be the poor accessibility of the probe target sites in the rRNA because of its secondary structure formation and because the probe binding sites are covered by

ribosomal proteins, which can block probe penetration (Fuchs *et al*., 1998).

Underestimations of the microalgal numbers in any sample can occur because they are too dimly visible after hybridizing with monolabeled FITC probes. This can be prevented by amplification of the fluorescence signal. The tyramide signal amplification (TSA) or the catalyzed reporter deposition (CARD) is an enzyme-catalyzed enhancement method used to overcome low fluorescence signals. An enzyme, horseradish peroxidase (HRP), is linked to the 5′-end of the oligonucleotide probe, and in the presence of small amounts of hydrogen peroxide it converts its labeled substrate, tyramide, into short-lived, extremely reactive intermediates. These activated tyramides rapidly bind covalently to electron-rich regions of adjacent proteins, such as tyrosines only at or adjacent to the probe target sites where the HRP-labeled oligonucleotide probe is bound to its target (Schönhuber *et al*., 1997, 1999; Pernthaler *et al*., 2002). Thus, multiple depositions of the labeled tyramides are introduced only at the hybridization site (Pernthaler *et al*., 2002).

The fluorochrome that is bound to the tyramide could be FITC, Cy5, or Alexa fluor conjugates, which consist of a series of rhodamine-based labels with different excitation and emission wavelengths (Shapiro, 2003). This indirect labeling method yields a far greater fluorescence intensity than would ever be possible with a direct label and enables the detection of species in low abundance or senescent cells in a sample. Given that the CARD FISH method is an enzymatic reaction, the hybridization is performed between 35 and 37 °C, and therefore, higher formamide concentration of the applied probe has to be applied to ensure probe specificity. Many controls are performed to ensure that the enhanced signals are not nonspecific. TSA in combination with FISH greatly increase the intensity of fluorescence and thus raise the limit detection and the signal-to-noise ratio (Not *et al*., 2002), which is critical for small cells. The result is an enhancement of the hybridized signal up to 20 times the normal FISH hybridization signal (Schönhuber *et al*., 1997).

The TSA system has been successfully used to detect bacteria (Schönhuber *et al*., 1997), cyanobacteria (Schönhuber *et al*., 1999; West *et al*., 2001) picoplankton cells (Not *et al*., 2002, 2004; Biegala *et al*., 2003), and bacteria associated with microalgae (Alverca *et al*., 2002; Biegala *et al*., 2002). Biegala *et al*. (2003) applied CARD FISH successfully for the identification and enumeration of phytoplankton cells by FCM. Töbe (2006) applied it for the detection of *Alexandrium* spp.

2.2.2.1.5 CARD FISH on a Filter or in Suspension for Solid-Phase Cytometry

Because of their limited sensitivity, epifluorescence microscopy and LFCs do not afford the detection of low numbers of target cells. Solid-phase cytometry (SPC) is the only technique with a detection limit of one cell per sample (Lemarchand *et al*., 2001). SPC combines the advantages of LFCs and image analysis (Kamentsky, 2001). SPC allows the rapid enumeration of several thousand cells with accuracy similar to LFCs (Darynkiewicz *et al*., 2001). As compared to epifluorescence microscopy and LFCs, it detects rare events (Lemarchand *et al*., 2001). Target cells are analyzed or counted with SPC while being fixed on a solid support, such as a glass slide or a filter membrane. In contrast to LFCs, where the cells are moved through a stationary laser for excitation, in SPC the laser is moved over the immobilized cells on their membrane support (Vives-Rego *et al*., 2000). The Chem*Scan*™ system (Chemunex, Ivry, France) is a SPC for the detection and enumeration of fluorescently labeled microorganisms on filter membranes (Mignon-Godefroy, 1997; Reynolds and Fricker, 1999). This system was initially developed for the fast detection of microorganisms in filterable products in industrial and environmental microbiology; it is therefore optimized for microbiological applications with standardized protocols (Vives-Rego *et al*., 2000), and it has recently been adapted for the detection of fluorescently labeled toxic microalgae with antibodies (West *et al*., 2006) and by FISH probes (Töbe, 2006; Töbe *et al*., 2006) and bacteria with FISH probes (Schauer *et al*., 2012). Microorganisms are collected by filtration onto a membrane, fluorescently labeled, and subsequently scanned with the laser. The fluorescent events on the membrane are detected by a series of detection units, and the computer applies various discriminants (e.g., the peak fluorescence intensity of an event) that allow the differentiation of positive fluorescing events to be counted from those rejected by the analysis (Roubin *et al*., 2002).

After applying the discriminants, only a fraction corresponding to the labeled algae, the true positive events, are retained from the total fluorescing events initially detected by the Chem*Scan*, which considerably reduces the number of fluorescent objects to be checked by the user. True positive counted algal cells are shown as colored spots on a display of the membrane in a scan map on a

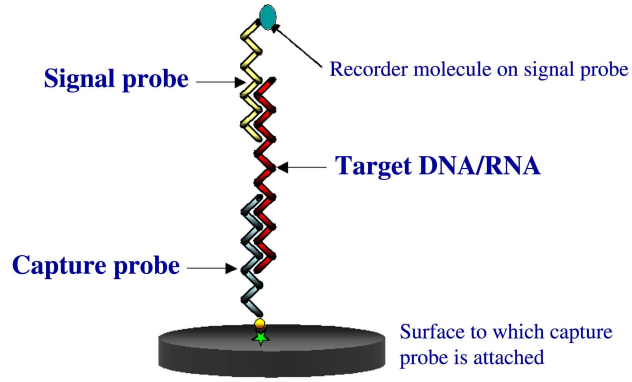

Figure 2.2 A typical construct for a sandwich hybridization. *Source*: Medlin, http://www.mdpi.com/1424-8220/17/5/1184. Licensed under CC-BY 4.0.

computer screen. After scanning, the positively counted cells can be visualized by transferring the membrane to an epifluorescence microscope, which is connected to the Chem*Scan* and equipped with a computer-controlled motorized stage. The stage can be racked to each positive data point after highlighting it for microscopical validation by the user (Reynolds and Fricker, 1999; Roubin *et al.*, 2002).

For a reliable automated detection of target cells with the Chem*Scan*, CARD FISH is required to facilitate the differentiation between target and nontarget autofluorescing cells/particles (Töbe *et al.*, 2006). The threshold for the peak fluorescence intensity as a discrimination pattern may need to be increased because the CARD FISH labeled cells reach very high peak intensities; however, SPC counting is only adequate for round and spherical cells and not for long filamentous cells, and a validation of the positive counted cells is recommended.

LFC and SPC are fast and sensitive methods appropriate for early warning of HAB when applied in combination with molecular methods, such as FISH techniques that can be used routinely for monitoring purposes. Both LFC and SPC are very expensive, but costs could be reduced in the future with increasing demand and the development of cheaper laser units. Both are limited by the number of species that can be detected with each experiment. Presently, only two different fluorochromes are routinely used for detection by these methods.

2.2.2.2 Cell-Free Format

If total nucleic acids are extracted from samples, then all cell morphology is lost. Also, free DNA from dead cells is taken as well. This has often been cited as the cause of differences in cell numbers from whole-cell methods as compared to those inferred from cell-free methods, which are usually higher (Anderson *et al.*, 2006). But despite these minimal drawbacks, several methods have been used that rely on high-quality DNA or RNA extracted from environmental samples and have been successfully applied to detect toxic algae from a number of different water types.

2.2.2.2.1 Sandwich Hybridization Assay (SHA)

In the SHA, target DNA or RNA is bound between a capture and a signal probe (Figure 2.2). Only one of the two probes needs to be specific for the target species. A capture probe is immobilized on a surface, which can be a membrane, an electrode, or a microtiter plate (Orozco *et al.*, 2011). If the target sequence binds to the immobilized capture probe in the first hybridization event, then its detection takes place during a second hybridization event with a signal probe linked to a recorder molecule (Zammatteo *et al.*, 1995; Rautio *et al.*, 2003), such as a fluorochrome or digoxigenin. An antibody to the recorder molecule is coupled to a HRP enzyme for signal amplification and forms the final complex. HRP converts inactive substrates to a product that can be detected electrochemically or colorimetrically. The colorimetric SHA offers the cheapest and fastest way to test the specificity of primer pairs (Diercks *et al.*, 2008c). Oligonucleotide probe detection assays involving the amplification of hybridization signals through enzyme tracer molecules have the potential advantage of being ultrasensitive. This assay format maximizes discrimination of the target sequences, and purification of target molecules (e.g., RNA) is not required. The SHA method has been widely used for the detection of toxic algae (Scholin *et al.*, 2003; Metfies *et al.*, 2005; Mikulski *et al.*, 2008)

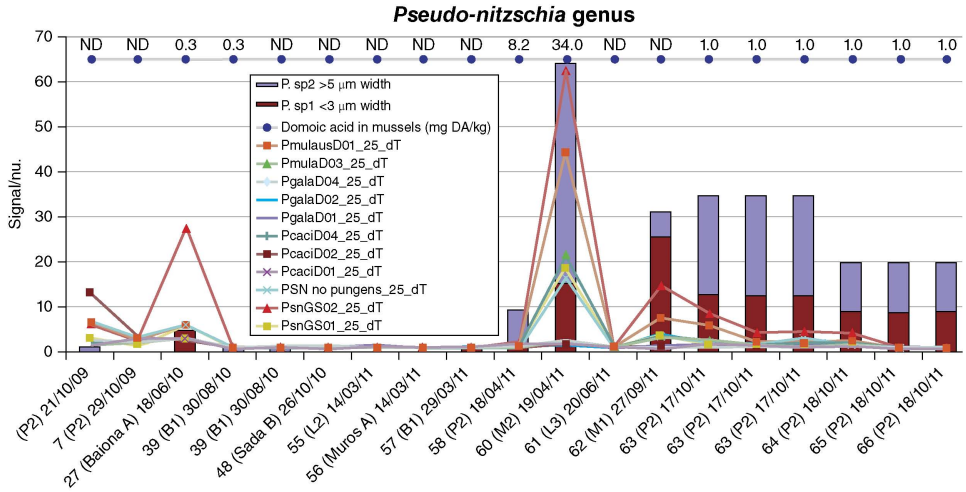

Figure 2.3 Comparison of microarray signals for *Pseudo-nitzschi*a spp. with cell counts for two size categories and mussel toxicity from the Galician Rias in Vigo, Spain. Data courtesy of Y. Pasos. Bars = cell counts. Lines over the bars are probe signal intensities. Mussel toxicity is expressed across the top of the graphs. *Source*: Figure reproduced from Medlin (2016).

and has been formatted for an automated Universal Assay Processor (Saigene Biotech, Inc.) that provides users with flexibility and control over various assay parameters (e.g., sequence, duration, and temperature of individual steps) (Marin and Scholin, 2010).

2.2.2.2.2 Microarrays (Slide-Based, Microelectrode-Based, Luminex, etc.)

A microarray consists of DNA sequences (barcodes) that are applied to the surface of a glass slide with special properties in an ordered array. It is based on a minimized form of a dot blot (Ye *et al.*, 2001; Gentry *et al.*, 2006). A DNA microarray experiment involves microarray production, sample isolation and preparation, hybridization, and data analysis. Prior to hybridization, the target nucleic acids are labeled with a fluorescent dye, which can be incorporated directly to the nucleic acid or via indirect labeling of other substances (Cheung *et al.*, 1999; Southern *et al.*, 1999; Metfies *et al.*, 2006). The hybridization pattern is captured via fluorescence excitation in a special device, the microarray scanner (Ye *et al.*, 2001). The DNA microarrays or so-called phylochips have been used to identify phytoplankton (Gescher *et al.*, 2008b; Metfies *et al.*, 2010), toxic algae (Metfies and Medlin, 2004; Ki and Han, 2006; Medlin *et al.*, 2006; Gescher *et al.*, 2008b; McCoy *et al.*, 2012, 2014; Barra *et al.*, 2013; Dittami *et al.*, 2013a, 2013b; Edvardsen *et al.*, 2013; Kegel *et al.*, 2013a, 2013b; Taylor *et al.*, 2014), bacteria (Loy *et al.*, 2002, 2005; Peplies *et al.*, 2003; 2004a, 2004b, 2006; Lehner *et al.*, 2005), and fish (Kappel *et al.*, 2003). An entire EU project, MIDTAL (www.midtal.com), was devoted to the construction of a universal microarray for the detection of toxic algae and is now commercially available (Microbia Environnement, Banyuls-sur-Mer, France). Some improvements in phylochip methodology were made in the MIDTAL project with the publication of a standardized method of hybridization, analysis, and calibration (Lewis *et al.*, 2012) to convert the signal to cell numbers for the monitoring of toxic algae. This is essential for monitoring because nearly all decisions on fisheries closure are based on cell numbers that trigger toxicity testing. This microarray was field tested for two years in five EU countries that regularly monitor for toxic algae (Figure 2.3), showing good correlations with standard cell-counting methods. Microarray analysis for environmental analysis has now received an ISO number (ISO 16578:2013 [en]) and thus is now a fully accredited method for determining the concentration of DNA in any environmental sample.

Microarrays for HAB species detection have also been formatted for multiplexed, bead-based arrays that employ flow cytometric detection of color-coded fluorescent bead populations (Xmap technology; Luminex Corp., Austin, Texas, United States). Each unique population of coded beads is dyed internally with a different ratio of two fluorophores and covalently functionalized with a species-specific capture probe that binds biotinylated target DNA. Hybridization of the target is detected using a reporter molecule (e.g., phycoerythrin coupled to streptavidin). Several groups

have explored this approach and generated encouraging results for several HAB species (e.g., Scorzetti *et al*., 2009; Diaz *et al*., 2010), but Luminex-based detection strategies are still considered research and development efforts and to our knowledge have yet to be adopted by formal HAB monitoring programs.

2.2.2.2.3 Biosensors

To overcome traditional detection and quantification limitations, biosensors are attractive candidates because they are simple, fast, and have led to the manufacture of compact and inexpensive devices (Diercks-Horn *et al*., 2011; Metfies *et al*., 2005). Electrochemical detection has low power requirements, which has made the approach sensitive, accurate, and versatile. Moreover, the ability of electrochemical sensors to identify nucleic acids directly in complex samples is a valuable advantage over other approaches, such as PCR that requires target purification and amplification (Liao *et al*., 2007) and is sensitive to enzyme inhibitors. Biosensors are powerful tools for species detection. Among them, those based on the direct electrochemical detection of nucleic acid target molecules have successfully applied the SHA method to detect toxic algae (Orozco and Medlin, 2011).

The reactions are rapid, easy to execute, and amenable to automation. Quantification of the target species can be performed by using smaller, portable, and less expensive instrumentation. Preliminary data from an innovative approach to harmful algal cell enumeration based on fiber-optic genosensors for *Alexandrium tamarense* (North American clade), *Pseudo-nitzschia australis*, and *Alexandrium ostenfeldii* have been introduced (Anderson *et al*., 2006). A faster, cheaper, and more user-friendly and reliable biosensor method, together with a handheld device for the detection and identification of the toxic dinoflagellates *A. ostenfeldii* and *Alexandrium minutum*, has been reported (Metfies *et al*., 2005). Development and adaptation of a multiprobe biosensor for use in a semiautomated device for the simultaneous detection of 14 target toxic algal species have been also recently devised (Diercks-Horn *et al*., 2011). This study has indicated that the electrochemical sensor approach has the potential for multiple species-specific detection of toxic algae. More recently, elucidation of the different steps of the biosensor fabrication process from the electrochemical point of view, proof of concept with different algal species, and evaluation of the influence of the transducer platform geometry and material in the biosensor analytical performance have been published (Orozco and Medlin, 2011; Orozco *et al*., 2011); however, a multi-analyte system not only to identify a broad spectrum of toxic algal species, but also to quantify very low concentrations of taxa without filtration of a large volume of water, is still an object of study. Proof of concept with different algal species demonstrated the feasibility of using electrochemical sensors for the detection of *Prymnesium parvum*, *Gymnodinium catenatum*, *P. australis*, *A. ostenfeldii*, and *A. minutum* (Orozco and Medlin, 2012). All components of the biosensor SHA assay have been optimized with calibration curves for 14 toxic algal species (Orozco *et al*., 2016).

2.2.2.2.4 qPCR

One of the most powerful technologies in molecular biology is the polymerase chain reaction (PCR). Nevertheless, there is no information about the quantity of starting material in the sample available with traditional qualitative "endpoint" PCR. By using fluorescent markers that are incorporated into each PCR product during amplification, data are collected over the entire PCR cycle. The quantity of the amplified product is proportional to the fluorescence generated during each cycle. This is monitored with an integrated detection system during the linear exponential phase of the PCR (Saunders, 2004). As the PCR amplicon is accumulated during each cycle, the amount of starting material is directly proportional to the change in fluorescence that is measured (Figure 2.4). qPCR can discriminate base pair differences so that closely related species or populations can be distinguished. For environmental samples, an external standard for quantifying the amplified DNA is needed, which could be a dilution of plasmids or DNA derived from laboratory cultures with a known concentration of the target template. Because of differences in DNA content per cell (Handy *et al*., 2006) a standard curve must be made for each target species to infer its concentrations in an unknown sample. One should also take into account that the copy number of the rDNA genes may vary among different strains of an organism and species (Erdner *et al*., 2010).

The SYBR Green approach is the most commonly used qPCR method. SYBR Green fluorescent dye binds to the minor groove of double-stranded DNA (dsDNA). This results in an increase of the fluorescence emission proportional to an increase after each cycle in the dsDNA PCR amplicon formation. To avoid primer-dimers, which would be counted as amplified DNA because of the nonspecific binding of SYBR Green to all dsDNAs, more attention should focus on

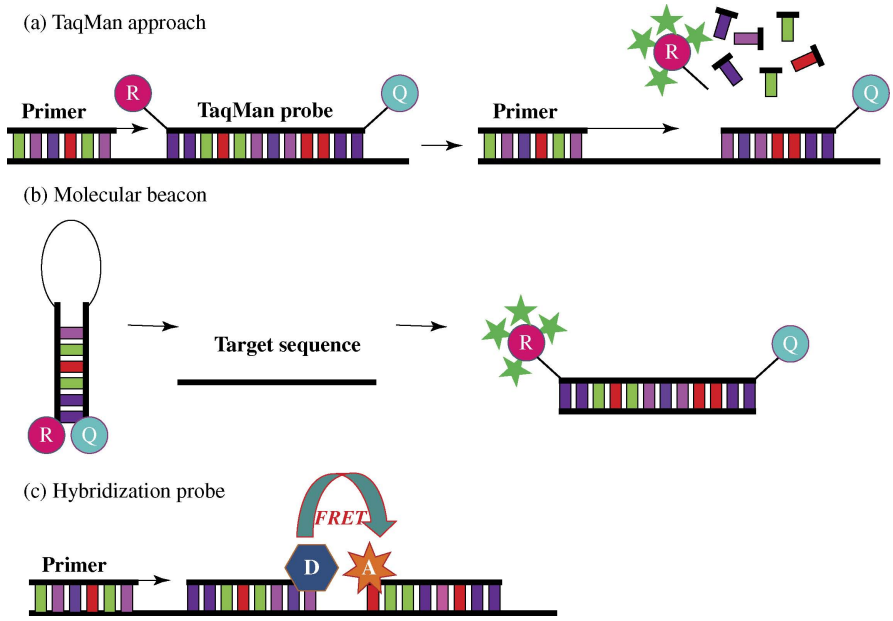

Figure 2.4 Three different types of qPCR approaches. (a) TaqMan: The fluorescence of the reporter (R) is suppressed by the intact quencher (Q). The TaqMan probe hybridizes to its target but is replaced by the Taq polymerase during product elongation. The reporter is separated from the quencher and fluoresces. (b) Molecular beacons: The probe possesses a reporter and a quencher, and the ends are self-complementary, forming a hairpin secondary structure. The reporter fluorescence is quenched, because of the directly adjacent quencher. After hybridization of the probe to its target sequence, the greater distance between the reporter and the quencher induces fluorescence of the reporter. (c) Hybridization probe technology: Two probes hybridize in close proximity to each other on the target. The narrow spatial hybridization between the donor (D) and the acceptor (A) initiates the fluorescence energy transfer (FRET) and fluorescence increases. *Photo credit*: K. Töbe.

primer design. Melting curve analyses identify primer-dimers because they have a lower melting temperature compared to that of the target amplicon (Bustin and Nolan, 2004).

In other more sensitive and specific qPCR methods, such as the TaqMan approach, and molecular beacon and hybridization probe assays, specific or nonspecific primers are paired with a specific fluorigenic oligonucleotide probe. Enhanced fluorescence upon binding of the specific probe to its target is achieved by fluorescence resonance energy transfer (FRET), which is the transfer of energy from an excited fluorophore, the donor, to another fluorophore, the acceptor (Cardullo *et al.*, 1988). A rapid and quantitative enumeration of several organisms within one sample (multiplex PCR) is achieved by using specific primers and oligonucleotide probes labeled with unique fluorescent dyes exhibiting different excitation wavelengths. The number of available fluorescent reporter dyes for the separate probes limits the number of detectable target genes in one sample. Currently, detection is limited at six species in one sample; however, multiplex qPCR experiments must be carefully optimized and often require an elaborate adaptation, notably with increasing target species in one assay (Kudela *et al.*, 2010).

In addition to organism detection, qPCR and multiplexed qPCR approaches have also targeted the genes responsible for toxin production, in cases where those genes have been identified and sequenced. An example of a multiplex (quadruplex) qPCR application was reported by Al-Tebrineh *et al.* (2012) for cyanobacterial taxa capable of producing the toxins microcystin, nodularin, cylindrospermopsin, and saxitoxin (STX). This application is now available as a commercial product (Phytoxigene™ CyanoDTec; Diagnostic Technology Pty. Ltd., Belrose, Australia). Although this approach of targeting detection of toxin genes does not provide any information on actual toxin levels present in a sample, it does yield important and useful information on the potential for toxin production. In this way, HAB subpopulations comprising potentially toxigenic strains can be distinguished from those that do not have the ability to synthesize toxins, and this information may be used to inform or trigger more targeted monitoring and management strategies that rely on detecting and measuring the toxins (Section 2.3).

Another method that is gaining popularity is the digital PCR method (Sykes *et al.*, 1992), which involves the Illumina® or 454 sequencing methods. Here, a sample is divided so that individual nucleic acid molecules within the sample are localized and concentrated within many separate regions, and this is most commonly achieved by dispersing the sample as an emulsion into microwell plates. Sample partitioning allows an estimation of the number of different molecules because the molecule population is assumed to follow a Poisson distribution. As a result, each part will contain "0" or "1" molecules (i.e., a negative or positive reaction, respectively). The template is prepared by fragmenting genomic DNA using DNase I to produce 2–4 kb fragments. The template mixture is made into droplets and paired with primer pair droplets, and both droplets enter a microfluidic chip at a rate of about 3000 droplets per second. Because the primer pair droplets are smaller than the template droplets, they move faster through the channels. Ultimately, they make contact with the preceding template droplet. Field-induced coalescence of these droplet pairs causes the two droplets to merge to produce a single PCR droplet, which is collected and processed as an emulsion PCR reaction (Tewhey *et al.*, 2009). By counting the regions that contain PCR end products as positive reactions after PCR amplification, nucleic acids may be quantified. To improve the diversity of the assay, different primer combinations can be allocated into the different plate wells. Te *et al.* (2015) compared qPCR and digital PCR (dPCR) for the simultaneous quantification of *Microcystis* and *Cylindrospermopsis* and found that the former was easier to use, but the latter was more sensitive and thus more accurate.

Potential drawbacks and limitations of qPCR could be that different DNA extractions yield different amounts depending on the extraction method used, and that the presence of humic substances could inhibit the PCR reaction. These problems can be resolved or minimized by applying a high-quality DNA isolation method. qPCR can be easily performed immediately after *in situ* sampling onboard ship or onshore, but preserved samples can also be used, although this may also be accompanied by inhibition problems. No preservation, or preservation using ethanol, coupled with freezing are preferred strategies, because it is still possible to detect and quantify target cells after three years from field samples processed in this way (Hosoi-Tanabe and Sako, 2005). Preservation with formalin and glutaraldehyde considerably lowers the sensitivity of qPCR. Lugol's iodine, another commonly used phytoplankton fixative, has been reported to lower the sensitivity of some qPCR experiments (Bowers *et al.*, 2000), but has also been successfully applied in others (Kavanagh *et al.*, 2010). In HAB studies, multiplex qPCR experiments are applied less frequently because of the extensive optimizations required to use different primers and/or probes together in one environmental sample. Handy *et al.* (2006) successfully tested multi-probing using a single primer set with species-specific probes in one assay versus multiplexing using specific primers and specific probes. They found that multiplexing was more efficient, albeit both methods were successful in detecting multiple raphidophyte species.

2.3 Toxin Detection

Algal toxins encompass a diverse array of chemicals, ranging from low-molecular-weight, water-soluble alkaloids (e.g., STX, anatoxin) and amino acids (e.g., domoic acid [DA]), to larger cyclic heptapeptides (e.g., microcystin, nodularin) and liphophilic polyethers (e.g., brevetoxin, okadaic acid [OA], ciguatoxin [CTX]). An overview of the major phycotoxin groups is presented in Table 2.3. In most cases, a given toxin class comprises a suite of similar but structurally distinct congeners. These chemical modifications can result in changes not only to the polarity and thus the solubility traits of these molecules, but also to their toxic potency, which can vary over several orders of magnitude (e.g., paralytic shellfish toxins [PSTs] or STXs). The mode of action is well established for most of the major toxin groups and does not tend to vary among derivatives of the parent compound; however, the primary biological target remains to be elucidated in certain cases (e.g., azaspiracids; Twiner *et al.*, 2014). The uniform mode of action within a toxin class can be exploited by detection methods that rely solely on biological function (e.g., receptor assays), whereas differences in chemical structure can be an impediment for techniques that depend on interaction with a recognition element, such as an antibody (Section 2.3.2).

The detection of HAB organisms (Section 2.2) provides important and useful information on bloom dynamics, including early warning of bloom development as well as details of changes in population density over time and space as an event proceeds through growth, maintenance, and

Table 2.3 Summary of algal/cyanobacterial toxin classes, their associated poisoning syndrome and toxigenic source organisms, the primary vector(s) for transmitting toxins to humans and wildlife, as well as the primary biological target(s) and activity for each toxin class.

Toxin class	Poisoning syndrome	Source organism(s)	Primary vector(s)	Biological target/activity
Saxitoxins	Paralytic shellfish poisoning (PSP)	*Alexandrium* spp.; *Gymnodinium catenatum*; *Pyrodinium bahamense*; *Anabaena circinalis*, *Aphanizomenon flos-aquae*, *Cylindrospermopsis raciborskii*, *Lyngbya wollei*, *Planktothrix* sp., *Raphidiopsis brookii*	Shellfish; crustaceans	Voltage-gated sodium channel; site 1; blocker
Domoic acid	Amnesic shellfish poisoning (ASP)	*Pseudo-nitzschia* spp.; *Nitzschia navis-varingica*	Shellfish; crustaceans; planktivorous finfish	Glutamate receptor; agonist
Okadaic acid and dinophysistoxins	Diarrhetic shellfish poisoning (DSP)	*Dinophysis* spp.; *Prorocentrum* spp.	Shellfish	Protein phosphatase (PP); PP1 & PP2A; inhibitor
Brevetoxins	Neurotoxic shellfish poisoning (NSP)	*Karenia* spp.	Shellfish; aerosol inhalation	Voltage-gated sodium channel; site 5; agonist
Ciguatoxins	Ciguatera fish poisoning (CFP)	*Gambierdiscus* spp.	Finfish (reef fish)	Voltage-gated sodium channel; site 5; agonist
Azaspiracids	Azaspiracid shellfish poisoning (AZP)	*Azadinium* spp.; *Amphidoma languida*	Shellfish	Primary biological target/receptor yet to be identified unequivocally
Yessotoxins and pectenotoxins		YTX: *Protoceratium reticulatum*, *Lingulodinium polyedrum*, *Gonyaulax spinifera* PTX: *Dinophysis* spp., *Protoperidinium* spp.	Shellfish	Primary biological target/receptor yet to be identified unequivocally
Microcystins		*Microcystis* spp.; *Anabaena* spp.; *Anabaenopsis* sp.; *Nostoc* spp.; *Planktothrix agardhii*; *Fischerella* sp.; *Phormidium* spp.; *Rivularia* spp.; *Arthrospira fusiformis*; *Tolypothrix distorta*; *Hapalosiphon* sp.; *Gloeotrichia echinulate*; *Plectonema boryanum*	Drinking water; swimming; dietary supplements	Protein phosphatase (PP); PP1 & PP2A; inhibitor
Cylindrospermopsins		*Cylindrospermopsis raciborskii*; *Aphanizomenon ovalisporum*; *Anabaena* spp.; *Raphidiopsis curvata*; *Umezakia natans*	Drinking water; swimming	Protein synthesis; inhibitor
Anatoxin-a		*Anabaena* spp.; *Aphanizomenon* sp.; *Planktothrix* sp.; *Arthrospira fusiformis*; *Phormidium* sp.; *Raphidiopsis meditteranea*; *Phormidium formosum*	Drinking water; swimming	Nicotinic acetylcholine receptor; agonist

termination phases. As outlined in the Introduction, however, the cellular toxicity of HAB organisms can vary markedly, reflecting differences in their genetic makeup combined with the effects of multiple environmental factors. This potential uncoupling of cell and toxin concentration can lead to severe over– or underestimates of risk associated with a bloom event. From a management perspective, it is thus essential to monitor bloom toxicity closely for proper management of impacts to fishery resources and drinking/recreational waters, and adequate protection of human and animal health. Identifying the environmental drivers that promote bloom toxicity represents a longstanding research goal, with the aim of informing predictive models needed to forecast the toxic impacts of HAB.

Apart from the need to measure toxicity associated with algal cells or dissolved in the surrounding water, algal toxins are well known for their ability to poison consumers of contaminated seafood, such as fish and shellfish (Rossini and Hess, 2010). Most countries have adopted a set of well-established regulatory guidelines that lay out requirements for the routine pre- and/or post-harvest monitoring of fishery products. Strict adherence to regulations is required to ensure the compliance and safety of products for commercial, recreational, or subsistence harvesting; sale on domestic or export markets; and ultimately human consumption. Therefore, detection of phycotoxins in complex matrices associated with various fishery products (e.g., bivalve and gastropod molluscs, crustaceans, finfish, etc.) is required.

Depending on if the target toxin(s) occurs in algae, seawater or freshwater, or some type of biological matrix, and whether detection is aimed at research, routine monitoring, or a specific regulatory use, each situation presents its own unique and often formidable challenges for the development and validation of reliable, robust methods. In the sections below, a variety of *in vivo*, *in vitro*, and analytical toxin detection methods are introduced. These methods exhibit a wide range of specificity, selectivity, and sensitivity, as well as different susceptibility to sample matrix interference and suitability for deployment in laboratory and/or field settings. The principle, rationale, and general conduct of the respective methods will be described along with examples of their use in research and management applications. In addition, a summary of toxin determination methods, the availability/status of a method for a given toxin class, the relative technical demand/training required to conduct a method, and the relative cost based on equipment/instrumentation and sample analysis are provided in Table 2.4.

2.3.1 *In Vivo* Assays

Because of the high acute toxicity of algal toxins to animals in general, a variety of animal assays have been developed and used. Some of these assays are more anecdotal though commonly used (e.g., feeding fish to cats to check for CTX contamination; Kemppainen *et al*., 2004), whereas others, such as the mouse bioassay (MBA) for PSTs (AOAC, 1995) have reached official validation status following interlaboratory trials. Several algal toxin groups have been studied for their effects on insects, in an effort to circumvent both ethical issues around mammalian assays and the use of toxins as model compounds for development of novel insecticides. Thus, several assays have been developed for DA and STXs using cockroaches as an animal model (e.g., Izawa *et al*., 1988; Ruebhart *et al*., 2011). Fly larvae have also been widely used for the detection of toxins, including CTXs, OA, STX, tetrodotoxin, and cyanotoxins (Labrousse and Matile, 1996; Denardou-Queneherve *et al*., 1999; Ruiz *et al*., 2010; Mosleh and El-Ela, 2011). The advantage of the fly larval assay is in the small consumption of toxin, as larvae only weigh 70 mg and die from small doses. Hence, this assay has been commonly used for screening purposes. Also, *Daphnia* has been evaluated for its sensitivity to shellfish toxins (e.g., OA; Marcaillou-Le Baut *et al*., 1994); however, aside from the ecotoxicity assays using *Daphnia*, none of the insect assays have been put forward for standardization or interlaboratory validation for algal toxins in shellfish, mostly because of matrix issues and, in the case of fly bioassays, limited availability of active larvae during winter time.

Fish embryos have been used for assessing toxicity of azaspiracid, showing that some toxins affect a wide range of genera (Colman *et al*., 2005). Mammals are all sensitive to algal toxins, ranging from small terrestrial mammals, such as mice, to larger mammals and even the largest mammal known – the blue whale (Lefebvre *et al*., 2002; Miller *et al*., 2010; Nolen, 2010; Bossart, 2011; Kirkley *et al*., 2014; Jensen *et al*., 2015; Wilson *et al*., 2016). Thus, a variety of assays have been developed using mammals, which is coherent with human shellfish poisoning; however, the endpoint in various assays is not necessarily related to human food poisoning – this is particularly true for mouse assays when the intraperitoneal route is used for exposure of the animals to the toxins. The

Table 2.4 Summary of toxin determination methods, availability and status of method for a given toxin class, relative technical demand and training required to conduct a method, and the relative cost based on equipment/instrumentation and sample analysis.

Method(s) of determination[1)]	Toxin class(es) (availability/ status of method)2,3	Relative technical demand/training[4)]	Relative cost[5)]		Selected references[6)]
			Equipment/ instrumentation	Sample analysis	
Mouse bioassay (MBA)	PST (E); BTX (E); DST (E); MC (P); NOD (P); CYN (P); ANTX (P)	S (requires training in care and handling of animals)	Range from L to M (depends on whether animal facility is on site)	L	PST: AOAC (1999); BTX: APHA (1970); DST: Yanagi *et al.* (1989), Yasumoto *et al.* (1978, 1985); MC, NOD, CYN, ANTX: Falconer (1993), Lawton *et al.* (1994)
Receptor binding assay (RBA) (plate)	PST (E); DA (P); BTX (P); CTX (P); ANTX (C, P)	Range from B to S	Range from M to I (depends on use of conventional vs. microplate scintillation counter)	L	PST: Van Dolah *et al.* (2012); DA: Van Dolah *et al.* (1996); BTX, CTX: Dechraoui *et al.* (1999), McCall *et al.* (2014b); ANTX: Aráoz *et al.* (2010)
Enzyme inhibition assay (EIA) (plate)	DST (E, C); MC (C, P); NOD (C, P); ANTX (P)	Range from B to S	Range from M to I	L	DST: reviewed by McLeod *et al.* (2015); MC, NOD: An and Carmichael (1994); ANTX: Mahmood and Carmichael (1987)
Cell-based assay (plate)	PST (P); BTX (P); DST (E); CTX (P); AZA (E); MC (P); NOD (P)	S	Range from M to I	L	PST: Manger *et al.* (1993); BTX: McCall *et al.* (2014a), Manger *et al.* (1995); DST: Ledreux *et al.* (2012); CTX: Manger *et al.* (1995); AZA: Ledreux *et al.* (2012); MC, NOD: Heinze (1996), Lawton *et al.* (1994)
Antibody-based assay (ELISA – plate and/or lateral flow device)	PST (E, C,); DA (E, C); DST (E, C, P); BTX (C, P); CTX (C, P); AZA (P); MC (C, P); NOD (C, P); CYN (C, P); ANTX (C, P)	Range from B to S	Range from L to M	L	PST: DeGrasse *et al.* (2014); DA: Kleivdal *et al.* (2007); DST: Jawaid *et al.* (2015), Johnson *et al.* (2016); BTX: Naar *et al.* (2002); CTX: Tsumuraya *et al.* (2012); AZA: Samdal *et al.* (2015); MC, NOD, CYN, ANTX: reviewed by Weller (2013)
Surface plasmon resonance (SPR) (single or multiplex; lab or field-portable instruments)	PST (P); DA (P); BTX (P); DST (P); MC (P); NOD (P)	Range from S to T	Range from I to H	Range from L to M	PST, DA, BTX, DST: reviewed by Vilariño *et al.* (2013); MC, NOD: Devlin *et al.* (2014)
Chromatography-based method (HPLC-UV; HPLC-FLD)	PST (FLD) (E); DA (UV) (E); DA (FLD) (E); DST (FLD) (P); BTX; CTX (UV, FLD) (P); MC (P); NOD (P); CYN (P); ANTX (P)	Range from S to T	I	Range from L to M	PST (FLD): Lawrence *et al.* (2005), AOAC (2005); DA (UV): Quilliam *et al.* (1995), AOAC (2000), EURLMB (2008); DA (FLD): Pocklington *et al.* (1990); DST (FLD): Uchida *et al.* (2014 & references therein); BTX (UV): Pierce *et al.* (2003); CTX (UV, FLD): reviewed by Caillaud *et al.* (2010); MC, NOD, CYN, ANTX (UV, FLD): reviewed by Moreira *et al.* (2014)

LC-MS or LC-MS/MS	PST (E, P); DA (E, P); DST (E); BTX (E, P); CTX (P); AZA (E, P); MC (E); NOD (E); CYN (E); ANTX (E)	Range from S to T	Range from I to H	Range from L to M	PST: Turner *et al.* (2015, references therein); DA: EURLMB (2010), McNabb *et al.* (2005; also for DST, AZA); DST, AZA: Braña-Magdalena *et al.* (2014), EURLMB (2015), van den Top *et al.* (2011); BTX: McNabb *et al.* (2012), Plakas *et al.* (2002); CTX: Lewis *et al.* (2009), Yogi *et al.* (2011); MC, NOD: USEPA (2015b), reviewed by Moreira *et al.* (2014; also for CYN, ANTX); CYN, ANTX: USEPA (2015a)
MALDI-TOF-MS or SELDI-TOF-MS	MC (P); NOD (P); ANTX (P)	T	H	Range from L to M	MC, NOD, ANTX: reviewed by Moreira *et al.* (2014)
Electrophoretic-based method (CE, CZE, MEKC; various detectors)	PST (P); BTX (P); MC (P); CYN (P); ANTX (P)	T	Range from I to H	Range from L to M	PST: Thibault *et al.* (1991); BTX: Shea (1997); MC, CYN, ANTX: Vasas *et al.* (2004)
GC-MS	ANTX (P)	T	Range from I to H	Range from M to H	ANTX: Rodriguez *et al.* (2006)

Note: Ranges have been provided for the estimated cost of equipment/instrumentation; however, given the fluidity and wide range of costs for the various components required to analyze a sample (i.e., supplies, reagents, labor, etc., needed for sample preparation and analysis), only costs relative to other methods are provided.

1) Abbreviations for methods: ELISA = enzyme-linked immunosorbent assay; HPLC = high-performance (or high-pressure) liquid chromatography; UV = ultraviolet detection (includes photodiode array detection [PDA]); FLD = fluorescence detection; LC-MS = liquid chromatography–mass spectrometry; LC-MS/MS = liquid chromatography–tandem mass spectrometry; MALDI-TOF-MS = matrix-assisted laser desorption/ionization–time of flight–mass spectrometry; SELDI-TOF-MS = surface-enhanced laser desorption/ionization–time of flight–mass spectrometry; CE = capillary electrophoresis; CZE = capillary zone electrophoresis; MEKC = micellar electrokinetic capillary chromatography; GC-MS = gas chromatography–mass spectrometry.
2) Abbreviations for toxin classes: saxitoxins (PST); domoic acid (DA); okadaic acid/dinophysistoxins (DST); brevetoxins (BTX); ciguatoxin (CTX); azaspiracids (AZA); microcystins (MC); nodularins (NOD); cylindrospermopsin (CYN); anatoxin-a (ANTX).
3) Abbreviations for method availability/status: E = method evaluated in single or multi-lab validation study *and/or* accepted as an Official Method (OM) or Standard Operating Procedure (SOP) (e.g., of the Association of Analytical Communities [AOAC], U.S. Interstate Shellfish Sanitation Commission [ISSC], American Public Health Association [APHA], and/or European Union Reference Laboratory for Marine Biotoxins [EURLMB]); C = commercial product; P = method appears in published literature, but no validation study conducted or OM or SOP designation.
4) Abbreviations for relative technical demand or training required: B = basic ability to follow simple protocol with no/minimal training; S = standard laboratory skills/competency with modest training; T = specialized technical training for conducting method and operating instrumentation.
5) Abbreviations for range of estimated cost of equipment/instrumentation (note: can vary from high-end laboratory instruments to low-end field-portable platforms): L = low (< $5000); M = moderate ($5000 to < $25,000); I = intermediate ($25,000 to <$100,000; H = high (> $100,000); and relative cost of sample analysis (includes supplies, reagents, personnel time required for sample preparation [e.g., extraction and clean-up], analysis, and data processing/reporting): L = low; M = medium; H = high.

(*Continued*)

Table 2.4 (*Continued*)

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animal used also represents a compromise between genetic similarity to humans (primates would be an ideal fit) and toxin consumption during the assay (the smaller the animal, the less toxin is required to conduct the assay). In coherence with general principles in toxicology, most progress has thus been made with small rodent assays (i.e., mouse and rat bioassays), which are considered in detail below.

2.3.1.1 Rat Bioassay

Following diarrhetic shellfish poisoning events in the Netherlands in the late 1970s and early 1980s, a rat bioassay, based on feeding shellfish hepatopancreas to rats, was developed (Kat, 1983). This assay was used in the Netherlands and some other EU countries for several years to protect shellfish consumers (e.g., Ireland; McMahon and Silke, 1996), but it was abandoned in the mid-1990s since the endpoint was considered too subjective for interlaboratory validation (endpoint is the consistency of feces of rats, classed into four categories from normally dry to liquid).

2.3.1.2 Mouse Bioassay

Compared to rats (ca. 120 g), mice (ca. 20 g) have the advantage of consuming less toxin to show toxic effects, and thus protocols using these animals require less sample, solvents, and other materials. All of the assays also work by injection of extracts into the stomach cavity of mice (intraperitoneal administration). This mode of administration typically requires less toxin than oral administration, as the toxins are rapidly distributed throughout the body without passing the intestinal barrier.

2.3.1.2.1 AOAC Mouse Bioassay for Paralytic Shellfish Toxins

The first systematic introduction of a MBA for detection of toxins in shellfish dates back to the 1930s, when Sommer and colleagues discovered the usefulness of this animal model to detect STXs (Sommer and Meyer, 1937; Sommer *et al*., 1937). These authors also related the toxin occurrence to dinoflagellates. This assay is based on the effects of introducing a dilute aqueous shellfish extract into the stomach cavity of a 20 g mouse. Even though the principles of the assay were developed in the 1930s, and even though initial isolation efforts of the toxin responsible started relatively early (Onoue *et al*., 1931), standardization could not take place until the 1950s when significant efforts were made to isolate STX, thereby allowing the assay to be calibrated properly (Mold *et al*., 1957; Schantz *et al*., 1957). Indeed, the response of toxic extracts in mice is dose dependent and can be calibrated for death times of 5–7 min. The protocol currently used for analysis of shellfish tissues has been validated and standardized as a method of the Association of Official Analytical Chemists (AOAC) International (AOAC, 1995). This assay was designed and validated to detect STX and its analogs, a group that is now known to contain more than 50 analogs (Wiese *et al*., 2010). It has not been reported as being susceptible to much interference from other compounds; however, tetrodotoxins have a very similar mode of action and will also be detected by this assay. The assay detection limit is around 350 to 400 µg of STX equivalents kg^{-1} of whole shellfish flesh, which is about half of the current regulatory limit of 800 µg of STX equivalents kg^{-1} of whole shellfish flesh. Because shellfish are a salty matrix and aqueous extracts contain significant amounts of salt, the method may underestimate true levels; for example, when levels of about 800 µg kg^{-1} mollusc flesh are detected by the PSP MBA, the actual concentration present may range from 1.2 to 2.0 mg kg^{-1}, due to salt effects (Anonymous, 2005). As recently shown for neosaxitoxin, not all toxins have the same relative toxicity or *toxicity equivalence factor* (TEF) in the intraperitoneal administration mode as in oral administration (Munday *et al*., 2013).

2.3.1.2.2 APHA Mouse Bioassay for Neurotoxin Shellfish Poisons

For a long time, the American Public Health Association (APHA) MBA protocol has been the only officially recognized method for assuring the safety of molluscan bivalves for neurotoxic shellfish poisoning (NSP) in the United States. It was also initially adopted for regulatory use in the management of NSP in New Zealand in 1993. The protocol for the NSP MBA is specified in the APHA procedure collection (APHA, 1970). The regulatory application of information derived from the MBA is based upon studies conducted in the 1960s that compared the incidence of human illness with the incidence of death in mice following intraperitoneal injection of crude residues extracted from shellfish in diethyl ether (McFarren *et al*., 1965). Brevetoxin structures and modes of action were not known in 1970, and the toxicity of the crude residues was expressed in terms of mouse units (MUs). One MU was defined as that amount of crude toxic residue that, on average, will kill 50% of the test animals (20 g mice) in

930 min. Any detectable level of toxin in shellfish tissue was considered potentially unsafe for human consumption. In practice, however, a residue toxicity >20 MUs per 100 g shellfish tissue was adopted, and remains the guidance level for prohibition of shellfish harvesting in the United States.

2.3.1.2.3 Mouse Bioassay for Lipophilic Shellfish Toxins

A method similar to the MBA for brevetoxins was developed by Yasumoto *et al.* (1978) for the detection of another lipophilic toxin group: OA and analogs. The protocol in this case is based on extraction of toxins with acetone. The water-miscible nature of this extraction solvent has led to many interferences, such as low levels of STX and DA; therefore, the protocol was later amended to include a solvent partition step between water and diethyl-ether to eliminate these water-soluble compounds from the crude acetone extract (Yasumoto *et al.*, 1985). The initial assay design had an observation time of 5 h; however, it was discovered relatively quickly that the assay needed to be prolonged to 24 h of observation for effective detection of fatty acid esters of the OA group (Yanagi *et al.*, 1989). This assay has never been subjected to a full interlaboratory validation; however, the procedure has been standardized as part of the efforts of the European Union Reference Laboratory for Marine Biotoxins (EURLMB, 2015). Although initially intended for detection of OA and dinophysistoxins, the efficacy of this procedure to detect azaspiracids at the current regulatory limit (160 $\mu g\,kg^{-1}$ whole shellfish flesh) has also been shown in a more recent study (Hess *et al.*, 2009). Nonetheless, these authors noted that the probability of detecting a positive at half the legal limit was only 5%.

2.3.1.2.4 Perspectives

As mentioned above, animal assays have one main advantage: they will detect all analogs of a toxin group if the lipophilicity of this group is appropriate to the extraction and cleanup procedure of the assay. They do not therefore require multiple standards and may be implemented early on after the discovery of a compound group. Also, the MBA for lipophilic toxins has been shown to be capable of detecting many toxin groups (OA, azaspiracids, brevetoxins, etc.). On the other hand, this large perimeter has also led to discovery of several compound groups that appear irrelevant to human health after all, such as pectenotoxins and yessotoxins (Lawrence *et al.*, 2011). Also, there is little room for improvement of sensitivity of animal assays, because the injection volume (1 mL) already exceeds veterinary recommendations twofold and samples cannot be concentrated easily to smaller volumes (Hess *et al.*, 2009). For such technical as well as ethical reasons, there is also a strong recommendation at the international level to diminish the use of animal assays (Hess *et al.*, 2006); however, even in EU legislation, the use of animal assays remains permitted for emerging toxins as the lack of calibrants tends to prevent the use of fully quantitative, specific methods of analysis (Anonymous, 2011).

2.3.2 *In Vitro* Assays

In vitro assays for algal toxin detection are normally grouped according to whether their response is based on signal generated via binding of the target compound (i.e., ligand) by its biological receptor (e.g., ion channel, enzyme) – *functional assays*; or results from the interaction of a natural or synthetic binder or recognition element (e.g., antibody, aptamer) with the analyte being measured – *structural assays*. Both assay categories exhibit clear advantages and disadvantages, depending on the desired application and deployment scenario (e.g., laboratory vs. field).

In general, functional assays (reviewed by Rossini, 2005; Botana *et al.*, 2009) provide information on toxic activity and can yield estimates of integrated toxic potency, because the assay response will reflect the cumulative intrinsic potency of those congeners present in the sample being tested. In other words, it will require a higher concentration of low-potency congeners to generate a signal similar to that produced by a lower level of highly potent analogs. In comparison to structural assays, this type of assay is considered to yield toxicity estimates more consistent with those reported by *in vivo* assays, although there are clearly caveats involved when attempting to extrapolate from the response of an *in vitro* assay to mammalian, whole-animal toxicity (Section 2.3.1). Nonetheless, the reliability and robustness of functional assays outside of the laboratory environment are often compromised because of the inherent instability of biological receptors when subjected to rugged field conditions. Structural assays employing antibodies (reviewed by Vilariño *et al.*, 2010; Reverté *et al.*, 2014) or, to a less frequent extent, aptamers tend to be considerably more stable than their functional counterparts and are frequently formatted in kits or as components

of biosensors that can accommodate or are targeted specifically for field-based applications. A frequently encountered drawback with structural assays is their inability to achieve consistently uniform recognition or cross-reaction across a suite of analogs within a toxin class, each with a related yet distinct chemical structure (as well as intrinsic potency). As a result, depending on the mixture of analogs present in a sample, the assay response is skewed toward those target compounds exhibiting a high level of cross-reactivity with the binder or recognition elements, even if they are in low relative abundance. In the case of immunoassays, this impediment can be addressed either by more careful selection of an antibody's target epitope that is in common across multiple toxin congeners, or by employing a "cocktail" or mixture of antibodies with different cross-reactivity profiles, with both approaches resulting in a broader assay specificity.

Below is a summary of various types of functional and structural assays, including examples for each category and how they have been applied to algal toxin detection across both research and regulatory environments. Certain benefits and limitations of a given assay are also identified in the context of management applications, although readers are referred to Chapter 10 (this volume) for an in-depth examination of HAB monitoring and management.

2.3.2.1 Functional Assays

2.3.2.1.1 Receptor Binding Assays

A majority of toxins comprising the primary phycotoxin classes target some type of biological receptor, including voltage-gated sodium or calcium channels, glutamate receptors, or nicotinic acetylcholine receptors. Although receptor assays were used initially to examine properties of ion-conducting channels and to characterize receptor–ligand interactions (e.g., Krueger *et al*., 1979), their utility for detecting toxins belonging to one or more classes that bind the same receptor was recognized quickly (Davio and Fontelo, 1984). Essentially, the only requirements for such assays were a crude or partially purified receptor preparation and a labeled form of the target toxin or functional analog that bound the same receptor site and served as a reporter for the outcome of the binding competition with or displacement by native toxin(s). The principal advantages of the receptor assay approach are: (1) the affinity of receptor–ligand (i.e., toxin) interactions generally matches or exceeds that of antibody–antigen interactions and is usually directly proportional to the *in vivo* toxic potency, although the latter can be influenced by a number of factors related to uptake and assimilation; (2) for samples containing multiple toxin congeners, each with its own intrinsic potency, the assay response will integrate across all toxins present to reflect total toxic potency, similar to *in vivo* assays; and (3) any structural modifications to toxin molecules (e.g., biotransformation, metabolism, etc.) that either enhance or reduce the affinity for their receptor will likewise affect the assay response – by comparison, an immunoassay, which relies on the structural integrity of an epitope for its recognition and response, may underestimate the toxin content of a sample because of a lack of cross-reactivity, even though the molecule may still bind its receptor (with a greater or lesser affinity) and cause a toxic effect.

Ion Channels The most common phycotoxin receptor is the voltage-gated sodium channel (VGSC), targeted by multiple toxin classes, including STXs (site 1), brevetoxins (site 5), and CTXs (site 5). In all cases, receptor binding assays (RBAs) have been developed and implemented to detect these toxins (Van Dolah and Ramsdell, 2000). In the case of brevetoxins and CTXs, a fluorescence-based RBA incorporating BODIPY-conjugated brevetoxin-2 as the ligand was developed recently (McCall *et al*., 2014b), although testing was limited to toxin reference materials and algal extracts, and did not include shellfish or finfish extracts. Nonetheless, efforts are underway to further develop and commercialize this and other fluorescence-based receptor assays for algal toxins (SeaTox, Inc., Wilmington, North Carolina, United States).

Presently, the most rigorously and extensively validated receptor-based assay targets STX and its PST analogs (Doucette *et al*., 1997; Van Dolah *et al*., 2009, 2012). This radiometric assay, which employs tritiated STX as the competing ligand, was recently recognized as an Official Method of Analysis (OMA 2011.27) by the AOAC and adopted subsequently by the U.S. Food and Drug Administration and the Interstate Shellfish Sanitation Commission (ISSC) as an "Approved Method for Marine Biotoxin Testing" for use in the U.S. National Shellfish Sanitation Program (NSSP, 2013). Moreover, the method was formally recognized as an alternative method to the MBA for detection of PSP toxicity in mussels (ISSC, 2013). The limits of detection and quantification determined in the AOAC collaborative study (for samples diluted one-tenth to remove any sample

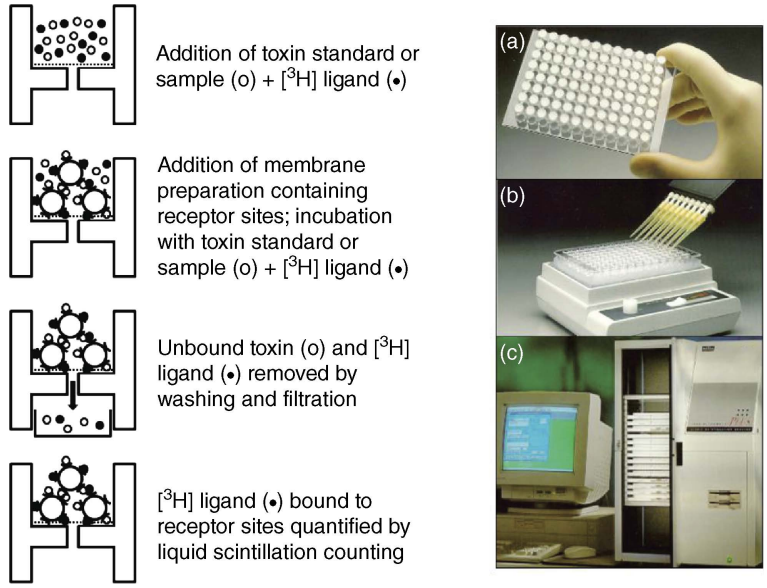

Figure 2.5 (Left) Diagram showing steps of generalized radio-receptor binding assay protocol. The images on the left represent a cross section of a single well in a filtration plate, with the dashed line indicating the filter membrane. (Right) (a) 96-well filtration plate showing white filter membranes occluding the bottom of each well. (b) Microplate mounted on a filtration manifold and multi-channel pipettor used to dispense reagents into the plate one column at a time. (c) Microplate scintillation counter containing multiple plates programmed for counting.

matrix effects) were 64 µg STX diHCl eq./kg and 131 µg STX diHCl eq./kg, respectively, both considerably below the regulatory action level of 800 µg STX diHCL eq./kg. In addition, this RBA has been employed extensively in a research context to examine toxin levels associated with laboratory cultures (Powell and Doucette, 1999) and natural populations of toxigenic algae (Turner *et al.*, 2000), and to investigate toxin trophic transfer in vector organisms (Doucette *et al.*, 2005) and upper-level predators (Doucette *et al.*, 2006).

The PST radio-receptor binding assay is conducted in a high-throughput, 96-well microplate filtration format. A generalized protocol for radio-receptor assays is shown in Figure 2.5, along with images of a filtration plate, filtration manifold, and microplate scintillation counter. For the PST assay, rat brain crude membrane preparation containing a finite number of receptors is incubated with ^{3}H-STX (American Radiolabeled Chemicals, St. Louis, Missouri, United States) and unlabeled PSTs contained in a standard or sample, followed by removal of unbound toxin by washing/filtration. The remaining receptor-bound ^{3}H-STX, which is inversely and quantitatively related to the amount of PST-like activity in a sample, is determined by microplate scintillation counting. The assay is calibrated using a STX diHCl standard (e.g., CRM-STX-f, Certified Reference Materials Program, National Research Council of Canada; and NIST RM 8642, U.S. National Institutes of Standards and Technology), and toxin concentrations, expressed in terms of STX diHCl equivalents, are generated from a calibration curve determined using a four-parameter logistic fit equation (i.e., a sigmoidal dose–response curve with variable slope or Hill equation).

Glutamate Receptor Rather than interacting with a type of ion channel, DA binds the kainite subtypes of ionotropic glutamate receptors (see Ramsdell, 2007), which have been used as the basis for developing a radio-receptor binding assay for DA detection. Initially, receptor preparations comprised frog brain synaptosomes (Van Dolah *et al.*, 1995), but these were later replaced with a cloned glutamate receptor (GluR6; Van Dolah *et al.*, 1996) that completely eliminated the use of animal components in this assay. This is currently an important consideration given the widely adopted constraints on use of live animals or animal components for testing product safety. Moreover, samples must first be digested with glutamate decarboxylase enzyme in order to eliminate ambient glutamate, which competes with DA binding to receptors in the assay which, can cause a false-positive response. Otherwise, conduct of the DA RBA and data processing are essentially the same

as those based on ion channel receptors described above, except that ^{3}H-kainic acid (PerkinElmer, Hopkinton, Massachusetts) is employed as the radioisotope, the assay is calibrated using a DA certified reference standard (CRM-DA-g; CRMP, National Research Council of Canada), and data are expressed in terms of DA equivalents. Although used extensively to study toxicity of algal cultures (Pan *et al.*, 2001) and natural populations (Scholin *et al.*, 1999; Trainer *et al.*, 2009; Parsons *et al.*, 2013), DA trophic transfer in marine systems (Kvitek *et al.*, 2008; Doucette *et al.*, 2012), and marine mammal mortality events (Scholin *et al.*, 2000), the DA RBA has yet to be fully validated and accepted for use in regulatory testing of shellfish samples.

nAChR (Nicotinic Acetylcholine Receptor) The cyanotoxin anatoxin-*a* represents yet another biotoxin demonstrated to bind a class of receptor (i.e., nicotinic acetylcholine receptor [nAChR]) that has been incorporated into receptor-based assays for this toxin (reviewed by Aráoz *et al.*, 2010). A commercially available 96-well microtiter plate-based receptor assay kit (Abraxis LLC, Warminster, Pennsylvania, United States) is available for detection of anatoxin-*a* in freshwater. The assay uses biotinylated α-bungarotoxin to compete with anatoxin-*a* for nAChR binding sites in material purified from *Torpedo* electrocyte membranes. Colorimetric detection of the bound biotinylated toxin employs a streptavidin–horseradish peroxidase (HRP)–tetramethylbenzidine (TMB) system, and the assay is calibrated using anatoxin-*a* standard solutions provided with the kit. The detection range of the assay is specified as 5–500 ng/mL, although the effective sensitivity can be increased by incorporating a solid-phase extraction (SPE) concentration step prior to testing a sample.

Saxiphilin Binding Protein Saxiphilin, a protein present in the blood or hemolymph of a wide array of terrestrial and aquatic invertebrate and vertebrate species, is distinct from the VGSC in terms of amino acid sequence and biochemical properties, and properly classified as a transferrin (Llewellyn *et al.*, 1997). Although transferrins are a family of proteins normally associated with the ability to bind iron, saxiphilin has been demonstrated to bind PSTs (but not tetrodotoxin), with certain isoforms exhibiting sub-picomolar affinity, one of which has been used to develop a radio-receptor binding assay to detect STX and its congeners in shellfish extracts (Llewellyn *et al.*, 1998). The assay is configured and conducted analogous to the receptor-based assay described above for PSTs, with saxiphilin substituted for the VGSC as the recognition element and tritiated STX serving as the competing ligand, and it exhibits comparable sensitivity (low nanomolar range for STX). Cross-reactivity among STX analogs is a function of the isoform(s) employed. Although the saxiphilin assay has proven reasonably robust for use with molluscan shellfish extracts (Llewellyn and Doyle, 2001) and results correlate well with other methods (e.g., HPLC, mouse bioassay, VGSC-based receptor assay; Llewellyn *et al.*, 2001), formal validation studies (single-laboratory or interlaboratory study) required for regulatory acceptance have not been published.

2.3.2.1.2 Enzyme Inhibition Assays

A second type of functional assay, in addition to the receptor-based methods outlined above, includes those that rely on the ability of a toxin or toxin class to inhibit specifically the activity of a given enzyme. Although enzyme inhibition assays do not employ a competitive binding format, as in the case of receptor assays, they nonetheless take advantage of a toxin's targeting the active site of an enzyme and thus its ability to interact effectively with a substrate. In most cases, the endpoint of an enzyme inhibition assay is either colorimetric or fluorometric depending on the nature of the substrate; however, radioactively labeled (^{32}P) substrate has also been incorporated into protein phosphatase inhibition assays (Xu *et al.*, 2000). Similar to the broad specificity among congeners of a given toxin class or functionally identical toxin classes (e.g., PSTs and tetrodotoxin) exhibited by receptor assays, an enzyme inhibition assay will detect all toxin classes and their variants that specifically target a given enzyme type in proportion to their binding affinity.

PPIA (Protein Phosphatase Inhibition Assay) A variety of marine and freshwater algal toxins are considered to exert their toxic effects via inhibition of serine–threonine protein phosphatases (PPs), which are responsible for catalyzing the hydrolysis of a phosphorylated serine or threonine moiety on a diverse array of regulatory proteins. Approximately 90% of all cellular PP activity is attributable to PP1 and PP2A, although a number of other PPs have been described. The marine toxins responsible for DSP, OA and dinophysistoxins (DTXs), have been documented as potent inhibitors of PP1 and PP2A, as well as PP4 and PP5, although the strength of

interaction for a given toxin congener with a specific PP can range over several orders of magnitude, and there have been questions raised recently as to whether PP inhibition is responsible for DSP symptoms in humans (Munday, 2013). As examples of differential activity, OA inhibits PP2A to a greater extent than PP1 (Takai *et al*., 1992) and DTX1 is a stronger PP inhibitor than OA, whereas DTX4 is about 500 times less active than OA (Hu *et al*., 1995b). Freshwater toxins exhibiting this activity include the hepatotoxins of cyanobacterial origin, microcystins (MCs) and nodularins (NODs), both of which inhibit PP1 and PP2A, as well as other selected PPs (Pereira *et al*., 2010). Notably, cylindrospermopsin, which is also hepatotoxic and produced by cyanobacteria, is an inhibitor of protein synthesis rather than a PP inhibitor and is not detected by PPIAs.

Numerous assays employing PP inhibition have been developed, each exhibiting varying degrees of efficacy in terms of accuracy, sensitivity, and susceptibility to sample matrix effects. Some of the earliest efforts to detect DSP toxins and the MC/NOD group of cyanotoxins using PPIAs were reported by Tubaro *et al*. (1996; isolated human red blood cell PP2A target) and An and Carmichael (1994; recombinant PP1 target), respectively, and adopted colorimetric endpoints. One of several fluorometric assays, developed by Mountfort *et al*. (2001), demonstrated detection of OA, DTX1, DTX2, and (if a hydrolysis step is included in the pretreatment of extracts) DTX-3 (i.e., 7-acyl derivatives of OA, DTX1, and DTX2), and other derivatives (e.g., DTX4 and DTX5) can also be detected for determination of total DSP toxins in this as well as other PPIAs. In terms of actual validation of PPIAs for regulatory application, only the 96-well plate-based assay (colorimetric endpoint) developed by Smienk *et al*. (2012, 2013) and commercialized by Zeulab (also marketed by Abraxis) has undergone both single-lab (SLV) and interlaboratory validation (ILV) studies. The SLV demonstrated a limit of detection and quantification of 44 and 56 μg/kg, respectively, both less than the EU action level of 160 μg/kg. This method has been recognized in the European Union as a supplementary method for detecting OA group toxins in shellfish (McLeod *et al*., 2015) and was under similar consideration in 2017 by the U.S. Interstate Shellfish Sanitation Conference (ISSC) Task Force I (Proposal No. 13-111).

Acetylcholinesterase Inhibition Assay The cyanobacterial toxin, anatoxin-*a*(s), although considered rare relative to many other cyanotoxins, is a potent alkaloid produced by certain *Anabaena* species and exhibiting a mode of action similar to that of synthetic organophosphate pesticides, irreversibly targeting cholinesterase enzymes (Mahmood and Carmichael, 1987). Analogous to the use of PPIAs for detection of algal toxins inhibiting PPs described above, inhibition of acetylcholinesterase (AChE) activity has been adopted for development of anatoxin-*a*(s) assays. Given that a number of widely distributed organophosphate insecticides and pesticides show a mechanism of action similar to that of anatoxin-*a*(s), specificity of *in vitro* assays based on AChE inhibition is a concern. In order to address this issue, Devic *et al*. (2002) employed an electrochemical biosensor incorporating a four-mutant set of AChE variants with variable sensitivities to anatoxin-*a*(s) and organophosphate – a strategy that the authors reported allowed specific detection of anatoxin-*a*(s) in environmental samples derived from cyanobacterial blooms. Although a novel and potentially effective assay, this method does not appear to have been adopted subsequently as a routine test for anatoxin-*a*(s). Currently, no *in vitro* assays with demonstrated specificity for anatoxin-*a*(s) detection are commercially available, and liquid chromatography–tandem mass spectrometry (LC-MS/MS) (e.g., Dörr *et al*., 2010) is the method of choice for measuring concentrations of this toxin in environmental samples.

2.3.2.1.3 Cell-Based (Cytotoxicity) Assays (CBAs)

CBAs, the third type of functional assay to be considered here, effectively integrate the events downstream from the receptor–ligand interaction to determine how a toxin or toxin class affects the growth and survival of a given cell type (Rossini, 2005). As a result, CBAs have been used extensively for characterizing the toxicity and investigating the mode of action of algal toxins, in addition to being adapted for detecting many of these compounds in various sample types. It is, however, important to note that the specificity of detecting a given toxin or toxin class tends to decline further downstream from the initial toxin–receptor interaction, leading to a situation where multiple toxin groups can elicit a similar effect on, for example, cell viability (Vilariño *et al*., 2010). Nonetheless, a wide range of eukaryotic cell lines has been employed for algal toxin detection, based on assessment of cell viability, metabolic activity, morphological changes, and apoptosis as determined through the application of various dyes and staining protocols. Much of this information as it relates to the detection of certain toxin classes has been reviewed by several authors

(Rossini, 2005; Vilariño *et al*., 2010; Reverté *et al*., 2014).

In terms of their practical application for detecting algal toxins in shellfish, algae, or seawater, the use of CBAs is largely restricted to laboratories housing cell culture facilities, given the need to employ live cultures. Notably, a shippable commercial product based on a CBA was developed for detection of PSP toxins (MIST™; Jellett *et al*., 1998), thereby alleviating the requirement for cell-culturing capabilities. Although its performance was reported to compare favorably with the AOAC MBA method for quantifying toxicity associated with PSTs in shellfish samples (Llewellyn *et al*., 2001), it was ultimately discontinued in favor of an antibody-based approach. Other cell-based technologies, such as neuronal networks, have been demonstrated to detect certain toxins (e.g., STX, brevetoxin-3) in seawater-based algal cell lysates (Kulagina *et al*., 2006), but remain to be tested in shellfish extracts and also require cell culture expertise. Another impediment to the use of CBAs in algal toxin detection is the effect of complex sample matrices that may themselves either elicit a cellular response (Malaguti *et al*., 2002) or potentially reduce the sensitivity of the assay. A strategy adopting HPLC fractionation or SPE-based fractionation to prepare shellfish or algal samples, respectively, for testing with the Neuro-2A CBA was used by Caillaud *et al*. (2009) to reduce matrix effects. Finally, as noted above, the issue of specificity for detecting and reliably identifying the presence of a given toxin or toxin class by CBA is a challenge that has been addressed by modifications to assays aimed at restricting the response of a cell line to compounds that target a certain functional component. Perhaps the best example of this approach is use of the Neuro-2A CBA as modified by standardized and critically timed additions of veratridine and/or ouabain for detecting VGSC blockers (PSTs), VGSC agonists (brevetoxin, CTXs), or Na^+, K^+-ATPase pump inhibitors (palytoxins) as outlined below. There are, nevertheless, advantages to nonspecific CBAs employing various cell lines, as pointed out by Ledreux *et al*. (2012), in their ability to detect toxicity of unknown compound(s) in shellfish responsible for atypical toxic events.

Neuro-2A Assay (Including Toxin-Specific Modifications) The Neuro-2A (N2A) mouse neuroblastoma cell line (ATCC, CCL-131) is generally considered the "workhorse" for CBA detection of algal toxins, and there exists an extensive literature (see reviews cited above) describing the numerous methods based on this cell line, some of which include toxin-specific modifications. In its most basic, nonspecific format, N2A cells maintained according to standard cell culture protocols are seeded into 96-well plates and allowed to grow for up to ~24 h, the culture medium is removed, and the cells are exposed to a test solution/extract for a given time period. Cell viability is then assessed, generally using a dye, such as MTT (1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan), which measures mitochondrial reductase activity as a proxy for cell number and produces a formazan precipitate. The formazan is dissolved, the absorbance of each well is measured using a microplate spectrophotometer, and the percent viability is derived from a standard curve produced using dilutions of reference material.

Toxin-specific versions of the N2A assay have been developed and refined for detection of several algal toxin classes. One of the earliest examples targeted detection of the VGSC-blocking toxins STX and tetrodotoxin (Kogure *et al*., 1988) by first treating cells with ouabain and then veratridine to enhance Na^+ influx and then introducing STX or TTX, which effectively rescued the cells by blocking this influx and maintaining viability in a dose-dependent manner as determined by visual scoring of each sample. Several improvements were made to the scoring of assay results (Jellett *et al*., 1992; Manger *et al*., 1993), and its application was extended to also include detection of VGSC-agonistic toxin classes, CTXs and brevetoxins, along with STXs, in seafood extracts (Manger *et al*., 1995), achieving limits of detection in the low to sub ng/mL range depending on the toxin class. Additional changes to the N2A assay for PST detection aimed at simplifying and increasing the sensitivity of the assay were reported more recently by Hayashi *et al*. (2006), who demonstrated reasonable correlation ($r = 0.9001$) with results of an HPLC-based method for extracts from a diverse assortment of shellfish species containing predominantly C-toxins or gonyautoxins. The N2A assay was four times more sensitive than the AOAC mouse bioassay, and testing of 12 samples from start to finish was completed in one day.

The N2A assay has found widespread acceptance for detection of CTXs, because of the ease of handling of this cell line, its high sensitivity to CTXs, and the accuracy for routine screening of these toxins in fish samples, and it has been suggested as a reference model system for this application. Although this application is based largely on the assay's original description by Manger *et al*. (1993), several adjustments have been

introduced and recommendations advanced for sample preparation and the maximum amount of matrix that should be applied for testing in order to avoid or minimize interference (reviewed by Caillaud *et al.*, 2010). This robust, high-throughput screening assay has been demonstrated to be highly sensitive with good repeatability and reproducibility for discriminating between toxic and nontoxic fish samples (Dechraoui *et al.*, 2005), as well as other seafood (Pawlowiez *et al.*, 2013), and for investigating ciguatera fish poisoning cases (Dickey, 2008). Moreover, a two-tiered strategy for analyzing potentially ciguatoxic fish samples has been proposed, whereby the N2A assay serves as the initial screen to assess toxicity and is followed by LC-MS/MS to confirm the molecular presence of CTXs (Dickey and Plakas, 2010). The authors noted that this tiered approach has "provided the most appropriate information for decisions of public health and economic importance," and its application is capable of supporting the current advisory levels of 0.10 ppb Caribbean (C)-CTX1 equivalent toxicity and 0.01 ppb Pacific (P)-CTX1 equivalent toxicity, as suggested in guidance provided by the U.S. Food and Drug Administration (USFDA, 2011, app. 5, table A-5). A novel modification of this assay, incorporating use of flow cytometry and fluorescent voltage-sensitive dyes, resulted in a significantly reduced assay time (minutes) while apparently retaining the level of sensitivity required for toxicity screening, although further experiments are needed to firmly establish performance characteristics with fish extracts (Manger *et al.*, 2014).

Further modification of the N2A assay has enabled the targeted detection of other toxin classes in addition to the VGSC-active toxins described above. Ledreux *et al.* (2009) demonstrated the suitability of this CBA for the toxin-specific detection of palytoxin (PlTX) and its analogs by pretreating the cells with ouabain, which reduced the effects of PlTX and enhanced cell viability as assessed via the MTT-based endpoint. The assay limit of detection was calculated to be 5 ng PlTX/mL of extract (equivalent to ~50 µg PlTX/kg shellfish tissue), and good correlation with expected values was obtained for mussel extracts spiked with 250 µg PlTX/kg shellfish tissue, corresponding to a provisional regulatory action limit (CRLMB, 2005). Moreover, these authors proposed an experimental design for the specific detection of four phycotoxin classes (i.e., palytoxins, saxitoxins, brevetoxins, and ciguatoxins) based on the N2A assay's differential response in the presence of veratridine and/or ouabain, as outlined in Figure 2.6.

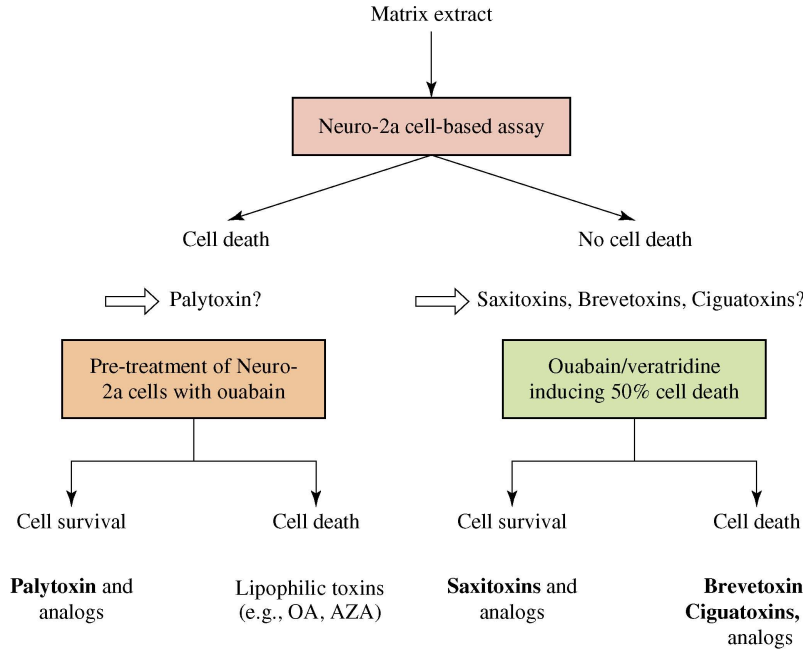

Figure 2.6 Schematic diagram of experimental design for the targeted detection of four phycotoxin classes using the Neuro-2a assay's response in the presence of veratridine and/or ouabain. Note that cell death following pretreatment with ouabain likely indicates the presence of lipophilic toxins (e.g., OA and AZA) (A. Ledreux, personal communication). *Source*: Adapted from Ledreux *et al.* (2009, Fig. 6).

Other Cell Types Apart from the N2A cell line, a variety of other eukaryotic cell types have been incorporated into CBAs for marine and freshwater algal toxin detection. Examples include: PC12 cells (ATCC, CRL-1721) derived from rat adrenal medulla cells; NG108-15 cells (ATCC, HB-12317), a mouse neuroblastoma × rat glioma hybrid; HEK-293 cells (ATCC, CRL-1573), derived from human embryonic kidney cells; rat hepatocytes; V79 hamster fibroblasts (ATCC, CCL-93); and fish cell lines PLHC-1 (ATCC, CRL-2406) and RTG-2 (ATCC, CCL-55) (see references in Cañete and Diogène [2008] and in Agrawal *et al*. [2012]). The comparative sensitivity of assays based on N2A versus NG108-15 cells for several algal toxins was examined by Cañete and Diogène (2008) in the presence or absence of ouabain and veratridine. Similarities and differences were observed in the response of these two cell lines to toxins representing diverse modes of action; nonetheless, both cell types were responsive to VGSC-active toxins and enabled quantification of STX, PbTx3, OA, DTX1, and pectenotoxin-2 under different CBA conditions. In certain cases, cell lines derived from tissue types considered primary targets for a given toxin or toxin class have been adopted with the aim of maximizing sensitivity and selectivity, generally in the absence of agonists/antagonists (e.g., ouabain, veratridine) designed to enhance a toxin-specific response as described in the preceding section. For example, based on prior knowledge of phycotoxin modes of action, Sérandour *et al*. (2012) and Ledreux *et al*. (2012) employed three cell lines – Caco-2 (human intestinal epithelial cells; ATCC, HTB-37), HepG2 (human hepatoma cells; ATCC, HB-8065), and N2A – to detect the lipophilic toxins, azaspiracid-1 (AZA1), OA, and pectenotoxin-2, in shellfish extracts. Their intra- and interlaboratory validations demonstrated the value of developing standard operating procedures (SOPs), although interlaboratory variability was quite high for spiked extracts (~50%) because of the AZA1-spiked material. Importantly, their work did include a careful appraisal of and accounting for shellfish matrix effects on these CBAs, which are essential for accurately interpreting results of these functional, *in vitro* assays. It should be noted that in these and other studies (reviewed by Twiner *et al*., 2014), the intestinal epithelial Caco2 cell line does not appear to show a clear cytotoxic response to AZA1, even though this toxin is well-known to affect such cells in mouse exposure models, demonstrating the potential disconnect between classical *in vitro* cytotoxicity and the *in vivo* response documented for live animals.

2.3.2.2 Structural Assays

2.3.2.2.1 Immunoassays

Immunoassays are based on antibodies recognizing and binding to one or more epitopes or antigenic determinants on a toxin molecule (i.e., antigen). A given antibody binding site may be chemically unique to a given toxin congener or in common to all structurally related congeners comprising a toxin class. The production of antibodies against algal toxins is challenging because of the inherent toxicity associated with these compounds and the fact that antibodies are generated by the immune response of a living animal that is exposed to a toxin yet is susceptible to its effects. Moreover, most algal toxin molecules are low-molecular-weight compounds that are too small to elicit an immune response (i.e., haptenic) and, as a result, must be conjugated to a larger antigenic carrier molecule (normally, a protein) for effective immunization; however, conjugation to a protein may not only reduce the effective potency of a toxin, but also offer a strategic means of manipulating how a toxin molecule is presented to the immune system by carefully directing the chemical reactions involved (e.g., Garthwaite *et al*., 1998). Changes to the conjugation chemistry or adopting a "multihapten" approach (e.g., Samdal *et al*., 2014) provides some control over the nature and characteristics of the antibodies produced against the toxin–protein conjugate. Carefully targeted screening can also be employed to identify those antibodies with the desired cross-reactivity profile and optimally to yield a profile aligning well with the TEF of the respective analogs, although achieving such an alignment is difficult (e.g., Stewart *et al*., 2009). Also, antibodies employed in immunoassays may be either polyclonal (i.e., derived from multiple B-cell lineages) or monoclonal (i.e., derived from a single B-cell lineage), binding multiple or single epitopes, respectively, on a toxin molecule. Each antibody type exhibits advantages and disadvantages for algal toxin detection depending on several factors (see Cembella *et al*., 2003).

As noted above, structural assays based on antibodies tend to be more stable than most functional assays (e.g., RBAs, CBAs) and are often incorporated into toxin-specific detection kits. Although often used in the laboratory, these robust methods are commonly deployed in field-based applications, especially for qualitative or semiquantitative screening of samples prior to confirmation using more expensive and technically demanding analytical methods. Antibodies can also be employed as toxin-specific recognition elements or binders

in various types of biosensors (Section 3.2.2.2), which are generally restricted to laboratory use. An excellent review of currently available immunoassays suitable for use in the field for testing shellfish samples along with a critical evaluation of their performance was published recently by McLeod *et al*. (2015). High-throughput, microtiter plate-based immunoassays for a variety of algal toxins are also commercially available and, generally, represent a cost-effective approach for rapidly testing large numbers of samples. In addition to the issues identified above related to differential cross-reactivity among structurally related congeners of variable toxicity, another drawback of immunoassays is the fact that an epitope recognized by an antibody may remain intact and available for binding even though the toxin's molecular structure, and thus its toxicity, has been modified. It is well-established that numerous biological, chemical, and physical factors can alter the chemical structure of a toxin, resulting in changes (i.e., increase, decrease, or elimination) to its intrinsic toxic potency. As a result, the relationship between an immunoassay's response and the toxicity of a molecule(s) generating a positive response on the assay can vary, which has clear implications for protecting public health.

ELISA (Enzyme-Linked Immunosorbent Assay)

The ELISA has been the format of choice for most commercial algal toxin detection kits. Normally provided in a 96-well configuration, some kits require that a full plate be run regardless of the number of samples being tested, whereas other more flexible, cost-efficient products include 12 eight-well inserts (equivalent of a single column) that can accommodate variable sample numbers without wasting a portion of the plate. ELISA kits are also available in a tube format for certain target analytes. ELISA-based methods exhibit several advantages, including good sensitivity, a long shelf life, a requirement for only basic laboratory skills and equipment (e.g., a microplate spectrophotometer or fluorometer, depending on the assay endpoint), and availability for all major toxin classes.

There are multiple ways to configure an ELISA (see Figure 2.7). However, the small size of the antigens of interest here (i.e., toxin molecules)

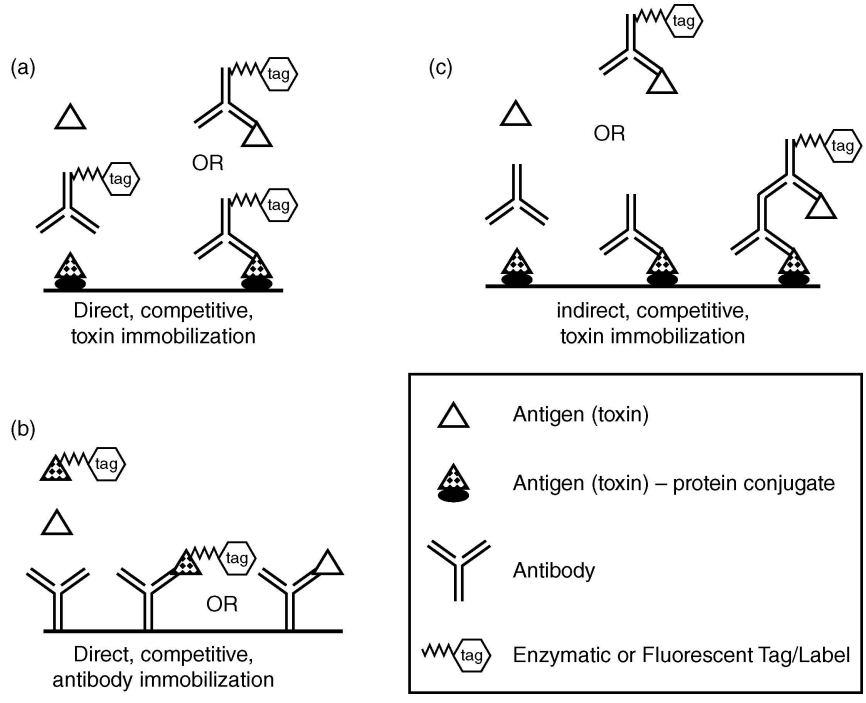

Figure 2.7 Schematic diagrams of several configurations for toxin immunoassays. (a) Direct, competitive ELISA with toxin–protein conjugate immobilized on solid surface. Immobilized toxin and toxin in sample compete for labeled primary (i.e., antitoxin) antibody. (b) Direct, competitive ELISA with primary antibody immobilized on solid surface. Labeled toxin and toxin in sample compete for immobilized antibody. (c) Indirect, competitive ELISA, with toxin–protein conjugate immobilized on solid surface. Same as in (A), except that labeled secondary antibody is used to visualize primary antibody bound by toxin–protein conjugate (primary antibody bound by toxin in solution is removed prior to introducing secondary antibody). Legend located in box at lower right.

generally requires adopting one of the following competition-based assays: direct, competitive, toxin immobilization (Figure 2.7A) – free toxin in a sample competes with toxin or toxin–protein conjugate immobilized on the plate surface for binding to labeled (e.g., enzymatic or fluorescent tag) antitoxin "primary" antibodies in solution, and the bound labeled antibody is visualized via enzymatic reaction (colorimetric or chemiluminescent) or based on fluorescence intensity; direct, competitive, antibody immobilization (Figure 2.7B) – the antitoxin primary antibodies are immobilized on the plate surface, free toxin in a sample competes with labeled toxin for binding to the antibody attached to the plate, and the bound labeled toxin is visualized as above; and indirect, competitive, toxin immobilization (Figure 2.7C), which is similar to the first direct method described above, but the antitoxin primary antibody is not labeled and requires an additional step involving binding by a labeled "secondary" antibody in order to visualize the amount of bound primary antibody. Note that formats involving binding of two primary antitoxin antibodies (labeled or unlabeled) to unique epitopes on a single toxin molecule are generally not feasible because of steric hindrance issues related to the small size of most algal toxins.

In all cases, the ELISA signal is converted to an "in-assay" toxin concentration using a calibration curve generated based on a reference standard supplied with the kit and generally fitted with a nonlinear four-parameter logistic curve-fitting model. Quantification of an assay result is limited to the linear range of the calibration curve, considered to be between the EC_{80} and EC_{20} values, or the signals representing 80 and 20% of the maximum assay response, respectively. Samples are often run at multiple dilutions, because values outside of the linear range cannot be quantified accurately and must be retested using lower or higher dilutions. A minimum dilution is frequently required in order to eliminate or minimize sample matrix effects. Toxin levels in the original sample are calculated taking into account the quantity of material processed and sample preparation details (e.g., concentration, dilution), and reported as concentrations in terms of the reference standard employed.

Commercial ELISA kits are available for most of the major algal toxin classes and are too numerous to identify here. An abbreviated list of companies manufacturing and/or marketing ELISA kits for algal toxins includes (in alphabetical order): Abraxis LLC; Beacon Analytical Systems, Inc.; BIOO Scientific Corp.; Biosense Laboratories AS; EnviroLogix, Inc.; Enzo Life Sciences, Inc.; MARBIONC LLC; Mercury Science, Inc.; Modern Water, Inc. (EnviroGuard®); and Zeulab (previously Zeu-Immunotec) S.L. Each kit normally includes detailed information outlining the test preparation and assay procedure, evaluation of results, and recommended sample preparation method. Important caveats and limitations of the product should also be identified, such as issues related to sample matrix effects and antibody cross-reactivity that may result in over– or underestimates of toxin concentration. Various products have been subjected to cross-comparisons with other kits for targeted applications (e.g., PSTs: DeGrasse *et al*., 2014; diarrheic shellfish toxins (DSTs): Johnson *et al*., 2016; and MC: Aranda-Rodriguez *et al*., 2015), as well as rigorous single-laboratory and/or multilaboratory validations (e.g., DA: Kleivdal *et al*., 2007a, 2007b; Litaker *et al*., 2008). The Abraxis Shipboard ELISA for PSTs (along with the Scotia [formerly Jellett] Rapid Testing kit; see below) were included in a successful pilot study of the Onboard Screening Dockside Testing Protocol (DeGrasse *et al*., 2014), leading to its adoption into the U.S. NSSP Model Ordinance and the subsequent reopening of part of Georges Bank to Atlantic surfclam and ocean quahog harvesting with use of this protocol.

LFIC, LFIA, or LFA (Lateral-Flow Immunochromatographic or Immuno-Assay) Commonly referred to as *dipstick assays*, lateral-flow assays generally comprise a test strip that may or may not be enclosed in a protective cartridge. At one end of the test strip is a sample pad to which the extract is applied. Following application, the extract is wicked downstream over an adjacent reagent pad containing labeled antitoxin antibody. Any toxin present in the extract competes with toxin immobilized on a test line for the labeled antibody, such that the color intensity of the test line is inversely proportional to the amount of toxin in the extract (i.e., the more toxin present in the extract, the less antibody available to bind on the test line, and the lower the signal produced). A control line, on which is immobilized an anti-species antibody (i.e., it will always bind the labeled antibody), is located downstream from the test line and should always bind a given amount of the labeled antibody and yield a strong signal as confirmation that the assay has functioned properly and that the result is valid. These types of assays are generally considered qualitative and are normally "tuned" to generate a positive result if the

toxin level matches or exceeds the regulatory action limit.

A number of LFIC/LFIA/LFA-based assays for some of the major algal toxin groups (i.e., DSP, ASP, and PSP) have been developed, and several of these are commercially available, primarily through either Scotia Rapid Testing Ltd. (formerly Jellett Rapid Testing Ltd.) or Neogen Europe Ltd. The PSP toxin assay manufactured by Scotia Rapid Testing was adopted by the U.S. ISSC as an "Approved Limited Use Method for Marine Biotoxin Testing" (i.e., it can be used to determine when to perform a MBA in a previously closed area, a negative result can be substituted for a MBA to maintain an area in the open status, and a positive result shall be used for a precautionary closure; note that this status refers only to use with the AOAC Mouse Bioassay [OMA 959.08] extraction protocol) for use in the NSSP (2013), whereas the Neogen DSP, ASP, and PSP toxin assays have undergone single-laboratory validation (Jawaid *et al.*, 2013, 2015a, 2015b). In addition, other investigators have conducted independent evaluations of certain products with results reported in the peer-reviewed literature (e.g., Vale *et al.*, 2009; Laycock *et al.*, 2010; Eberhart *et al.*, 2013; DeGrasse *et al.*, 2014; Turner *et al.*, 2015a, 2015b; Johnson *et al.*, 2016).

Luminex xMAP Given that multiple algal toxin classes can co-occur in a single shellfish sample because of spatiotemporal overlap of the toxigenic source organisms, it is indeed desirable to develop capabilities for multiplex toxin detection. A microsphere-based liquid array-formatted (Luminex xMAP; Luminex Corp.) immunoassay has proven capable of achieving simultaneous measurement of several toxins in a single sample (i.e., STX, OA, and DA; Fraga *et al.*, 2013), although its use is restricted to a laboratory setting. Briefly, this technology relies on the flow cytometric analysis of a mixture of fluorescent microspheres, each group or class coded uniquely based on their intrinsic fluorescent spectral signal and their surface functionalized via immobilizing a specific target analyte (i.e., toxin molecule or class). The mixture of toxin-functionalized microspheres is then introduced into a competitive immunoassay, whereby the immobilized toxins compete with free toxins in solution for toxin-specific antibodies, also in solution. Antibodies bound to the microspheres are then reacted with fluorescently labeled anti-species antibodies, and the signal intensity associated with each spectrally unique, toxin-specific class of microspheres is measured using FCM. Toxin concentrations are quantified based on calibration curves developed with certified reference materials. Some preliminary testing has been done using mussel and scallop matrices, and although results are promising with recovery rates at or above 80% for single extracts containing STX, OA, and DA, use of the Luminex method is currently limited to research applications. Nonetheless, this novel, high-throughput approach clearly shows promise for multiplex screening of several algal toxin classes in a single sample, as is also the case for multiple congeners comprising one toxin family (e.g., AZAs; Rodríguez *et al.*, 2014).

MBio SnapEsi® System The capability for multiplex detection of algal toxins is also provided by the planar waveguide-based SnapEsi System (MBio Diagnostics, Inc.), a semi-automated, fluorescence-based microarray analysis system designed for the rapid quantification of target analytes within a fluidic sample (Figure 2.8). A proprietary LightDeck® total internal reflection fluorescence (TIRF) core optical module provides highly sensitive detection on a small-footprint, field-portable platform that is robust and easy to use, in comparison to the more technically demanding, laboratory-based Luminex xMAP system described above. Algal toxin assays adopt a competitive immunoassay-based approach conducted on a self-contained, single-use SnapEsi cartridge, with assay times as short as ~15 min, not including sample preparation. The cartridge incorporates a protein array in a fluidic channel that operates under passive flow and can accommodate multiple fluid additions (samples, washes, detect reagents, etc.) via a single sample inlet port while storing all waste onboard.

Initial demonstration of the SnapEsi system for single-plex toxin detection focused on single-lab validation of PST determination in algal cells (Meneely *et al.*, 2013) and of microcystins in both cells and water (Devlin *et al.*, 2013). In the case of PSTs, the assay detection capability was assessed at 20 pg STX/mL in matrix. Of particular note, these authors validated use of a rapid, fully portable extraction method using the BagPage® + 80 system (full surface micro-perforated filter blender bags, 280 μm filter; Interscience, France), yielding a cell lysis rate of 99.6 ± 0.2% and a toxin recovery rate from five *Alexandrium* strains ranging from 71 to 91% (compared to a bead-beating method; Devlin *et al.*, 2011). For the microcystin assay, the detection capability was determined at 1 ng MC-LR/mL for the free toxin, whereas a detection level of 0.1 ng MC-LR/mL was achieved

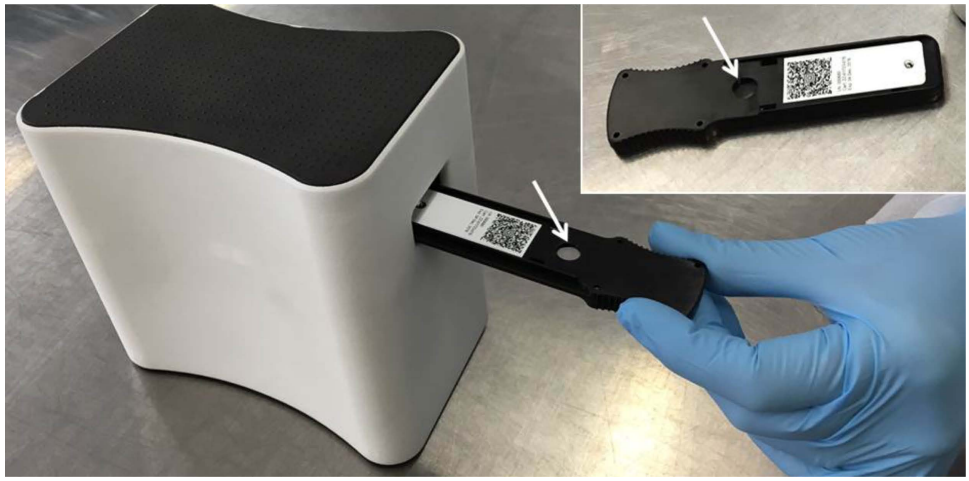

Figure 2.8 MBio Diagnostics LightDeck® Technology consists of a simple fluorescence reader (shown here) and a self-contained, single-use disposable cartridge (top right insert). The cartridge incorporates a protein array in a fluidic channel that operates under passive flow and can accommodate multiple fluid additions (samples, washes, detect reagents, etc.) via a single sample inlet port (arrows) while storing all waste on-board. *Photo credit*: M. Lochhead.

for intracellular toxin based on a 10× concentration step included in the latter sample preparation method. This novel, field-portable sample preparation method employed glass beads used in combination with a handheld milk frother to achieve ~100% toxin recovery from cells in 10 min as compared with a laboratory-based bead beater method (Devlin *et al*., 2011). Finally, the feasibility of multiplex toxin detection with the SnapEsi system was demonstrated by McNamee *et al*. (2014), whereby five algal toxins (DA, OA, STX, cylindrospermopsin [CYN], and MC) were detected simultaneously in single water and culture samples. Detection limits for this rapid (15 min) assay were reported at 0.37, 0.44, 0.05, 0.08, and 0.40 ng/mL for DA, OA, STX, CYN, and MC, respectively. Results for 12 culture samples as compared with values generated using conventional analytical methods (HPLC and LC-MS/MS) showed a reasonable correlation ($r^2 = 0.83$). It should be noted that each of the antibodies employed in this assay exhibits a different sensitivity level as well as a unique cross-reactivity profile for analogs within the targeted toxin class – factors that must be taken into account when interpreting results and comparing with analytical data. Overall, the SnapEsi system shows excellent potential as a rapid, sensitive, and field-portable approach for semiquantitative algal toxin detection (including multiplex capability) in water and algae; however, a full validation and interlaboratory trial remain to be completed. Additional studies are also required for toxins occurring in shellfish matrices in order to evaluate possible use as a screening method for seafood monitoring and regulatory applications.

2.3.2.2.2 Molecularly Imprinted Polymers (MIPs)

A robust, fully synthetic type of structural recognition element, sometimes referred to as *artificial antibodies*, MIPs are produced via the polymerization of one or several monomers and a cross-linker around a target toxin template. Following removal of the template (which can be difficult), specific molecular recognition sites complementary in shape, size, and chemical functionality to the target remain and serve to bind selectively only the template molecules. Although computational design can aid in optimizing composition of the MIP, a solely computational approach to polymer design can also be adopted, which does not involve use of a physical template and thus avoids issues arising from incomplete template removal post-synthesis. The primary use of MIPs targeting algal toxins has been as a sorbent used in SPE cartridges for isolation, cleanup, and concentration of toxins, such as DA present in various matrices (e.g., seawater, urine [Piletska *et al*., 2007], and shellfish extracts [Nemoto *et al*., 2007]). MIPs have also been incorporated as recognition elements into detection technologies, such as piezoelectric (Chianella *et al*., 2003) and surface plasmon resonance (SPR) (Lotierzo *et al*., 2004) sensors; however, because of a number of limitations (e.g., Weller, 2013) that can compromise their overall performance, MIPs have not gained wide acceptance as binders for algal toxin detection.

2.3.2.2.3 Aptamers

Another class of binder that mimics the molecular recognition features of antibodies includes RNA or single-stranded DNA oligonucleotides referred to as *aptamers*, which are identified using various enrichment/selection techniques to recognize specifically a target analyte with potentially high affinity. Among the many advantages of aptamers over antibodies are high stability, relatively low cost, low immunogenicity, an ability to target low-molecular-weight compounds (e.g., algal toxin molecules), and the potential for optimization using a variety of molecular-level modification strategies (reviewed by Hong and Sooter, 2015). Aptamers have been discovered and employed as biorecognition elements since the 1990s, and more recently have been adapted for use as diagnostic and therapeutic tools as well as probes for biosensors (aka *aptasensors*). There are now multiple examples of aptamers directed at diverse algal toxins, including STX (Handy *et al*., 2013), MC (Ng *et al*., 2013), OA (Eissa *et al*., 2013), and brevetoxin (Eissa *et al*., 2015). A recent study by Zheng *et al*. (2015) demonstrated the use of techniques, such as site-directed mutagenesis and truncation (i.e., targeted shortening), to achieve a 30-fold increase in the affinity of a previously designed (Handy *et al*., 2013) aptamer for STX. In several cases, aptamers have been used to replace antibodies in electrochemical biosensing applications and exhibited very acceptable sensitivity (pg/mL to ng/mL) for algal toxin detection. Although rigorous testing in algal or shellfish matrices has yet to be reported, there is a strong potential that aptamer-based methods can be developed successfully for such applications, based on the many unique features and flexible design/modification of these recognition elements.

2.3.2.3 Biosensors

A biosensor is an analytical device incorporating a biorecognition element intimately associated with or integrated within a transducer that converts the binding response into an electrical signal that can be used to measure the amount of ligand (e.g., toxin) present in a sample (Vilareño *et al*., 2010). In the case of algal toxin detection, these devices are predominantly either optical or electrochemical biosensors, with the majority of these being optical biosensors based on SPR technology. Biosensors can employ a variety of biorecognition elements or binders, including either functional or structural types (e.g., antibodies, receptors, MIPs, and aptamers), but antibodies have been adopted most frequently and detection methods are available for a range of algal toxins. In-depth reviews of biosensor technologies were provided recently by Vilareño *et al*. (2013) and Reverté *et al*. (2014).

Methods based on SPR employ a toxin-specific sensor chip upon which is immobilized the target toxin, or representative compound for a given toxin class (e.g., STX for the PSTs, OA for DSTs, etc.). A mixture of the binder and standard solution or sample extract is flowed over the chip surface. Immobilized toxin competes with toxin in solution for binding by the recognition element (e.g., antibody), and the degree of binding to the chip surface is inversely related to the amount of toxin in solution. Binding of the recognition element at the chip surface results in a localized increase in mass, which is detected as a change in the angle of reflectance of a polarized light beam directed from beneath the chip. This response can be calibrated using an appropriate reference material to provide a measurement of total toxin concentration in solution based on the calibrant. SPR-based methods for conduct on laboratory instrumentation have been developed for the detection of toxins associated with PSP, DSP, ASP, as well as palytoxin, yessotoxin, tetrodotoxin, and microcystins (see Vilareño *et al*., 2013; Devlin *et al*., 2014).

In terms of single-laboratory and interlaboratory validation studies, SPR-based methods for PST detection have received the most attention. K. Campbell *et al*. (2010) conducted a single-laboratory validation using mussels and cockles, and reported an acceptable detection limit of 120 μg STX-diHCl/kg in mussels (regulatory level is 800 μg/kg), albeit with a low cross-reactivity for (and thus an underestimate of) certain PSTs that may represent a large proportion of total PSTs in shellfish depending on geographic region. A follow-on interlaboratory validation (seven laboratories and 20 shellfish samples; van den Top *et al*., 2011b), including both spiked and incurred samples, generated HorRat values <1, indicating acceptable precision or reproducibility of the method (mean recoveries for low [$94.6 \pm 16.8\%$] and high [$98.6 \pm 5.6\%$] spiked samples were also acceptable).

The feasibility of multiplex SPR-based detection of algal toxins was introduced recently by Campbell *et al*. (2011), who demonstrated the ability of a prototype instrument to measure concentrations of DA, OA, STX, and neoSTX on a single chip. Additional development efforts on this same prototype SPR platform have led to a proposal of the artificial or "optoelectronic" mouse concept, whereby diverse toxin classes (DA, OA, and

STX) and an emerging toxin (palytoxin) were targeted for simultaneous detection (Campbell *et al.*, 2014). A single-laboratory validation study established the potential for this technology to be used as a screening method for the regulated toxins in shellfish, with detection capabilities of ≤10 mg/kg, ≤160 µg/kg, and ≤400 µg/kg reported for DA, OA, and STX, respectively. Further optimization is required for OA in order to reduce the detection capability, which was near the regulatory action level; unfortunately, a limited amount of palytoxin and its antibody precluded a complete analysis of this toxin. Notably, development of a single-step extraction protocol (50% aqueous methanol; 4 : 1) that achieved an acceptable recovery rate of >80% for all four toxins was also reported.

In addition to the more conventional laboratory-based instrumentation, a field-portable SPR device, now commercially available as the SPIRIT™ instrument (Seattle Sensor Systems, Corp.), was used to develop a DA biosensor that performed acceptably for detecting this toxin in a shellfish (Pacific razor clam; *Siliqua patula*) matrix, with a limit of detection at 3 ppb and a strong correlation ($r^2 = 0.99$) with HPLC analysis of six razor clam extracts (Stevens *et al.*, 2007); however, the SPIRIT DA assay is not currently marketed as a "kit," and no complete validation studies employing this mobile SPR system for DA analyses have yet been reported. Interestingly, a subsurface SPR-based instrument for *in situ* measurement of dissolved DA in seawater was reported recently to exhibit a 0.1 ng/mL limit of detection (Colas *et al.*, 2016). Rigorous validation of this system's performance in the field remains to be completed, and it should be considered in the research and development phase as several important modifications to the instrument are recommended by the authors. Nonetheless, there is reasonable potential for *in situ* operation in the future, although the inability to detect particulate, cell-associated toxin will need to be taken into consideration when interpreting results in the context of monitoring and management applications.

Apart from the SPR-based biosensors described above, those employing electrochemical detection are most commonly reported and have successfully incorporated structural as well as functional recognition elements (reviewed by Vilareño *et al.*, 2013). A wide range of electrochemical immunosensors have been developed for multiple algal toxins, including OA, DA, and brevetoxins, as well as MC. These methods can generally be described as exhibiting similar advantages and disadvantages as for other antibody-based biosensors. As an alternative to immunosensors, a functional strategy employing the PP2A enzyme has been adopted for electrochemical detection of the DSP toxins OA and derivatives, with multiple assay configurations examined (e.g., Volpe *et al.*, 2009) and certain approaches showing sensitive detection in shellfish extracts (Campàs *et al.*, 2008a). A PP2A-based MC sensor has also been developed, with certain signal amplification techniques (e.g., Campàs *et al.*, 2008b) demonstrated to considerably enhance sensitivity and greatly expand the dynamic range of detection. None of the electrochemical devices referred to above, some of which are quite novel and would be considered as prototypes, have undergone the single-laboratory or interlaboratory validations with shellfish samples considered to be a prerequisite for advancing toward regulatory application.

2.3.3 Analytical Techniques

In vivo tests, such as the rat and mouse bioassays, have been used for many years for regulatory analysis of various toxin groups. Although in many ways these techniques have been effective, there are also multiple concerns. The commonly used AOAC MBA for PSP toxins is subject to sensitivity issues and interference from salts and metals (McCulloch *et al.*, 1989; Anonymous, 2005), whereas the DST MBA has similar sensitivity issues, requires long observation periods (Yanagi *et al.*, 1989), and suffers from false positives as well as false negatives (Suzuki *et al.*, 1996; Fernandez *et al.*, 2003). Considering these performance issues and obvious ethical concerns with the use and sacrifice of large numbers of test animals (Hess *et al.*, 2006), significant effort has been directed to developing alternative chemical analytical methods.

Depending on the application and requirements of the analysis, development of chemical analytical methods has been brought to various stages of completion, from initial proof of concept through to rigorous, interlaboratory validation. For the latter, stringent parameters must be evaluated to establish that methods are robust, can deal with challenging matrices, and are highly selective in terms of toxin detection. This section provides an overview of the principal chemical analytical methodologies used for toxin analysis without exhaustively referencing all methods reported to date. Methods developed initially were based on HPLC with optical detection using either ultraviolet (UV) or fluorescence (FLD) techniques. Advances in robust electrospray ionization (ESI), which

enabled coupling of liquid chromatography (LC) and mass spectrometry (MS), led to a surge in development of LC-MS methods. Various techniques will be summarized considering originally developed and follow-on methods, and where relevant their validation and regulatory status.

2.3.3.1 High-Performance Liquid Chromatography with Optical Detection (UV or FLD)

Early analytical approaches developed as alternatives to *in vivo* bioassays were based principally on HPLC with UV or FLD detection. HPLC is a very powerful adaptation of traditional column chromatography in which sample mixtures are injected into a solvent (mobile phase) that is pumped under pressure through a column filled with chromatographic packing material (stationary phase). The potential for modifying HPLC methods via selection of mobile phase, stationary phase, pH, and temperature makes HPLC a highly versatile technique with broad applicability. HPLC methods for algal toxin analyses are typically based on reverse-phase liquid chromatography (RPLC), employing a nonpolar stationary phase (generally C8 or C18 bonded silica) and a polar mobile phase (generally a mixture of water and organic solvents with buffers). The choice of method parameters depends on the chemical and physical properties of toxins being analyzed. In RPLC, there is not a strong interaction between polar compounds in a sample and hydrocarbon chains attached to the silica (i.e., stationary phase), and therefore they generally elute earlier in the chromatographic run. Nonpolar compounds form attractions to the stationary phase, which causes a delay in their passage through a column resulting in longer elution times. Other modes of separation that have been used for algal toxins include ion-exchange chromatography (IEC) and hydrophilic interaction liquid chromatography (HILIC). IEC is a separation technique based on charged functionalities of the analytes, while HILIC is a "multimodal" separation process using hydrophilic stationary phases with mobile phases similar to those used for RPLC.

The careful selection of LC conditions enables separation of analytes from related and nonrelated components in the sample mixture prior to direction toward a detector. Choice of detection method (e.g., UV or FLD) depends on the properties of target analytes. The primary LC methods for algal toxins that use conventional UV and FLD detectors are outlined below. The well-established HPLC methods for DA and PSTs will be described in detail, whereas a brief summary of methods reported for other toxin classes will also be provided.

2.3.3.1.1 Domoic Acid

Following the DA shellfish poisoning crisis in eastern Canada in 1987 (Wright *et al*., 1989), HPLC methods for DA were developed rapidly (Lawrence *et al*., 1989a; Quilliam *et al*., 1989a). Analysis was based on boiling aqueous extraction of shellfish tissues with RPLC separation and detection by UV. DA is highly suited to detection by UV due to the presence of a strong chromophore absorbing at 242 nm. These methods demonstrated good sensitivity (Quilliam *et al*., 1989a), ruggedness (Lawrence *et al*., 1989a), and reasonably quick analysis times, all required traits for routine and regulatory monitoring applications. The method developed by Lawrence *et al*. (1989a), which included extraction in 0.1 M HCl and analysis by LC-UV, underwent a collaborative study (Lawrence *et al*., 1991a) and was designated as an AOAC official method (OMA 991.26; AOAC, 2000). These protocols formed the basis of methods that have been used in screening and regulatory analysis of DA in shellfish for nearly three decades. A variety of subsequent studies evaluated extraction, cleanup, and derivatization methods for improved DA determination in seafood as well as clinical samples (Lawrence *et al*., 1989b; Hatfield *et al*., 1994; Quilliam *et al*., 1995). The work by Quilliam *et al*. (1995) was comprehensive in its evaluation of extraction and cleanup methods, importantly demonstrating that extraction with 50% aqueous methanol provided best recoveries for the HPLC-UV method and overall excellent method performance for DA analysis in shellfish tissues. The utility and robustness of the procedure led the European Union Reference Laboratory for Marine Biotoxins (EURLMB) to adopt it as a SOP for determination of DA in seafood (EURLMB, 2008). The method also compared well with MS-based techniques (Hess *et al*., 2005). The success and broad application of the HPLC-UV method in routine monitoring programs are due to the suitability of DA for UV detection, relatively minor levels of DA-related analogs, and the availability of reference materials at an early stage of method development (Hardstaff *et al*., 1990).

2.3.3.1.2 Paralytic Shellfish Toxins

As noted above, more than 50 structural analogs of PSTs have been reported (Wiese *et al*., 2010). Unfortunately, these toxins do not contain a

chromophore or a fluorophore suitable for trace-level analysis, so most techniques for their analysis are based on LC coupled with FLD (LC-FLD) using an oxidation reaction to yield fluorescent derivatives. Since the early work on developing chemical analysis methods for PSTs (Bates *et al.*, 1978), two primary approaches have evolved: pre-column oxidation (pre-cox) and post-column oxidation (p-cox).

The pre-cox method was refined over several years (Lawrence and Menard, 1991; Lawrence *et al.*, 1991b, 1995) and involves acidic extraction of PSTs, SPE cleanup of sample extracts, alkaline conversion of toxins to highly fluorescent purine oxidation products using hydrogen peroxide or periodate, and RPLC separation prior to detection by FLD. The method underwent a single-laboratory validation (Lawrence and Niedzwiadek, 2001), followed by an extensive interlaboratory validation study (Lawrence *et al.*, 2004, 2005). Following AOAC acceptance (OMA 2005.06; AOAC, 2005), the method was designated an official alternative method for PST analysis in the European Union (Anonymous, 2006). Since then, the procedure has been widely adopted and further improvements have been made, including extending the scope to a wider range of PSTs and shellfish species, improving efficiencies via automation of sample cleanup components, and achieving shorter runtimes by using ultra-high-pressure liquid chromatography (UPLC) systems (Turner *et al.*, 2009; Ben-Gigirey *et al.*, 2012; Turner and Hatfield, 2012; Harwood *et al.*, 2013; Hatfield *et al.*, 2016).

The p-cox method differs from pre-cox in that chromatographic separation occurs before the oxidation step. Early work showing that under basic pH conditions PSP toxins could be oxidized to fluorescent purines facilitated development of a selective method where LC column effluent is mixed with oxidant and passed through a reaction system for derivatization. Acid is then added to form stable oxidation products that can be detected by FLD. The Oshima p-cox method (Oshima, 1995) involved isocratic (i.e., single mobile phase) analysis of PSTs in three separate groups, with the saxitoxin (net charge: 2+) and the gonyautoxin groups (net charge: 1+) separated using 1-heptanesulfonate as an ion-pairing agent for reverse-phase chromatography, whereas tetrabutylammonium phosphate was used to separate the C toxins. The Oshima method was adopted and used widely with various modifications and improvements (Lawrence and Wong, 1996; Bire *et al.*, 2003). Thomas *et al.* (2006) optimized a gradient separation that enabled p-cox analysis of the STX and GTX congeners in a single run, thereby allowing analysis of all toxins (including C toxins) in two separate analyses instead of three. For higher throughput analyses, Rourke *et al.* (2008) modified the LC conditions and introduced a different cleanup procedure. This version of Oshima's method underwent a single-laboratory validation (Van de Riet *et al.*, 2009) followed by an AOAC collaborative study (Van de Riet *et al.*, 2011), leading to its acceptance as an AOAC official method (OMA 2011.02; AOAC, 2011).

Several studies have compared performance of pre-cox and p-cox methods for PST analysis (Rodríguez *et al.*, 2010; DeGrasse *et al.*, 2011). Overall, the pre-column oxidation method requires simpler equipment, is relatively fast, and has many aspects that can be more easily automated, and its fitness for purpose has been demonstrated through validation in a number of laboratories (Lawrence and Menard, 1991; Lawrence and Niedzwiadek, 2005); however, significant training is required to become proficient with the method, and some toxins yield the same oxidation product, which complicates data interpretation (Lawrence *et al.*, 1995). The post-column oxidation (LC-PCOX-FLD) method is straightforward, sensitive, and robust, and has been validated for use in routine testing (Rourke *et al.*, 2008; Van de Riet *et al.*, 2009, 2011). Nonetheless, this method requires more complicated instrumentation requiring frequent maintenance and involves conducting two separate chromatographic analyses for each sample to quantitate all the regulated PSTs.

2.3.3.1.3 Other Toxin Classes

Additional HPLC-based methods for algal toxin analysis have been reported that require derivatization to introduce a fluorophore or a chromophore to the analyte prior to LC separation. Fluorometric methods for DA were developed to improve sensitivity and selectivity in analysis of algal and shellfish samples, including methods using derivatization with 9-fluorenylmethylchloroformate (Pocklington *et al.*, 1990) and 4-fluoro-7-nitro-2,1,3 benzoxadiazole (James *et al.*, 2000). These procedures are not used widely due largely to the availability of simpler and more robust HPLC-UV methods, which do not require a derivatization step (Quilliam *et al.*, 1995).

Chemical derivatization has been applied principally to the lipophilic classes of marine algal toxins, which generally do not contain chromophores or fluorophores suitable for direct UV or

FLD detection. Such methods have been developed for OA and DTXs enabling analysis by HPLC-FLD. A range of coumarins (Marr *et al*., 1994) and other compounds (e.g., Nogueiras *et al*., 2003) have been investigated as derivatizing agents, with varying performance. Nonetheless, procedures based on derivatization with diazomethane reagents have been adopted most widely. The method developed originally by Lee *et al*. (1987) using 9-anthryldiazomethane (ADAM) involved shellfish extraction with aqueous methanol, chloroform partitioning, the derivatization step, an SPE cleanup, and finally RPLC analysis with FLD. The method performs reasonably well; however, issues were reported with reagent stability (Marr *et al*., 1994), which led to development of a novel procedure to generate the ADAM reagent *in situ* during the derivatization process (Quilliam *et al*., 1998). Improvements have also been made to sample cleanup protocols for the procedure (Quilliam, 1995; Aase and Rogstad, 1997). The procedure was applied broadly in analysis of shellfish and algal samples prior to the introduction and routine use of LC-MS methods. Recent innovations have included column-switching protocols to improve throughput (Uchida *et al*., 2014). For determination of fatty acid acyl esters of OA and DTX group toxins, base hydrolysis of extracts prior to derivatization is generally necessary. The ADAM method for OA and DTXs has been evaluated in single-laboratory validations (Van de Riet *et al*., 1995; Uchida *et al*., 2014). It was studied collaboratively and considered as a European Committee for Standardization (CEN) protocol, but this was not pursued as LC-MS-based methods evolved rapidly as the preferred approach (Turner, 2014).

Post-derivatization HPLC-FLD methods have not been applied as broadly to other classes of regulated lipophilic algal toxins. Although analysis of YTXs is possible by direct HPLC-UV analysis due to the presence of a conjugated diene (Lee *et al*., 1989), the method's low sensitivity compromises its usefulness. Derivatization using the dienophile reagent DMEQ-TAD has enabled detection of YTXs in shellfish (Yasumoto and Takizawa, 1997), yet use of the technique has been limited and no formal method validation efforts have been reported. The ADAM method has also been applied for a range of PTX analogs (Yasumoto *et al*., 1995), but the selectively of the analyses is complicated by the frequent co-occurrence of OA and DTXs produced by the same organisms that produce PTXs. Other derivatization reagents have been tested for PTXs, but use of derivatization procedures is not common and no validation studies have been conducted (Turner, 2014). An HPLC method based on ADAM derivatization was developed for azaspiracids (AZAs), facilitated by the availability of reference materials AZAs (Perez *et al*., 2010; McCarron *et al*., 2015). A study examining approaches for derivatization of either the amine or carboyl functions on AZAs revealed that the *in situ* ADAM derivatization method was most effective (McCarron *et al*., 2011a). Although reaction and cleanup procedures were optimized, the protocol is laborious in comparison to LC-MS methods, and no formal method validation exercises have been conducted.

No targeted HPLC methods using optical detection have been reported for cyclic imine toxins, including spirolides, gymnodimines, and prorocentrolides. Some HPLC procedures have been reported for compounds such as palytoxins, brevetoxins, and ciguatoxins, but no extensive validation efforts have been performed (Turner, 2014).

2.3.3.2 Liquid Chromatography–Mass Spectrometry (LC-MS) and Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS)

Since the early development of ESI (Yamashita and Fenn, 1984), methods using liquid chromatography coupled with mass spectrometry (LC-MS) have become some of the most powerful and widely adopted techniques for algal toxin analysis. Compared to conventional methods based on HPLC-UV or HPLC-FLD (described above), LC-MS and LC-MS/MS methods have emerged primarily as a result of addressing initial challenges associated with accessibility to and costs of MS platforms and, to a lesser extent, perceived technical challenges. Increased availability of algal toxin reference materials (RMs) also facilitated progress in adopting LC-MS as a widespread approach (Hardstaff *et al*., 1990; Perez *et al*., 2010; Beach *et al*., 2016a; McCarron *et al*., 2016). LC has formed the basis of many detection methods for algal toxins. The choice of LC conditions is a critical consideration, keeping in mind both the physical and chemical properties of the analytes and the requirement for compatibility with MS. Optimization of MS conditions for sensitivity is also a key part of method development. Matrix effects associated with diverse sample types constitute a major challenge and can result in either suppression or enhancement of signal caused by co-extractives and sample components interfering with ionization efficiency in the source. Numerous studies have investigated matrix effects

as well as mitigation strategies (Kilcoyne and Fux, 2010), with principal approaches based on sample cleanup, extract dilution, matrix-matched calibration, or even classical standard addition (Gerssen *et al*., 2009a; McCarron *et al*., 2011b; van den Top *et al*., 2011a).

A wide range of LC-MS and LC-MS/MS methods have now been reported for algal toxins. Methods reported initially targeted a single analyte or single toxin group using what are considered by modern standards as basic MS platforms (e.g., single quadrupoles for single-ion monitoring); however, the real power of this technology lies in its ability to monitor multiple toxin analytes simultaneously with a high degree of selectivity. Advances in MS platforms available, principally tandem (MS/MS) and high-resolution MS (HRMS) with fast scan speeds and diverse scan functions, have played a major role in evolution of the technique. One of the first reports of LC-MS for marine algal toxins was for hydrophilic analytes including DA, STX, and TTX (Quilliam *et al*., 1989b). Early methods were based on single-ion monitoring and lacked specificity due to the possibility of isobaric species interfering in the analyses. A series of toxin group-specific methods have been reported, including methods for DA (de la Iglesia *et al*., 2011), OA group toxins (Quilliam, 1995), PTXs (Goto *et al*., 2001), YTXs (Draisci *et al*., 1998), and AZAs (Ofuji *et al*., 1999). Focus gradually switched to development of multitoxin methods. The remainder of this section considers the evolution of separate methods for regulated lipophilic toxins (sometimes including DA) and PSTs, as well as limited applications for other toxin classes.

2.3.3.2.1 Lipophilic Toxins

As noted above, choice of LC conditions is a major consideration when developing multitoxin methods. The first multitoxin method for shellfish toxins was reported by Quilliam (1998). Samples were extracted in methanol, and the method was capable of analyzing simultaneously DA, spirolides, OA, DTXs, PTXs, AZAs, and acyl esters of OA group toxins. Based on this early concept of multitoxin analysis, efforts then focused on making the method more practical. RPLC is used almost exclusively for lipophilic toxins analysis by MS and initially incorporated an acidic pH mobile phase (Quilliam *et al*., 2001) using a C8 column. Key to the success of any LC-MS method is good chromatographic separation and good selectivity and sensitivity in the MS. Issues with adopting acidic methods for multitoxin work include problems with peak shapes for YTXs and retention time overlap for toxins preferentially analyzed in opposing ionization modes for optimum sensitivity (e.g., AZAs and PTXs in positive, OA and YTXs in negative). A method based on neutral pH improved peak shape for YTXs and enabled analysis of toxins in groups according to ionization mode with optimum sensitivity (Stobo *et al*., 2005). Subsequently, a method using a basic pH mobile phase was developed, which also separated toxins in groups according to optimum ionization efficiency, and while peak shape was impacted for some toxins (e.g., AZAs), good sensitivity was reported (Gerssen *et al*., 2009b). Several comparisons of lipophilic toxin LC-MS methods have been conducted, with method selection based on the application (McCarron *et al*., 2011b; Garcia-Altares *et al*., 2013). With the advent of smaller particle size columns and LC instruments capable of withstanding the associated high back pressures, UPLC-MS methods for algal toxins have been reported with extremely short runtimes of under 10 mins for >25 toxin analogs (Fux *et al*., 2007). Such high-speed methods require MS systems with faster scanning speeds, which has been accommodated by newer MS platforms capable of rapid polarity switching. Rapid polarity switching enables simultaneous analysis of toxin groups preferably ionized in opposing ionization modes, solving some of the issues addressed previously by adjusting mobile phase pH.

New multi-analyte methods targeting lipophilic toxins are now reported regularly with applications ranging from shellfish (Wu *et al*., 2014; Turner and Goya, 2015) to algae (Suzuki and Quilliam, 2011) to marine sediments (Chen *et al*., 2016). Solid-phase adsorption toxin tracking (SPATT [Section 2.4.5]; MacKenzie, 2010) has been used extensively in combination with MS-based methods for monitoring the presence and distribution of algal toxins in seawater (Fux *et al*., 2009; Li *et al*., 2010; McCarthy *et al*., 2014; Mashile and Nomngongo, 2016). Methods for analysis of lipophilic shellfish toxins using high-resolution mass spectrometry (HRMS) are increasingly prevalent (Blay *et al*., 2011; Domenech *et al*., 2014; Zendong *et al*., 2015). These techniques have built on chromatographic developments made on lower resolution instruments and show great promise for nontargeted screening of multiple toxin analogs in diverse matrices (Gerssen *et al*., 2011).

The first significant method validation study for algal toxins by LC-MS focused on the range of

regulated lipophilic toxins and DA. The method demonstrated satisfactory performance using acidic pH (McNabb *et al.*, 2005), and the method was used for regulatory shellfish monitoring in New Zealand. Several validation studies conducted in the European Union varied in specific prescription of method conditions, such as that based on the alkaline method reported by Gerssen *et al.* (2010) using matrix-matched calibration to correct for matrix effects (van den Top *et al.*, 2011a). Other studies did not prescribe LC conditions (These *et al.*, 2009). Whereas no multitoxin LC-MS methods for lipophilic shellfish toxins have been the subject of a full AOAC collaborative study, acceptable results of the exercises conducted led to a change in EU regulations for shellfish toxin monitoring. Since 2011, LC-MS has replaced the MBA as the official method for regulatory monitoring of regulated lipophilic algal toxins in shellfish (Anonymous, 2011).

2.3.3.2.2 Paralytic Shellfish Toxins

The general move toward LC-MS methods has not been as rapid for PSTs as for the regulated lipophilic toxins due to availability of validated HPLC-FLD methods and to a number of challenges associated with analysis of this toxin group by LC-MS: a large number of PST analogs, chromatographic challenges with polar toxins, in-source fragmentation of some PSTs yielding products with mass similar to parent ions of other analogs, and interferences and ESI matrix effects caused by sample co-extractives. With modern instrumentation capable of analyzing multiple analogs simultaneously and with TEFs established for many of the regulated analogs (Oshima, 1995), development of LC-MS methods has focused on establishing good chromatography and mass spectrometric detection parameters, and developing robust sample cleanup procedures. Because of the hydrophilic nature of PSTs, methods are based primarily on hydrophilic interaction liquid chromatography (HILIC). Whereas RPLC is used for the established HPLC-FLD methods, the corresponding ion-pairing agents are not compatible with optimal MS detection. HILIC requires a polar stationary phase, and analytes are retained based on polarity, with elution controlled by increasing the polar aqueous content of the mobile phase. Importantly, HILIC enables separation of polar toxins using mobile phases that are compatible with ESI-MS. The LC-MS analysis of PSTs was reported first by Quilliam *et al.* (2001), where a TSK-gel Amide-80 column was used for separation, and MS detection was by single-ion monitoring. Boiling acid extraction similar to that used for LC-FLD was employed. The method was improved further to utilize the additional selectivity of tandem MS and was used to analyze PSTs in algae and shellfish (Dell'Aversano *et al.*, 2005). Further adaptations of the HILIC method for PSTs included use of alternative column chemistries (Diener *et al.*, 2007), as well as a focus on developing sample cleanup protocols to reduce interferences and matrix effects (Zhuo *et al.*, 2013). A rapid HILIC method using a small particle size column (<2 μm) and HRMS to improve selectivity of detection has been reported by Blay *et al.* (2011). Boundy *et al.* (2015) reported development of a fast method using a small particle size column with tandem MS detection. Interferences and matrix effects were mitigated through use of a carbon SPE cleanup, and the method has been evaluated in a single laboratory validation for PSTs in shellfish (Turner *et al.*, 2015a, 2015b). As yet, there is no interlaboratory validated method for PST analysis by HILIC-MS. Nonetheless, continued development of robust and reliable PST methods will be facilitated by increased availability of reference materials (Reeves *et al.*, 2006; Turner *et al.*, 2013).

2.3.3.2.3 Other Toxin Classes

Methods based on LC-MS are now a primary technique for determination of algal toxin classes in addition to those that are currently regulated. LC-MS played an important role in the identification of cyclic imines, including gymnodimines (Miles *et al.*, 2010; de la Iglesia *et al.*, 2013), spirolides (Hu *et al.*, 1995a; Aasen *et al.*, 2006), and pinnatoxins (Selwood *et al.*, 2010; McCarron *et al.*, 2012). The sensitive detection of these compounds in positive ionization mode due to their amine functions enables analysis by LC-MS in a range of matrices, including algae, seawater, and shellfish. LC-MS methods have also been reported for toxins associated with ciguatera fish poisoning (Yogi *et al.*, 2011; Robertson *et al.*, 2014) and in the identification and measurement of palytoxins in algae and seafood (Ciminiello *et al.*, 2011; Pistocchi *et al.*, 2012); however, the very limited availability of standards for ciguatoxins and palytoxins limits broader application of LC-MS for detecting these compounds, even prompting use of cleavage chemistries to facilitate detection and quantitation of the latter (and associated analogs) by LC-MS (Selwood *et al.*, 2012).

LC-MS is an important tool in the analysis of toxins produced by a broad range of freshwater cyanobacteria (Codd *et al*., 2005), including microcystins, anatoxins, cylindrospermopsins, and lyngbya toxins. Microcystins present a significant challenge as they comprise a diverse array of congeners with varying chemical structures (Roegner *et al*., 2014a). Accordingly, the multianalyte capabilities of LC-MS methods are highly valued for toxin identification and detection. Although the World Health Organization's (WHO) drinking water guideline limit of 1 μg/L applies to microcystin-LR, LC-MS methods for microcystins typically target multiple analogs (Spoof *et al*., 2010). To overcome challenges associated with measuring multiple microcystin analogs, and because it is not practical to obtain a complete set of reference standards, some methods report total microcystin concentration following oxidation of the sample (Harada *et al*., 1996). Microcystins are generally analyzed using RPLC and because of the wide range of analogs, tandem MS or HRMS is preferable for selective analysis (Neffling *et al*., 2009). The USEPA recently published methods for determining cylindrospermopsin and anatoxin-*a* (USEPA, 2015a) as well as microcystins and nodularins (USEPA, 2015b) in drinking water by LC-MS/MS. Oehrle *et al*. (2010) reported the detection of seven microcystins, nodularin, cylindrospermopsin, and anatoxin-*a* in natural water samples in under 8 min using UPLC-MS/MS, with a lower limit of detection of about 0.5 μg/L for each of the target analytes without the need for sample cleanup/enrichment. The proliferation of toxic cyanobacterial blooms has led to an increased need for use of LC-MS methods to monitor for cyantoxins in drinking water sources and in products harvested from freshwater bodies (e.g., dietary supplements) (Hollingdale *et al*., 2015).

2.3.3.3 Other Analytical Methods: Capillary Electrophoresis (CE), Matrix-Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF), and Laser Ablation Electrospray Ionization (LAESI)

Whereas the majority of chemical analytical methods for algal toxin analysis use LC with either optical or MS detection, a number of alternative and supplementary techniques are available. These include methods based on capillary electrophoresis (CE) or ambient ionization techniques. CE is applicable to charged species, and methods for algal toxins have been reported since the early 1990s (Pleasance *et al*., 1992), including applications to DA (Zhao *et al*., 1997), PSTs (Buzy *et al*., 1994), and lipophilic toxins (de la Iglesia and Gago-Martinez, 2009). The limited use of CE in comparison to HPLC-UV/FLD and LC-MS methods is due in large part to widespread adoption of the more established methods. Nonetheless, as interfaces for CE and MS improve, there is significant potential for broader application of this technique.

Improvements in ambient ionization technologies for MS detection are yielding more applications for algal toxins, and offer the potential for direct analysis from the surface of samples, including water and possibly complex matrices, such as algae or even shellfish. Because of its sensitivity and range, MALDI coupled with ToF MS has received significant attention for microcystin analysis. The technique was used initially to screen microcystins in water samples and cyanobacteria (Erhard *et al*., 1997; Welker *et al*., 2002), and this was followed by some quantitative applications (Howard and Boyer, 2007; Puddick *et al*., 2012). To overcome impediments associated with poor reproducibility for quantitative analysis in seawater, Roegner *et al*. (2014b) developed a method utilizing synthesized internal standards for targeted microcystins that resulted in significantly improved sensitivity without the need for sample preparation. By comparison, there have been few reports of ambient ionization techniques for analysis of marine algal toxins. Laser diode thermal desorption (LDTD) ionization MS has been used to detect anatoxin-*a* directly from contaminated water (Lemoine *et al*., 2013). Roy-Lachapelle *et al*. (2014) reported quantification of total microcystins (free and bound) in water using the oxidation product 2-methyl-3-methoxy-4-phenylbutyric acid (MMPB), with a 15 sec analysis time via LDTD-APCI-MS/MS (limit of detection = 0.2 μg/L). Nonetheless, the nonvolatile and thermally labile nature of most algal toxins may limit the utility of thermal desorption for other toxins.

A method was reported recently for rapid and high-throughput analysis of DA in shellfish tissues using laser ablation electrospray ionization (LAESI) (Beach *et al*., 2014, 2016b). Ionization is carried out by charge transfer from electrospray droplets (not by the laser); therefore, ionization specificity of LAESI is comparable to that of ESI rather than laser ablation techniques. By permitting direct analysis of shellfish tissues, analysis times were reduced from ~20 min using conventional HPLC-UV methods to less than 20 sec per sample. Although this method demonstrated its utility solely in a screening role, such procedures

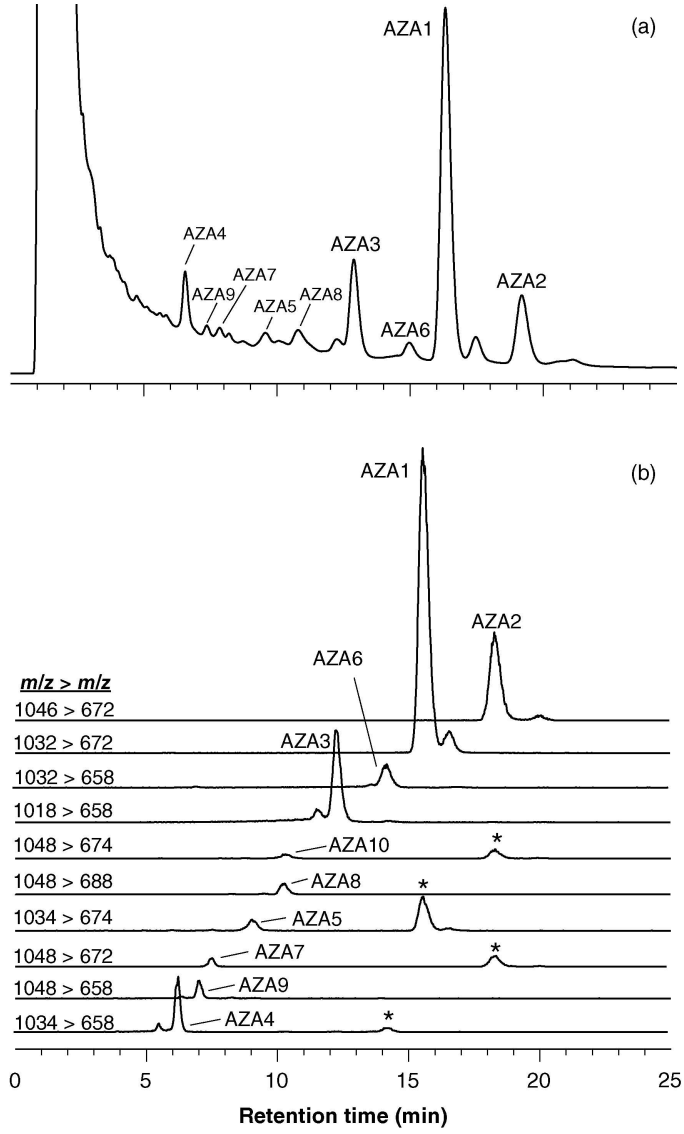

Figure 2.9 HPLC-FLD (a) and LC-MS (b) analysis of AZA-contaminated mussels after ADAM derivatization. Peaks marked with an asterisk (*) are due to ^{13}C isotope signals from other AZAs SRM transitions. *Source*: Reproduced from McCarron *et al.* (2011a) with permission of Elsevier.

hold promise for the future where increased sample loads will demand higher throughput screening capabilities.

2.3.3.4 Perspectives

When comparing methods that involve derivatization and sample cleanup for limited toxin groups and use of nonselective detection, such as FLD (Figure 2.9), it is clear that with modern HRMS methods capable of analyzing multiple toxins simultaneously (Figure 2.10), significant advances have been made in chemical analytical capabilities for algal toxins. This progress owes much to improvements in the technology, increased availability of RMs, accessible proficiency testing exercises, and concerted efforts to move away from bioassays. As techniques continue to evolve, efforts may focus on comprehensive screening methods capable of monitoring simultaneously multiple toxin groups, including the combined analysis of groups, such as PSTs and lipophilic toxins, which has thus far been limited based on chromatographic impediments. The broader availability of HRMS systems to facilitate nontargeted screening

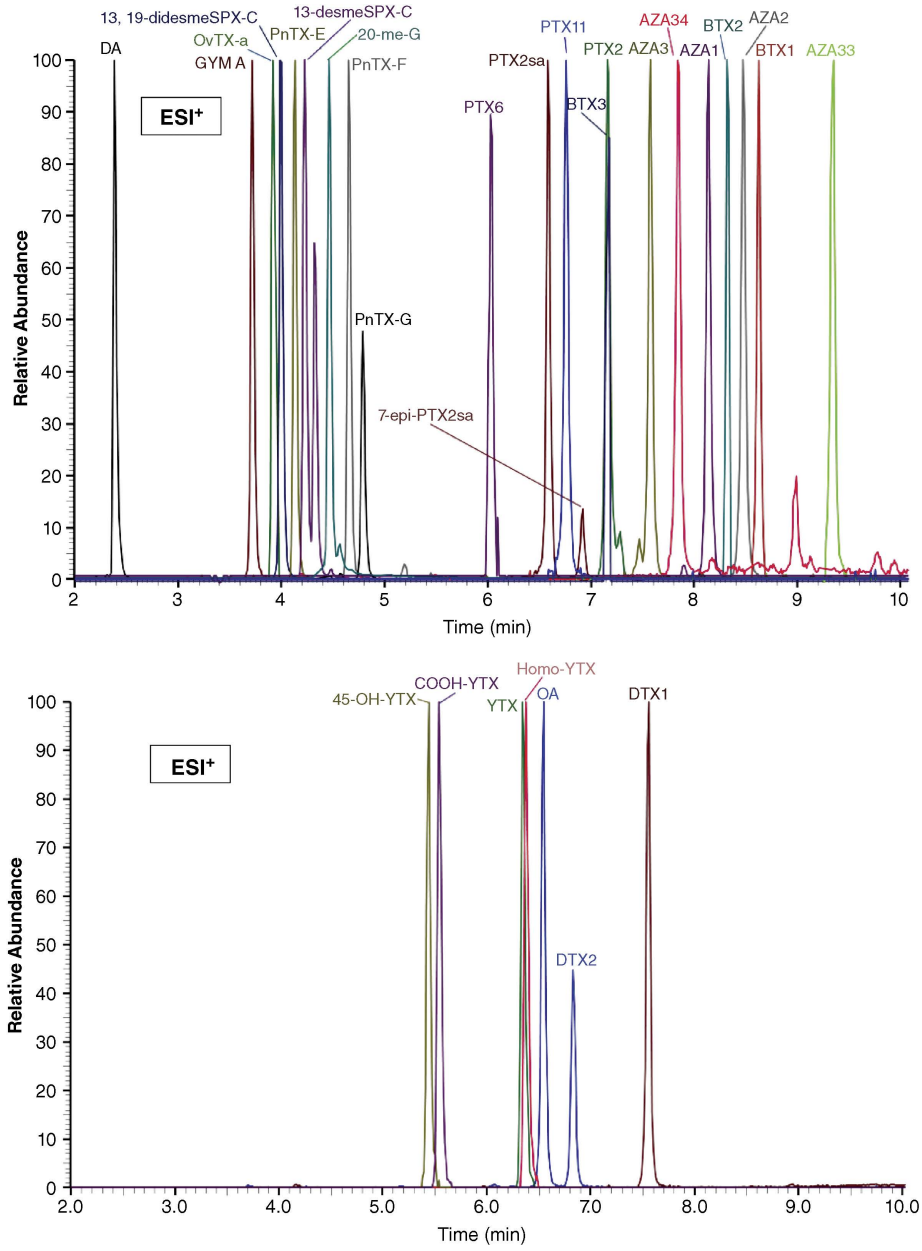

Figure 2.10 LC-HRMS analysis of mixture of DA and lipophilic shellfish toxins. *Source*: Reproduced from Zendong *et al.* (2015) with permission of Elsevier.

methods in combination with comprehensive toxin mass and spectral libraries will be an important resource in the pursuit of such objectives. Continued development of novel techniques to improve and supplement the selectivity of analysis, including the use of ion mobility (Beach *et al.*, 2015), will result in further enhancement to technologies and methods available to be transitioned into management and regulatory applications.

2.4 Autonomous, *In Situ* Technologies

The approaches to HAB species and toxin detection described in the preceding sections each have their advantages and disadvantages in terms of sensitivity, specificity, sample throughput, cost-effectiveness, ease of use, and portability. These

and other performance specifications effectively dictate whether a method is best suited for monitoring, management/regulatory, and/or research applications; however, a major limitation in virtually all cases is the inability of these methods, together with the required sample acquisition and preparation, to be conducted autonomously in a remote location (either *in situ* or dockside) and transmit results in real time or near-real time to end users that may include resource managers, public health officials, or researchers. Such timely access to data on HAB species and toxin concentrations provided by autonomous instruments deployed (or sampling) subsurface is viewed as critical for decision makers charged with protecting the health of humans, wildlife, and local economies. Moreover, although predictive models used to forecast HAB events rely heavily on input of satellite-based remote sensing data, the ingestion of reliable real-time or near-real-time biological observations is generally acknowledged as crucial for continuing to improve the accuracy and information content of forecasts.

Over the past decade, considerable progress has been made in the development of autonomous instruments capable of detecting the presence of HAB species and/or toxins and in certain cases generating quantitative measurements of cell and/or toxin concentrations (see reviews by Babin *et al.*, 2005; Anderson *et al.*, 2013; Seltenrich, 2014). Several of these instruments are now commercially available, whereas others are either ready to transition to the commercial market or have been at least demonstrated to function in a real-world setting. These instruments, their operating principles, and their detection capabilities are described below, along with selected examples of how they have been applied in the field.

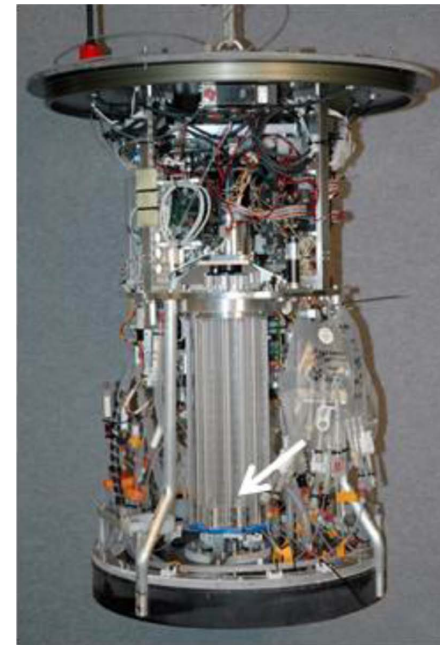

Figure 2.11 Second-generation (2G) ESP manufactured by McLane Research Instruments, Inc. (MRL); arrow points to carousel containing silver-colored titanium "pucks" that hold various filter media for conducting sample collection and molecular assays; flexible bags containing liquid reagents are visible to right of carousel. *Photo credit*: T. Walsh © 2009 MBARI.

2.4.1 Environmental Sample Processor (McLane Research Laboratories)

The Environmental Sample Processor (ESP; Figure 2.11) is a fully autonomous, electromechanical fluidic system designed originally by the Monterey Bay Aquarium Research Institute (MBARI) to collect discrete water samples, concentrate particulates, and automate application of molecular analytical technologies (Scholin *et al.*, 2009). It is capable of acquiring and processing sample volumes of milliliters up to several liters at depths to 50 m. For subsurface deployments, the instrument is contained inside a pressure housing and integrated with a fixed mooring system that is custom-designed according to the siting location (e.g., marine coastal area, offshore/shelf region, or shallow lake basin) and provides for sampling, power supply, and communications (Figure 2.12). The ESP is generally co-deployed with contextual sensors, which can include a CTD (conductivity, temperature, and depth) sensor, nutrient sensor, and *in vivo* fluorometer that provide physico-chemical and biological (e.g., Chl-*a*) data at the location and depth of the instrument. The ESP has also been deployed on a drifter at a fixed depth to provide a degree of passive mobility and is amenable to shore-based/pier deployments with a hard-wired power supply and water pumped to the intake from the surface or a certain depth.

This commercially available second-generation instrument (2G ESP; McLane Research Laboratories, Inc.; Figures 2.11 and 2.12) can implement several molecular analytical functions downstream of common sample-processing operations that include concentration of particulates and application of target (e.g., nucleic acid, toxins/metabolites, etc.) extraction chemistries to generate a sample for testing. Detection chemistries employ membrane-based

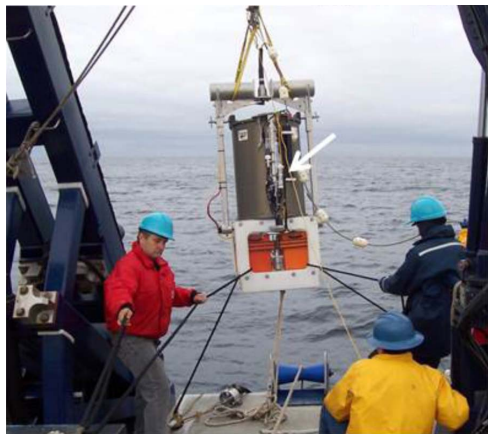

Figure 2.12 Offshore deployment of second-generation (2G) ESP contained in pressure housing showing battery pack (orange) and co-deployed contextual sensors (e.g., CTD, nutrient sensor, in-vivo fluorometer; see arrow). *Photo credit*: © 2006 MBARI.

DNA probe and protein arrays. The ESP can also archive samples for laboratory analyses post-recovery, and a qPCR capability has been demonstrated for microbial targets (Preston *et al.*, 2011). DNA probe arrays reveal target HAB species based on rRNA sequences using a SHA format for the simultaneous detection of multiple organisms in a single sample (Greenfield *et al.*, 2008). The protein arrays utilize a competitive ELISA technique for detection of toxins produced by HAB species (Doucette *et al.*, 2009). Biological data generated by the ESP are transmitted to ship or shore in near-real time via radio modem, cell phone, or satellite-based communications. Direct communication with the ESP is also possible, allowing for on-the-fly modifications to the timing and frequency of sampling as well as changes in sample volume and dilution of lysates/extracts prior to onboard testing. These adjustments allow for adaptation to changing conditions (e.g., increasing/decreasing target concentrations) and conservation of assay supplies/reagents during a deployment, which can last up to several months.

The 2G ESP has been deployed successfully many times in various geographic regions targeting several HAB species and toxins, including the Gulf of Maine (*Alexandrium* and paralytic shellfish toxins), Lake Erie (microcystins), and the California and Washington coasts (*Pseudo-nitzschia* and domoic acid). For several deployments off the California coast, this technology has been integrated into an ocean observing network comprising a variety of assets, including fixed ocean moorings, water column profilers, autonomous underwater vehicles/gliders, and satellite remote sensing (e.g., Ryan *et al.*, 2011, 2014). In these examples, the 2G ESP provided near-real time *in situ* data on the presence and abundance of multiple HAB species (with a primary focus on *Pseudo-nitzschia*), as well as concentrations of DA, that were interpreted in the context of environmental variability and processes as potential drivers of bloom formation and toxicity. Moreover, as noted by Ryan and coauthors (2011), their results illustrated "the importance of mobilizing HAB detection on autonomous platforms that can intelligently target sample acquisition as a function of environmental conditions and biological patch encounter." This requirement is being addressed in the form of the next-generation or third-generation ESP (3G ESP), as described below.

Although the 2G ESP is a uniquely powerful surveillance platform, deployments restricted to a fixed mooring (or pier) or to a drifter, as noted above, clearly impede its ability to sample accurately the spatial variability of target populations over time. In order to address this shortcoming, the 3G ESP, currently in the prototyping stage of development at MBARI (Figure 2.13), has been engineered to fit into the payload of a long-range autonomous underwater vehicle (LRAUV; Figure 2.14), while retaining much of the 2G ESP functionality (Pargett *et al.*, 2015). Moreover, software algorithms of the 3G ESP enable active detection, tracking, and sampling of target features over space and time (Zhang *et al.*, 2011). The utility of these algorithms for studying HAB ecology has been demonstrated previously on a short-range AUV (Ryan *et al.*, 2014). Deployments of the 3G ESP/LRAUV may be up to three weeks' duration and cover about 1800 km. Sample processing is carried out on single-use, self-contained cartridges (~60 total) arranged as "fins" on a movable ring assembly (Figure 2.13). Use of these sample processing cartridges maximizes efficient use of space and flexibility in lysis/extraction chemistry. Analytical capabilities being developed include onboard digital PCR and metabolite/toxin detection via reusable miniature SPR sensor chips. Several field trials of the 3G ESP have been completed successfully in Monterey Bay, California, including an end-to-end test of the SPR-based measurement of domoic acid during one flight in early 2015 (G. Doucette, B. Ussler, and C. Scholin, unpublished data). Although transition of the 3G ESP to a commercial product will likely take several years, realizing the potential capabilities of this technology will provide an unprecedented ability to interrogate HAB

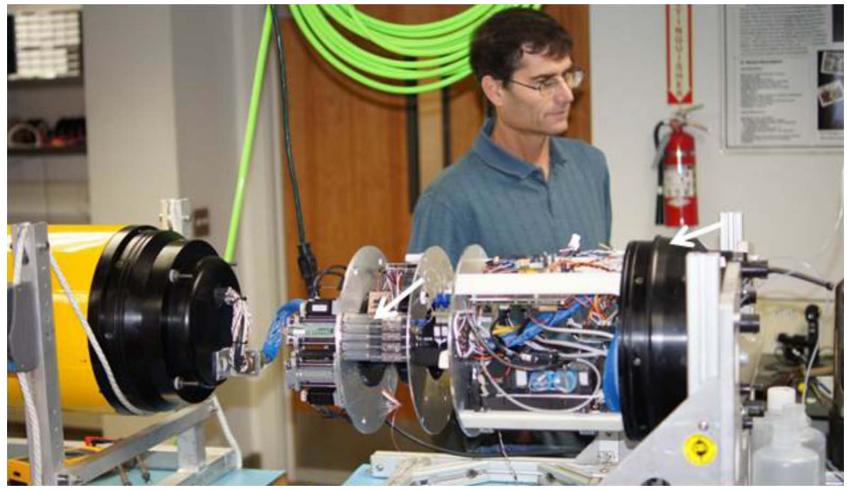

Figure 2.13 Engineers assembling a prototype of third-generation (3G) ESP inside the hull of MBARI long-range autonomous underwater vehicle (LRAUV); several self-contained sample processing cartridges are shown mounted on the prototype (arrow). *Photo credit*: J. Birch © 2015 MBARI.

populations at the molecular/metabolic level over space and time. From a management perspective, contributions of 3G ESP data streams to modeling and forecasting the growth and toxicity of bloom events and their potential impacts will be far-reaching in the context of science-based decision making.

2.4.2 Imaging Flow Cytobot (McLane Research Laboratories)

The Imaging Flow Cytobot (IFCB; Figure 2.15) is a submersible, imaging flow cytometer designed to acquire images of suspended particles in flow streams obtained from aquatic environments. The IFCB combines flow cytometric and video technology to autonomously generate high-resolution images (~3.4 pixels/μm) of particles ranging in size from <10 to 150 μm. Measurements of laser-induced fluorescence and light scattering from individual particles are used to trigger targeted image acquisition. Ambient water is sampled continuously at a rate of 15 mL/h, and ~30,000 images/h can be acquired depending on the target population. Optical and image data are transmitted in real time to a shore laboratory. The IFCB is available from McLane Research Laboratories, and operation currently requires a hard-wired power source, which is consistent with its deployment scenarios, including pier-based applications (L. Campbell *et al*., 2010) and integration into

Figure 2.14 MBARI LRAUV with third-generation (3G) ESP in payload (arrow) prepared for field deployment from R/V Paragon. *Photo credit*: J. Birch © 2015 MBARI.

Figure 2.15 Imaging Flow Cytobot (IFCB) manufactured by McLane Research Laboratories (MRL). *Photo credit*: Y. Honjo, MRL.

subsurface, cabled observatories (Olson and Sosik, 2007; Figure 2.16). Also of note is the incorporation of anti-fouling technology and periodic standard analysis to assess performance, permitting unattended deployments of up to six months. Efforts are presently underway to reduce the instrument's footprint and power requirement with the aim of integrating the IFCB into various mobile platforms for enhanced spatiotemporal coverage.

Figure 2.16 IFCB contained in pressure housing for in-water deployment. *Photo credit*: E. Taylor Crockford, WHOI.

The key capability of the IFCB is its ability to continuously monitor the planktonic community by external implementation of an automated image classification system that enables near-real-time identification of HAB species to the genus or potentially species level with accuracy comparable to that of trained microscopists (Sosik and Olson, 2007). Classification software is "tuned" to reflect the composition of the local HAB assemblage, updated as necessary, and validated rigorously in order to maintain the required level of accuracy for organism identification (e.g., Campbell *et al*., 2013). Moreover, data for the non-HAB components of the planktonic community (phytoplankton, ciliates, etc.) are extremely valuable in providing context to HAB observations. For example, information obtained on species successional patterns as well as potential prey abundance in the case of mixotrophic taxa (e.g., *Dinophysis*) prior to or following bloom events can provide critical insights into processes or factors that may regulate bloom populations' dynamics (L. Campbell *et al*., 2010). The IFCB has facilitated the identification and early warning of seven HAB events along the Texas coast, thereby providing timely information to resource managers effective in the prevention of human illness associated with these blooms (L. Campbell, personal communication). Development of a network of IFCBs is planned for the Texas coast along with their integration into existing and planned HAB monitoring and observing systems (e.g., HABIOS; http://gcoos.tamu.edu/documents/HABIOSPlan-Sept2015.pdf). Additional instruments are being deployed in multiple geographic regions – an acknowledgment of this technology's effectiveness for HAB-related applications (e.g., San Francisco Bay, California; R. Kudela, personal communication).

2.4.3 Optical Phytoplankton Discriminator (aka BreveBuster; Mote Marine Laboratory)

The Optical Phytoplankton Discriminator (OPD) is an autonomous sensor that collects spectral information on the ambient phytoplankton community to produce a photosynthetic pigment-based "fingerprint" of the material being

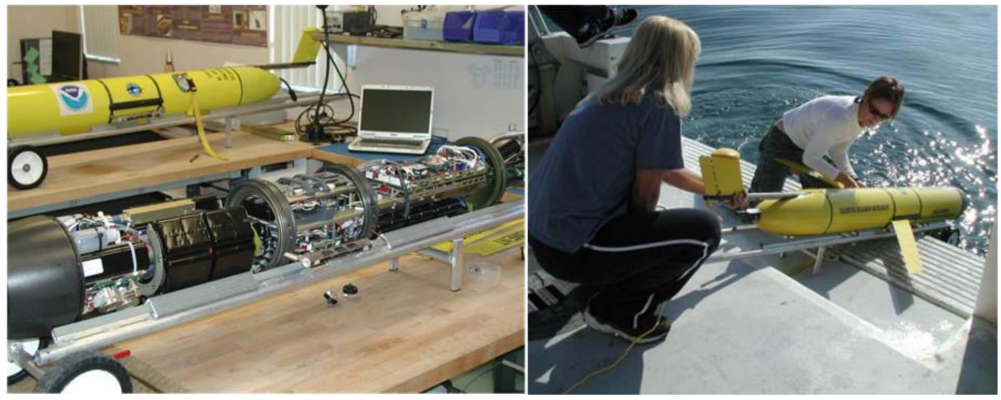

Figure 2.17 Left panel. Optical Phytoplankton Discriminator (OPD; aka BreveBuster) shown in laboratory (foreground) as configured for payload of WRC Slocum electric glider (background). *Photo credit*: G. Kirkpatrick, MML. Right panel. Field deployment of WRC Slocum glider carrying OPD. *Photo credit*: G. Kirkpatrick, MML.

interrogated. A detailed review of the development, applications, and future development of the OPD technology was published recently by Shapiro *et al*. (2015). The OPD has been deployed numerous times in a variety of configurations, including on research vessels, piers, subsurface fixed installations, and AUV-based platforms (e.g., the Slocum Glider [Teledyne-Webb Research Corp., N. Falmouth, Massachusetts; Figure 2.17] and REMUS [Hydroid, Inc., Pocasset, Massachusetts]). In a more general sense, the OPD can be used to assess overall phytoplankton community composition based on group-specific pigment signatures of the algal classes present (Kirkpatrick *et al*., 2008). In the context of HAB detection and surveillance, the basis for target specificity or discrimination of HAB taxa relies on the presence of a signature pigment that can be detected as a biomarker unique for a given species or potentially toxigenic genus. Examples of such an application include use of the accessory pigment gyroxanthin diester to distinguish *Karenia brevis* and other toxic or potentially toxic *Karenia* species within a phytoplankton assemblage (Kirkpatrick *et al*., 2000), as well as preliminary tests involving cyanobacterial HAB and identification of causative organisms to the genus level (Sullivan and Boyer, unpublished data). Several OPD installations are currently supported as part of the Gulf of Mexico Coastal Ocean Observation System (GCOOS, a regional association of the U.S. Integrated Ocean Observing System), with the aim of integrating this technology into an expanded HAB monitoring system. The OPD is not presently considered a commercially available technology; however, inquiries on custom orders can be directed to Mote Marine Laboratory (http://mote.org).

2.4.4 CytoBuoy (CytoBuoy b.v.)

CytoBuoy has placed the CytoSense benchtop scanning flow cytometer inside a submersible pressure housing configured for integration with a surface buoy for near-real-time continuous monitoring of phytoplankton and real-time data transmission (Figure 2.18). This unique system combines classical FCM data with silico-images of the measured particles and targeted video imaging. The instrument is equipped with two lasers that scan over the entire surface of a phytoplankton cell instead of striking the cell once, as

Figure 2.18 Preparation of CytoSense (arrow) integration with a surface buoy for offshore deployment (insert). *Photo credits*: G. Dubelaar, CytoBuoy b.v.

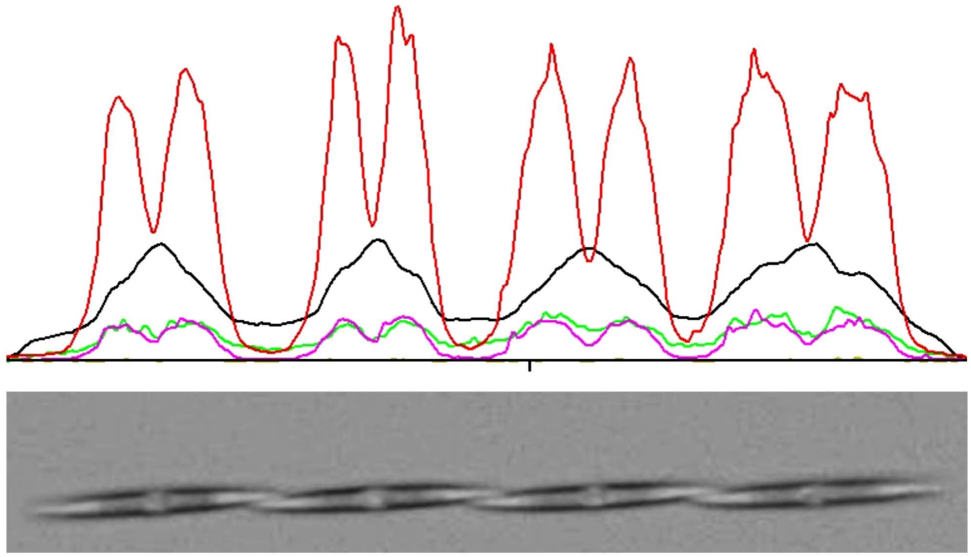

Figure 2.19 Example of a *Pseudo-nitzschia* chain as it passes through the laser of a buoy-mounted CytoSense flow cytometer. Top: Laser profiles (red = chlorophyll fluorescence; black = forward scatter; length of chain is 174 µm) generated by CytoSense software. Bottom: Matching target image (10× objective) obtained by internal camera. *Image credit*: G. Dubelaar, CytoBuoy b.v.

do most lasers in cytometers, to provide information on size and pigment signatures. This technology allows the acquisition of highly detailed information and morphological data, including better shape definition and determining the number of cells in a chain (Figure 2.19). A photocapturing system has also been incorporated into the CytoSense instrument to provide a photographic record and hence identification of cells in the flow path. The CytoSense flow cytometer has been deployed in a research mode for autonomous monitoring of the phytoplankton community, including HAB species (e.g., Dugenne *et al.*, 2015). Examples of integrating the *in situ* CytoBuoy into moored platforms for coastal monitoring systems have also been reported (Pereira *et al.*, 2016), highlighting its potential contribution to decision making within integrated coastal zone management strategies, including HAB early warning.

2.4.5 SPATT Passive Samplers

In addition to the *in situ* detection technologies described above, it is important to also briefly highlight the availability of a simple yet very powerful means of autonomously obtaining samples for laboratory-based toxin analysis. SPATT passive samplers were first described by MacKenzie *et al.* (2004) to monitor for the presence of low-polarity lipophilic marine algal toxins (e.g., DSTs, pectenotoxins) in water. This approach, which involves placing one of several different porous synthetic resins in a fine-meshed bag that is deployed suspended in the water column, is aimed at simulating contamination of filter-feeding bivalves and thereby serving as a sentinel for the appearance of toxin(s) at a monitoring site. Use of SPATT-based sampling strategies has since been adopted for applications targeting a wide range of marine and freshwater toxins, including more polar compounds, such as domoic acid and saxitoxin (Lane *et al.*, 2010), as well as cyanobacterial toxins, such as microcystin (Gibble and Kudela, 2014). Extracts of SPATT resins are amenable to testing by either *in vitro* or analytical methods, whereas interpretation of results in terms of extrapolating back to in-water toxin levels presents a greater challenge, and standardized protocols (e.g., resin type, quantity of sorbent, deployment period, extraction procedure, etc.) are needed in order to ensure intercomparability of data. Nonetheless, SPATT passive samplers have proven effective in providing early warning of shellfish contamination by algal toxins in various geographical regions; however, in cases where blooms develop in remote locations and are transported rapidly into a local area, SPATT signals may develop simultaneously with resource contamination (reviewed by MacKenzie, 2010).

2.5 Conclusions and Future Prospects

Detection and surveillance of the species responsible for HAB and the toxins produced by these increasingly ubiquitous and problematic organisms are essential on multiple levels, ranging from research to monitoring, management, and regulatory applications. The performance requirements of methods specific to each of these applications cover a wide spectrum in terms of the type of data generated (i.e., qualitative, semiquantitative, or quantitative), sensitivity, specificity, speed of analysis, logistical constraints (e.g., lab and/or field applications), technical expertise, and cost-effectiveness. As a result, the method(s) adopted for a given application should be "fit for purpose" from the standpoint that performance requirements are aligned as closely as possible with the desired outcome or purpose of the application. For example, if the intent is to implement a rapid, field-based, qualitative/semiquantitative screen for the presence of a target organism, toxin, or even type of toxic activity, then only a given subset of the methods and technologies identified in this chapter would be appropriate and fit for purpose. It is therefore critical that end users, whether research scientists, resource managers, public health officials, or regulatory agencies, first determine what type of information they require and on what spatiotemporal scale, and use these criteria to evaluate carefully the available methods or technologies in order to determine which ones are best suited for their needs.

In certain cases, more classical approaches to HAB organism detection (e.g., light microscopy) may be entirely adequate and highly cost-effective for surveillance requirements, especially in cases where detailed taxonomic resolution (i.e., species-level identification) and strictly quantitative data are not required. A positive result could then trigger application of molecular-level analysis to provide accurate identification of species and assessment of their concentration. Similarly, for surveillance of toxin presence in the water column, tiered application of a rapid, high-throughput detection method (e.g., LFIA) to screen samples in the field can be sufficient to alert resource managers to a potential hazard, which can then be followed up by more detailed analytical evaluation using methods, such as LC-MS/MS for the chemical confirmation and concentration measurement needed to assess more accurately the risk associated with a bloom event. Conducting a detailed cost–benefit analysis (e.g., equipment purchase and maintenance, level of operator technical expertise, cost per sample, time per sample, etc.) should also be considered when designing a surveillance strategy for detecting toxic HAB for the purpose of mitigating and managing their impacts.

It is important to reiterate the fact that the toxicity of HAB species can be highly variable such that toxicity can become uncoupled from organism presence depending on environmental factors and the resulting physiological status of the algae. This can lead to situations where cells of a given HAB species may be present or even abundant, yet little or possibly no toxicity is associated with these cells. Clearly, the data required to inform accurately the proper management response need to include information on both toxin and cell concentrations, and in certain cases detection of toxin genes, and methods should be adopted accordingly. This may involve the use of a tiered approach where a positive test (perhaps at some threshold level) for the presence of cells belonging to a given HAB species and/or toxin genes triggers the application of testing for the actual toxin(s) associated with that organism. The co-deployment of toxin- and organism-specific sensors on an *in situ* instrument (e.g., ESP) can also provide concurrent information on cell and toxin concentrations useful in estimating the amount of toxin per cell and thereby aid in assessing bloom toxicity.

In terms of future prospects and gaps to be addressed, although considerable progress is being made on the development of new methods and technologies for HAB species and toxin detection, significant needs remain at both ends of the technological spectrum. Powerful yet small, user-friendly, and cost-effective devices that can be implemented in the field (e.g., from shore or small boats) by monitoring programs will maximize spatiotemporal coverage as well as facilitate adaptive sampling in response to the rapid changes that frequently characterize HAB events and associated contamination of fishery resources. Moreover, an emphasis should be placed on the development of multiplexed technologies that are capable of targeting multiple HAB species, toxin genes, or toxin classes (including major congeners) in a single sample, because there is frequently overlap in the distribution of these targets within a geographic region. Given that the resources available to support monitoring programs for phytoplankton and/or fishery resources are frequently quite limited, such capabilities are essential to maintaining the coverage required to ensure

public safety and minimize the potential economic impacts to wild and farmed shellfish and finfish harvesters. It is also crucial that developers interact closely with the user community in order to ensure from the outset of the design and development process that the resulting detection technology will be fit for purpose and achieve the desired aims.

Advances are also needed in the area of sample preparation to support the use of field-portable devices targeting either organisms or toxins, noting that the toxins being targeted can be associated with either algal cells or tissue (i.e., shellfish, crustaceans, fish, etc.) as well as occurring in dissolved form (especially a concern for drinking water treatment). Frequently, the focus of developers is solely on modifying or improving the detection technology, whereas issues associated with sample preparation can limit the usefulness of a device for a given application. Processes, such as particle (i.e., cell) harvesting and concentration, cell lysis/tissue homogenization, target (i.e., nucleic acid, toxin) extraction, cleanup, and/or concentration, may be required depending on the type of sample required by the detection device. The sensitivity of detection is often proportional to the extent of sample preparation; in other words, achieving a high level of sensitivity (or low limit of detection) frequently requires extensive sample cleanup and preconcentration of the analyte. In the area of medical diagnostics, the ability to conduct these latter processes "on-a-chip" has been a priority, as has been the development of sensors that operate at the same miniaturized scale; however, for environmental applications, and specifically for HAB species and toxin detection, the sample volume required (e.g., a drop of blood vs. a liter[s] of seawater vs. milliliters of tissue homogenate) generally precludes such small-scale processing. Nonetheless, efforts to identify and design unique and novel approaches to address the challenges associated with sample preparation should be considered essential and capable of yielding important advances that will directly benefit HAB monitoring and management.

At the other end of the technological spectrum, progress in the development, refinement, and application of chemical analytical methods and their associated instrumentation has been quite remarkable, as discussed above (Section 2.3.3); nevertheless, there are several outstanding and/or continuing requirements that are critical for successfully implementing such methods – especially in a monitoring or regulatory environment. In particular, chemical analytical methods and their utility in regulatory applications rely on the development and acceptance of *toxicity equivalence factors* (TEFs; Botana *et al*., 2010). TEFs are normally applied in the context of control or surveillance systems, where the presence of a toxin group needs to be defined by a single value that provides a reliable measure of a sample's total toxicity. In order to convert quantitative values for concentrations of a given toxin or toxin group into an overall toxic level based on the intrinsic toxicities of each analog present in a sample, TEFs of all analogs present should be summed to yield a single value representing the total toxicity of a sample. This value is generally expressed as equivalents relative to a reference compound (e.g., STX equivalents), which requires availability of pure reference material. Moreover, determination of TEFs for individual toxins is highly dependent on the route of administration used and can vary accordingly (e.g., i.p. vs. oral; Munday *et al*., 2013). In the case of reference materials, which are required for determination of TEFs, as well as a range of other applications including method development, evaluation of sample matrix effects, and accurate calibration of instruments, considerable progress is being made for many of the toxin classes of regulatory concern. These include both pure reference materials and, importantly, those contained in a shellfish matrix (e.g., McCarron *et al*., 2016). Moreover, as technology continues to advance and researchers develop methods and approaches to take advantage of these new capabilities, it will be essential to implement rigorous interlaboratory validation studies in order to transition their application into the regulatory environment.

Finally, several examples were highlighted in which autonomous, *in situ* detection technologies have been (or are planned to be) deployed as components of observing networks capable of generating near-real or real-time data for HAB species and/or toxins. Such observations have intrinsic value in providing early warning of HAB events, assessing bloom toxicity, identifying potential drivers of HAB growth and toxin production, initializing models, and validating airborne/satellite observations and model outputs; however, in the context of observing systems, it is expected that building out of this architecture will continue at a rapid pace as assets are added in order to enhance data collection capabilities and information content, which are especially lacking for biologically targeted sensors as compared to those designed for physicochemical measurements. Anderson *et al*. (2013) pointed out the need to integrate HAB-specific instrumentation

into expanding observatory infrastructure in order to co-locate assets in areas or at sites that are most useful for HAB detection (e.g., well-established "hot spots" for bloom initiation). Additionally, these authors, as well as Kudela *et al.* (2013), emphasized the importance of observing data for HAB species and toxins in the context of ingesting this information into predictive models that are essential to generate accurate forecasts. The latter authors also point out the need for observing systems to include shore-based observing stations located in proximity to HAB-sensitive targets in addition to carefully selected offshore mooring sites that can serve as a proxy for regional conditions and also generate early warning of potential bloom activity. As in the case of weather forecasts that rely on distributed observing networks for assimilating accurate, real-time, high-resolution data, the quality, reliability/robustness, timeliness, and spatiotemporal resolution of cell and toxin observations will influence the accuracy and thus the effectiveness of forecasts for managing and mitigating HAB impacts in the future.

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Modeling Marine Harmful Algal Blooms: Current Status and Future Prospects

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3.1 Introduction

A model is a simplification of reality, and the purpose of this chapter is to explore the limitations and potentials for such simplifications to serve useful roles in the management and mitigation of harmful algal blooms (HAB). Others, such as Glibert *et al.* (2010), have provided overarching reviews on factors that may actually be associated with predicting events; here, the emphasis is upon assessing the state of the art, and how to advance it. Some of the challenges identified stem from issues specific to HAB science, while others apply to plankton research in general; challenges in both have arguably hindered progress in the development of HAB forecasting capability and management tools. These challenges can best be addressed by closer collaboration among researchers conducting laboratory, field, and modeling work. Improved interactions among these communities can be facilitated by clarification of terminology used in the various subfields (for discussion and an attempt to provide some clarity, see Flynn *et al.*, 2015b). Indeed, models can provide useful dynamic test beds for exploring and testing hypotheses, guiding future iterations of field and laboratory investigations, and providing an improved overall level of understanding.

Simplification in modeling can be extreme, as represented by a statistical fit of a regression line through data; and, in some cases, such models can be entirely adequate. At the other end of the spectrum, models may purport to describe temporal dynamics of dozens of organism types within 3D spatial scenarios. While it may be argued that all models are imperfect and that models are designed specifically to tackle individual questions, such views malign the real value and potential of adequately constructed models in informing us about the real world, how we think it works, and how our understanding may be in error. Errors may reside at conceptual levels as well as in the conversion of understanding into equations and parameter values. Nevertheless, both statistical/empirical and mechanistic models can provide tools for scientific investigation as well as prediction. Choice of approach depends on the specifics of the application and purpose of the model in that context.

The more complex models typically are built upon (and thence should enhance) mechanistic understanding. Complexity does not refer here to factors such as spatial resolution or pure computation load, but rather to the degree of conceptual complexity that underpins the description. For biological components, complexity refers more to the level of physiological detail applied to each organism grouping (ecological functional type; Flynn *et al.*, 2015b); complexity does not relate simply to the number of groups, each of which could contain the same very simple conceptual structure differing only in the value ascribed to traits such as organism size or maximum growth rate.

Typically, model components describing physiological features of organisms are empirical; that is, they describe behavior that accords with empirical data (i.e., that which is observed). At the extreme, empirical descriptions may relate factors that in reality are only distantly related to each other. Care

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must be taken when using such relationships, especially in a predictive mode. On the other hand, empirical approaches can help identify the relative importance of multiple factors relevant to HAB phenomena, therefore contributing to knowledge of the underlying dynamics. At the other extreme are systems biology approaches that are akin to dynamic biochemistry pathway descriptions. One may argue that feedback processes akin to those controlling the biochemistry (ecophysiology) of the individual organism types should be a feature of mechanistic models (Flynn *et al.*, 2015b); the behavior of the modeled organism is then an emergent property of the interactions between various processes, mimicking reality. In practice, however, even the most mechanistic of models includes empirical components that do not contain such feedbacks. A parallel between such "empirical" and "mechanistic" descriptors as applied to ecosystem models can be seen. At one extreme, empirical models could relate bloom events to climatic features by statistical fits to data, and at the other extreme mechanistic models could describe temporal dynamics of detailed interactions between named organisms in a 3D description of watery space. A rigorously constructed and tested mechanistic description (at both the autecology and ecology levels), built upon a high level of understanding, has potential to provide a firmer basis for prediction into an uncertain future, such as that presented by climate change. From such models, robust empirical simplifications may be built to ease computational burdens, but such a route differs greatly from an *a priori* empirical simplification based upon approaches such as statistical fits between data from past events. Critically, however, sufficient scientific understanding is needed to be able to build such mechanistic models, and we need to appreciate that even mechanistic models may have limited predictive power in regimes where the dynamics are intrinsically chaotic (Benincà *et al.*, 2009).

Here, emphasis is placed upon descriptions of simulators of HAB that describe systems dynamics, and thus contain time as a dimension. Deployment of models in management ranges from short-term forecasting, often driven in part by external data from remote sensors, while other approaches use fully computational simulators in a what-if predictive mode, for example in consideration of proposed coastal engineering or of sewage outfall design. The construction and testing of dynamic models are severe tests of our understanding of the real system. Even after decades of research, our understanding of the underpinnings of HAB events remains incomplete. Indeed, our understanding of growth dynamics, loss processes, excystment and encystment, and factors promoting toxicity for individual species is wanting. Understanding is promoted by attempts to build models from a conceptual basis (akin to flow diagrams or food web schematics), and comparing the output of such models to empirical evidence. Confidence in the behavior of models under all plausible conditions promotes increasing confidence in the value of using such models in a predictive setting, whether that be for toxic HAB (T-HAB) or for algal blooms that cause aesthetic and/or ecosystem damage (ecosystem-disruptive HAB, or ED-HAB).

From here onward in this chapter, the term T-HAB refers to bloom events linked to biotoxins. The bloom of the T-HAB species itself may be of minor consequence (cryptic) from a total plankton biomass perspective, and the toxins often have their impact far from the sphere of algal trophic dynamics (i.e., on mammals and birds, rather than on their zooplanktonic grazers). Furthermore, the causative organisms need not necessarily be toxic all the time, and toxicity can develop significantly with limited concurrent biomass growth. The term ED-HAB is used to describe ecosystem-disruptive mass growths of organisms that developed at least in part because growth was not constrained by grazers. ED-HAB events may develop because the algae are *de facto* unpalatable to the usual grazers of microalgae (hence, the typical trophic interactions are blocked). Alternatively, ED-HAB may develop where the grazers cannot contain the algal production, perhaps because those grazers are themselves contained by the activities of higher trophic organisms, such as planktivorous fish or ctenophores. When mass growths die, their decay frequently causes ecosystem disruption due to deoxygenation of the water column and/or of the benthos. (The term *ecosystem disruptive algal bloom*, or EDAB, as proposed by Sunda *et al.*, 2006, for specific reference to blooms of algae unpalatable to grazers, falls within our term ED-HAB.)

While various aspects of T-HAB and ED-HAB overlap, the causative organisms and the events themselves typically differ greatly in detail and scale, and thence also in the ways in which one may elect to model their development and progression. That said, the proliferation of any species (be it cryptic or dominant in biomass) is a function of the rates of growth and losses of that particular species set against those of competitors and

predators. It may thus be expected that studies (and models) of HAB species alone cannot provide mechanistic understanding of the events; a more holistic understanding and simulation capability is required of planktonic (if not also benthic) systems.

If there were confidence that HAB events ran along a set pattern, that future events could be mapped against past events, then statistical models could be safely deployed (noting that one should not use regression statistics to predict results outside of the data range used to configure the model fit). However, set against the uncertainties of climate change and the vagaries of human activities that affect nutrient release into aquatic systems, removal of fish, modification of coastal topography, and so on, conditions enabling or supporting future HAB events, and especially T-HAB events, may well not conform to past events. The need to develop mechanistic understanding and deploy that within the framework of computational modeling thus becomes strengthened. This is not, however, to minimize the importance of short-term forecast-mode HAB modeling, which operates over time scales of days to weeks, coupled with weather forecasting and data collection in real or near-real time (e.g., Raine *et al.*, 2010). Such programs provide early warnings to resource managers and users to enable them to take what mitigating action they can (e.g., Applied Simulations and Integrated Modeling for the Understanding of Toxic and Harmful Algal Blooms [ASIMUTH]; see www.asimuth.eu; Anderson *et al.*, 2015, sect. 17.5.3).

3.2 Building Models to Describe Ecological Events

In broad terms, studies of plankton can be divided at the extreme between those conducted in the laboratory (in which variations in the abiotic environment and the biological composition are both controlled) and those conducted in the field (where the abiotic system is not controlled and the biotic composition is often highly complex). By the same token, modeling studies may be divided along similar lines, into those that are relatively highly detailed physiologically and those that allocate computational resources more toward descriptions of the physical environment and thence use simple descriptions of biology. Depending on their complexity, studies in mesocosms align more or less with laboratory or field studies.

A schematic of idealized interactions between laboratory and field research efforts is shown in Figure 3.1 and described in the associated legend. The reason for conducting physiological experiments is to provide a better understanding of how individual biological and trophic interactions function, with studies run under guidance from those working in the field to identify the organisms of interest and the types of events (e.g., transients in temperature, nutrient availability, etc.) for which detailed information is lacking. From the understanding developed through such biological studies, models can be constructed and run to test hypotheses under different environmental conditions.

One line of hypotheses particularly worthy of consideration is to explore which parameters, and which model components, exert most leverage on model performance. This is of use in two ways. Firstly, components or features are identified that warrant the most attention for both future model and experimental (laboratory/field) work. Secondly, those components that may be safely simplified or even deleted from computationally expensive models can be dealt with accordingly. This complex-to-simple approach (akin to an engineering approach of overbuilding and then testing for weakness and redundancy) is, however, not typically undertaken in biological modeling work. While flasks contain complex organisms growing in simple physics, the seas never contain simple organisms growing in complex physics. Acknowledging this situation presents an important reality check when considering the status of different generations of ecosystem models (Figure 3.1).

Two other points are worth making at this juncture. Plankton ecosystem models have many of their roots in biogeochemical studies. As such, they tend to place comparatively little emphasis upon the physiologically and ecologically complex food webs that encompass HAB events. Indeed, the modeling of zooplankton (noting that many HAB are mixotrophs, and also that algal blooms can only develop in the absence of effective grazing pressure) is well known to be weak (Mitra *et al.*, 2014a). For many applications to HAB, the current basis of plankton ecosystem models may thus appear less than optimal. The other point is that, although specific subcomponents used in these ecosystem models are often informed by laboratory measurements (e.g., phytoplankton growth rate as a function of temperature and light), the models have rarely if ever been actually tested against robust data series as generated in laboratory conditions. Some attempts have been made to

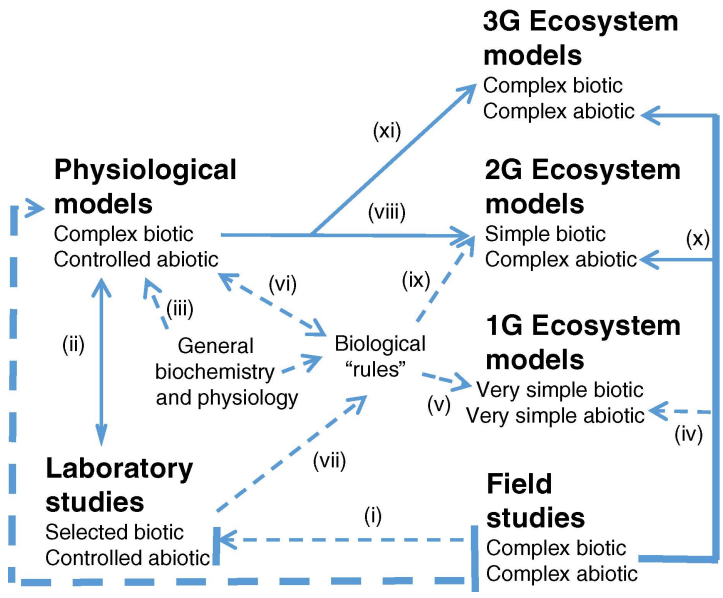


Figure 3.1 Schematic for the development of ecosystem models. Conditions and biological composition at field sites inform the laboratory study of selected organisms grown under controlled conditions (i) Information, and data, from laboratory studies (ii), together with generic biochemical and physiological understanding (iii), enable the construction and testing of complex systems biology–style models describing the physiology (autecology) of organisms, and thence coupled models of simple trophic systems. Typically, the flow of information (ii) is from experimental to modeling research, although models can be used to design *in silico* experiments to aid hypothesis setting for further rounds of laboratory studies. First-generation (1G) ecosystem models, as typified by Fasham *et al.*'s (1990) type NPZ models, contained much-simplified representations of the abiotic system (iv), together with very simple models of the biota configured from biological rules (v) built from general and theoretical principles (vi) such as Monod and Holling kinetics, perhaps including concepts developed from physiological models, and data such as maximum growth rate estimates from laboratory studies (vii). The current, developing, second-generation (2G) ecosystem models contain greatly enhanced abiotic descriptions; however, the biotic descriptions typically do not make use of advances from physiological models (viii) but deploy enhanced developments from biological rules (ix). Future (third-generation, or 3G) ecosystem models may be expected to describe abiotic systems with ever greater fidelity, with the aspiration that these will also serve as platforms for placement of systems biology–style physiological models (xi) within high-resolution abiotic simulators.

use mesocosm experiments for this purpose (e.g., Aksnes *et al.*, 1994). Whether models are fit for purpose is gauged by comparison of model output, typically in terms of areal biomass, against spot sample points (oceanographic stations) or against satellite images of events at the sea surface. The use of field data carries with it the burden of transformations between pigment abundance and biomass, between cell and organism counts in different volumes of water, and so on. Those interested in T-HAB and ED-HAB need to ask whether they consider models originally constructed for biogeochemistry (rather than ecology) as representing a suitable basis for best progress.

Taking all the above into account, the schematic of Figure 3.1 describes a research effort that is in reality all too often dispersed and isolated, rather than coupled. For the most part, conceptual detail on the physiology of plankton, let alone on HAB species, gained from laboratory experiments does not make it to ecosystem models. While many scientists may (with justification) worry that experiments with laboratory cultures cannot replicate events in reality, not least because of the potential adaptation of cultured organisms to artificial conditions during long-term laboratory growth, it is difficult to see how the underpinning biochemical and physiological framework would be so overturned that laboratory results are not of value. The utilization of "biological rules" in ecosystem models, which include concepts of allometric scaling and "trait trade-offs," may be viewed as of particular concern for the task at hand, because these do not appear to be applicable to many of the planktonic organisms associated with HAB or indeed of planktonic predator–prey interactions in general (Hansen *et al.*, 1994; Flynn *et al.*, 2015b). There are also some obvious important aspects of plankton ecology that are underemphasized, if not absent, in most models. An example concerns

descriptions of encystment and excystment, although for the most part comprehensive data on the death rates of cysts and the triggers for excystment are also lacking (Hense, 2010). Another important avenue that is underexplored and hence poorly considered in models is the role of micronutrients and of allelopathic interactions (Pohnert *et al.*, 2007).

3.3 Limitations to What Models Can Do, and Why

3.3.1 Building Models

How useful HAB models may be depends on how well the model describes reality. Models can be used for various purposes. Conceptual models help the formulation of ideas, to identify at a phenomenological level the strengths and weaknesses in knowledge; however, it is only at conversion of the conceptual model into a mathematical model that a quantification develops of what is known, and what is not known. For each of the interactions, one may commence by configuring a response curve between the driver and consequence. For example, one may generate relationships between satiation in a consumer and its feeding rate; as satiation develops (gut becomes full), feeding is slowed. Response curves may have a negative or positive slope; they may be linear, curvilinear, sigmoidal, or of more complex form. In all instances, organisms can upregulate or downregulate aspects of their physiology depending on the environment, thereby introducing plasticity into the parameters of such response curves (Flynn *et al.*, 2015; Kana and Glibert, 2016). Establishing the form of the curve is the first step in converting, for example, the conceptual model of a food web diagram into a dynamic model.

Relatively little is known about the nonlinearities in these response curves. Two examples of importance to the topic of modeling HAB relate to the key role of grazers in permitting or controlling bloom development. This is of particular concern for ED-HAB (Mitra and Flynn, 2006; Sunda *et al.*, 2006). The decline in food quality when phytoplankton exhaust nutrients does not necessarily have the simple linear consequence one may expect from stoichiometric ecology (Sterner and Elser, 2002); rather, it may have a distinctly nonlinear response resulting in prey rejection at low levels of nutrient stress leading to formation of an ungrazed bloom (Mitra and Flynn, 2005, 2006). Understanding just how important subtle changes in biochemical stoichiometry may be, for example how changes linked to ocean acidification may have far-reaching consequences on plankton ecology, has just begun (Flynn *et al.*, 2015a; Cripps *et al.*, 2016). Another feature of grazers commonly modeled as linear relates to assimilation efficiency (AE); this is typically held constant in zooplankton models, although it is well known to vary with quality and quantity of phytoplankton prey (Mitra *et al.*, 2014a). Modeling to account for changes in AE generates very different predator–prey dynamics that can see a much more rapid removal of a bloom than would otherwise be expected from simple models (Flynn, 2009). For the formation of ED-HAB, on account of insufficient grazer control due to the success of planktivorous higher trophic levels, such challenges in modeling the activity of consumers extends beyond microzooplankton and copepods. While closure terms may often be deployed in such instances, this approach is not a substitute for adequate understanding of the role of trophic cascades in ED-HAB ecology.

3.3.2 Model Complexity

A fundamental challenge in modeling centers on the issue of a "simplification of reality." In a more ideal world, where resources and thence data and computational power were less limiting, complex models would be built and their behavior explored to identify how best to achieve simplifications by progressively deleting or otherwise simplifying components. Indeed, some modelers now use a complex-to-simple approach; this provides a route to generate empirical models from mechanistic models (see "Introduction," this chapter). There are many modelers who quake at the number of parameters in complex biological descriptions, concerned as to how these will all be estimated; however, an appropriately formulated mechanistic model actually does not have that many real free parameters for adjustment. Most parameters are used to describe the shape of response curves, and model behavior is largely insensitive to their exact value. Fasham *et al.* (2006) give an example of a complex phytoplankton model placed in an ecological setting; they discuss the (non)issue of the parameter count.

More often, however, the starting point for model construction is a discussion regarding which minimum set of parameters and equations is needed to confront a specific issue; only if this

simple model fails are additional complexities added. The challenge in the simple-to-complex approach is deciding what constitutes a failure (Franks, 2009) that perhaps then warrants increasing complexity to include additional factors. Statistical approaches such as *maximum likelihood* offer methods to frame model–data comparisons in terms of a hypothesis test, thereby allowing quantification of the confidence with which one model fits the observations better than another (Stock *et al.*, 2005, 2007). Examples of errors that develop during initial simplification include using single rather than multiple variable stoichiometries, and incorporating inappropriate functional type descriptions and associated food web linkages. The last mentioned is particularly problematic given growing appreciation for the role of mixotrophy in aquatic protist ecology (Flynn *et al.*, 2013; Mitra *et al.*, 2014b), and linked to the fact that many (protist) HAB species are mixotrophic (Burkholder *et al.*, 2008). The extent of this particular failing runs across all parts of the HAB research spectrum, from issues of field monitoring (are chlorophyll and inorganic nutrient levels really the best indices for the presence and activities of mixotrophic protists?) to defining conceptual and thence mathematical models.

A consequence of the drive for simplification is the need to group organisms together; it would be impractical to describe the dozens up to perhaps hundreds of individual species present in a real ecosystem. In ecology, organisms are typically grouped (irrespective of phylogenetic origin) according to the way that they interact with environmental factors (Gitay and Noble, 1997), thus forming "functional type" grouping. Plankton functional types (PFTs) appropriate for modeling HAB may be expected to be quite different from such groupings intended for biogeochemical modeling (with their emphasis on "diatoms," "coccolithophorids," etc.). In biogeochemistry applications, little emphasis is placed on competition and predator selection processes, or on features such as consideration of mixotrophs that acquire their photosystems from prey (the nonconstitutive mixotrophs; Flynn and Hansen, 2013; Hansen *et al.*, 2013; Mitra *et al.*, 2016). These factors may be of critical importance to describe the types of events that lead to (or block) development of T-HAB or ED-HAB events. Understanding the causal basis for coexistence or mutual exclusion of species on the run up to, during, and then after plankton blooms appears fundamental to the task at hand.

It is at this point worth considering the interface between molecular biology and modeling. The application of molecular biology to HAB and general plankton research has brought to our attention the great variety of life forms, and the presence of different species and subspecies. There is thus a stark contrast between molecular and mathematical biology, because while modeling inevitably merges the activity of organisms together and is a topic driven by trophic dynamics, molecular biological research represents almost a diametric contrast. Linkage of omic signatures to physiological status and toxicity could, however, be of great value to modelers, generating data for validation. The use of automated molecular tools may also help in building PFT groupings as well as for the detection and monitoring of HAB (Scholin *et al.*, 2009).

From the foregoing, it may be tempting to conclude that empirical approaches, based on statistical methods or expert systems, may be no less robust than attempting to deploy dynamic mechanistic-based models. There is, however, one fundamental problem; as mentioned in this chapter, it assumes that future patterns of behavior have already been seen in previously collected data series. With the permutations of potential change (natural fluctuations as well as anthropogenic forcing), it seems likely that future conditions will be outside the envelope of variations in the recent past. This is perhaps not so much an issue for short-term management of existing coastal systems (although extreme weather conditions may become more common with climate change), but it is an issue in considerations of the design of coastal engineering projects and watershed management, with a need for risk analyses played out over decades. In consequence, there is a need to try to encapsulate understanding of all the factors that impinge upon HAB events within models. Like weather forecasts, there is a need to appreciate that, at best, capabilities for predicting HAB are limited, deal with probabilities, and most likely will depend on inputs from different model types and approaches. Indeed, the corollary drawn with weather forecasting is particularly apposite given that the weather plays such an important role in the initiation and termination phases of HAB events, and indeed of plankton growth in general.

3.3.3 The Need for Data

Data availability is important, and of equal importance is the form of the data. Conceptual food web diagrams, and simple models such as Lotka–Volterra predator–prey descriptions, have no need for data with specific units. However,

systems dynamics models have an absolute need to correctly account for units; most are based upon a single or multiple currencies. Classic marine biogeochemical models use nitrogen (N) as the sole currency (Fasham *et al.*, 1990); nutrients and biomass are defined as mol N m^{-3}, with rates as mol N m^{-3} d^{-1}. Allied to this usage of a single currency is the assumption of fixed Redfield ratios for C:N:P:(Si). More complex models employ variable stoichiometries and multiple functional types within trophic levels (C:N:P; Baretta *et al.*, 1995). Given what is known about HAB, the bases for development of toxicity and poor palatability for grazers, and the ability of microalgae to use and hence compete for different nutrients including prey (Flynn *et al.*, 2013), multiple variable stoichiometric models can be seen to present various advantages over single-currency models (Flynn, 2010a). That is all the more so when one considers that, in the future, the nutrients limiting growth may differ from those that do so at present due to the damming of rivers and changes in land use, fertilizer applications, and rainfall patterns (Rabalais *et al.*, 2009). Correctly modeling the usage of different nutrients is important as it affects the potential to predict the nutrient limitation of phytoplankton successions (Flynn, 2005, 2010b).

The need for data of a certain type presents a modeler with various challenges, as transforms (with associated assumptions) are then required to interconvert data types. As an example, algal biomass is typically estimated in terms of chlorophyll (and that often as *in vivo* fluorescence of the bulk population), while zooplankton are often estimated as numbers per unit volume with some level of taxonomic detail. In contrast, the representation of these groups in models may be as N-biomass, with the phytoplankton and zooplankton each described as one or just a few functional types. Decisions upon such matters, nutrient currency and how best to collate or group data, affect the modeling activity and thus scope for use of the final product.

3.3.4 Validating Models

Models should be constructed and tuned through reference to one set of data, and then validated against another separate data set. That is to say, the model is typically run against real data and selected (constant) parameters adjusted to enable the best fit of the model output to data. The model is then run again under a new set of conditions, in line with the drivers for a different documented scenario, and its output compared to the new real data series. Too often, data series are not available to support both tuning and validation. It is thus important to appreciate the limitations of modeling; sufficient knowledge of the biotic and abiotic system is often lacking to achieve more than a phenomenological fit of model to data. A good outcome is if model output satisfactorily aligns with the validation data series, ideally with respect both to timings of events and to magnitude. Getting the model to replicate the timing of an event is often considered more important than simulating the magnitude correctly, but for HAB management both are important.

3.4 Modeling T-HAB and ED-HAB Events

There are fundamental differences between describing T-HAB versus ED-HAB dominated blooms, and versus blooms dominated by benign organisms (accepting that any bloom could become so large that it could cause damage to the ecosystem upon its death through deoxygenation – at which point it would conform to what is termed here a form of ED-HAB). Cyanobacterial T-HAB and *Phaeocystis* ED-HAB may be dominated by these organisms growing in near-monospecific blooms, while blooms of T-HAB dinoflagellates may contain the organism of interest (e.g., *Alexandrium*) growing as only a small proportion of total primary producers. Understanding what enables the growth of a particular HAB organism in competition with that of other organisms, and against losses due to abiotic (typically out-mixing or washout events) or biotic (grazing) processes, lies at the heart of any mechanistic attempt to explain bloom growth. There is also the important issue of bottom-up and top-down influences. The top-down influences may be considered as just grazers upon the HAB species themselves (Irigoien *et al.*, 2005; Stoecker *et al.*, 2008), but actually they also include their activity upon their competitors (Flynn *et al.*, 2008), and for mixotrophs also their prey (Adolf *et al.*, 2008; Glibert *et al.*, 2009; Hansen *et al.*, 2013). Thus, proliferation of one species may occur not because of its competitive advantage in growth rate or nutrient acquisition, but because it is not the subject of such great grazing pressure (Mitra and Flynn, 2006; Flynn, 2008). The course of such developments will likely change if the activity of the next grazer up the food web is altered, with potential for ED-HAB formation. (Grazers include benthic

organisms such as bivalves, and not just zooplankton.) Models are ideal for exploring such cascade events, although clearly the predictions can only be as robust as the data and knowledge used to build the model.

Much of the conceptual bases for describing ED-HAB events driven by eutrophication is present in extant modeling platforms; these provide linkage between physics, nutrient load, and light (including self-shading as the bloom develops) to primary production in an environment where the simulated grazers of those primary producers are themselves typically subjected to a density-dependent closure term (Mitra, 2009). It should be possible to use suitably constructed multi-nutrient models (see Flynn, 2005) to conduct hypothesis testing of what types of nutrient loads and ratios (noting that the former are more important than the latter – Flynn, 2010a) are likely to raise risks of ED-HAB events; however, allelopathic interactions are also recognized as important features of HAB plankton interactions (Pohnert *et al.*, 2007; Granéli *et al.*, 2008). And, like feedbacks from grazing, allelopathic interactions have potential to generate positive feedbacks where the increasingly dominant organism rapidly overpowers its competitors due to the escalation of cell-density-dependent interactions. Physical processes, and behavioral traits such as vertical migration, have clear potential to affect allelopathic interactions by bringing organisms together or conversely by dispersing them. While allelopathic interactions may well be important features of ED-HAB events, they are typically absent from ecosystem simulators.

Modeling the growth of cryptic T-HAB species presents a different, if not greater, challenge to that for ED-HAB. How necessary is it to model the growth of the biomass-dominant species in addition to that of the T-HAB species, and at what level of detail? If there is a close coupling to other species (as for the mixotrophic T-HAB *Dinophysis* for the supply of acquired photosystems from a specific sequence of other plankton; Hansen *et al.*, 2013), then a line of exploration for model complexity can be developed. Ultimately, work can only progress using the information at hand. Theoretical/conceptual models may help here, in exploring the likely sensitivity of different trophic interactions and processes, and hence guide field and laboratory studies. Models of these, as much as for any system, can usefully act as platforms for generating and testing hypotheses as well as guiding empirical research (Figure 3.1).

3.5 How Good Are Current HAB Models?

Predictive HAB models take a variety of forms, including conceptual, empirical, and numerical approaches (McGillicuddy, 2010). As the sophistication of such models has increased and the data sets used to evaluate them have expanded, the metrics by which their skill can be assessed have begun to receive more attention (Lynch *et al.*, 2009). Examples of the various approaches to HAB prediction are provided (Table 3.1) and the means by which they have been evaluated. See Anderson *et al.* (2015) for a more complete review of recent and ongoing predictive modeling efforts.

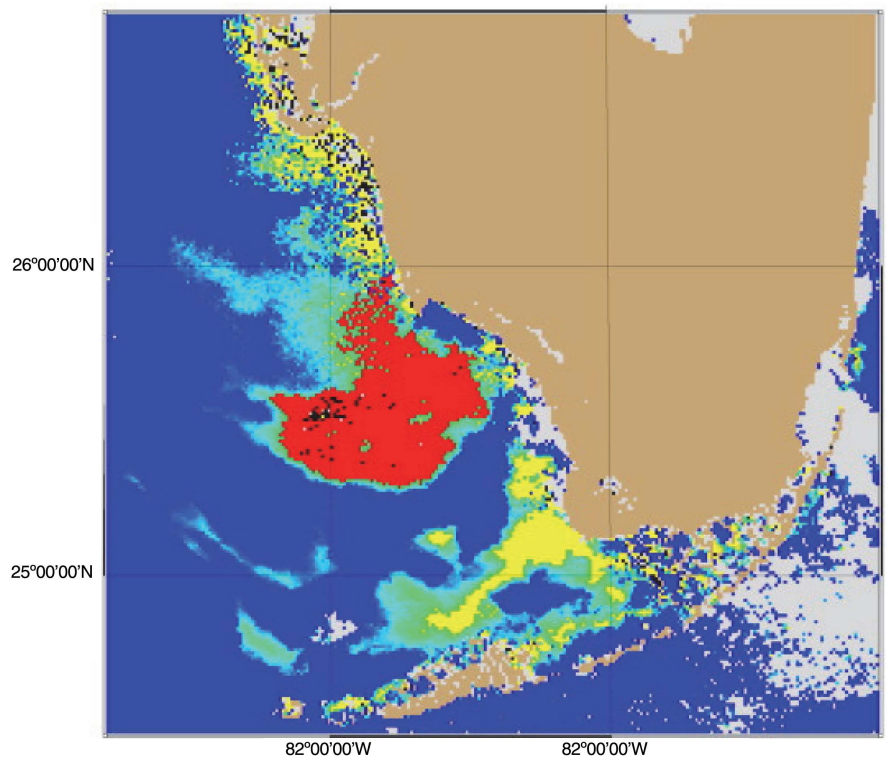
Empirically based models have shown predictive skill in a variety of contexts. For example, Blauw *et al.* (2010) related nuisance foam events in Dutch coastal waters to *Phaeocystis globosa* ED-HAB blooms, predicting their occurrence on the basis of relationships with environmental parameters such as mixed layer irradiance and nutrient availability using a "fuzzy logic" approach. In a hindcast of the period 2003–2007, the model predicted 93% of the observed foam events – an impressive record of "true positive" outcomes; however, there were also many "false positives" in which the model predicted a foam event but none occurred. Of course, it is also of interest to quantify "true negatives" and "false negatives" for a more complete assessment of model skill. From a management perspective, the relative importance of different types of error may differ. For instance, in protecting public health from exposure to toxins, a false positive may be more tolerable than a false negative. From the viewpoint of the tourist trade, however, false positives for HAB can prove highly costly.

In some regimes, remote sensing is a valuable input into HAB predictive systems. In the eastern Gulf of Mexico, T-HAB of the toxic dinoflagellate *Karenia brevis* are dense enough to be detected in satellite imagery (Figure 3.2, top). Not only does such imagery provide a means for bloom identification following ground truthing, but also it can feed forecasts of bloom transport, extent, intensification, and impact (Stumpf *et al.*, 2009). Each of these aspects has been evaluated in the context of an operational forecasting system, with accuracies in the range of 73–99% (Figure 3.2b, bottom). It is important to note that the resolution of the forecast and validation data are not sufficient in this example to yield skill at scales finer than 30 km, and considerable patchiness of the *K. brevis* population and

Table 3.1 Summary of predictive modeling approaches discussed in the text.

Example	Inputs	HAB models	Model outputs	Duration, spatial dimension
Phaeocystis foam events in Dutch coastal waters; Blauw *et al.* (2010)	T, S, DIN, DIP, solar irradiance, K_d, wind	Empirical (fuzzy logic)	Foam event identification, magnitude	0D; hindcast
Karenia brevis in the eastern Gulf of Mexico; Stumpf *et al.* (2009)	RS Chl	Empirical (rule-based decision tree)	Bloom identification, magnitude, extent, impact	2D; 3-day forecast twice weekly
	RS Chl, wind	Transport	Bloom magnitude, extent, impact	
Pseudo-nitzschia along U.S. west coast; Anderson *et al.* (2011)	RS Chl, R_{rs} (3 wavelengths), *T, S from hydrodynamic model*	Empirical (generalized linear model)	Cell abundance, pDA, cDA	2D; nowcast
Various H/ED-ABs in Chesapeake Bay (Figure 3.4); Brown *et al.* (2013)	Month, *T, S, DO, Chl, DIN* NH_4, *TON, TSS,* K_d *from physical-biological-biogeochemical model*[*]	Empirical (logistic regression, neural network, hierarchical decision tree)	Probability of occurrence; relative abundance (*Karlodinium veneficum* only)	2D; 3-day forecast
Cyanobacterial blooms in the Baltic; Roiha *et al.* (2010)	ICs and BCs for hydrodynamic and biogeochemical models (notably, wintertime DIN and DIP), climatology, *atmospheric forcing*	Physical-biological-biogeochemical[*]	Bloom probability	2D; seasonal ensemble
Alexandrium fundyense in the Gulf of Maine; McGillicuddy *et al.* (2011)	Cyst distribution, *hydrodynamic ICs and BCs from global model*, nutrient climatology, river fluxes, *atmospheric forcing*	Physical-biological, population dynamics	Cell concentration	3D; seasonal ensemble, weekly 7-day forecast

BCs, boundary conditions; cDA, cellular domoic acid; Chl, chlorophyll; DIN, dissolved inorganic nitrogen; DIP, dissolved inorganic phosphorus; DO, dissolved oxygen; HAB, harmful algal bloom; ICs, initial conditions; K_d, diffuse attenuation coefficient; pDA, particulate domoic acid; R_{rs}, remote sensing reflectance; RS Chl, remotely sensed chlorophyll; TON, total organic nitrogen; TSS, total suspended solids. In the inputs column, observations are in regular font, and model-based quantities are in italics.
[*]Models that explicitly include non-HAB phytoplankton and zooplankton.

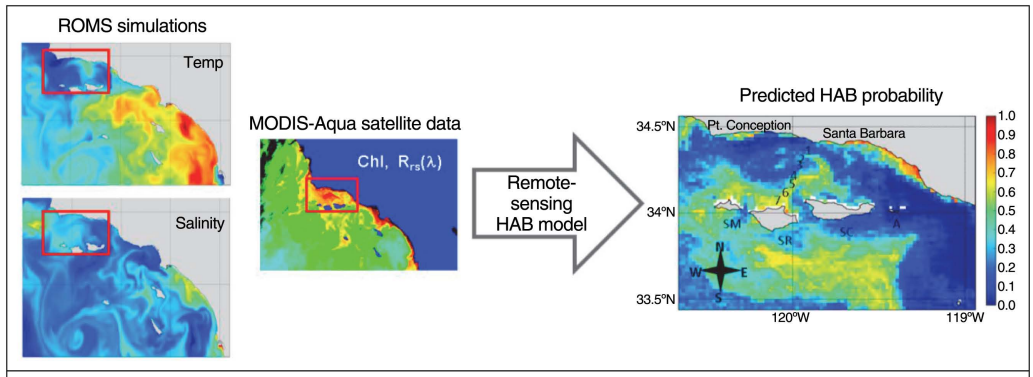


Forecast	Number of forecasts	Assessable (%)	Accuracy (%)
Identification	10	100	80
Transport	184	67	90
Extent	48	56	77
Intensification	84	63	73
Impact	209	49	99
Combined	525	58	89

Figure 3.2 (Top) SeaWiFS satellite image from November 21, 2004. Yellow areas indicate where the chlorophyll anomaly based on Stumpf *et al.* (2003) exceeded 1 μg L^{-1}; cyan and green show anomalies between 0 and 1; blue indicates no positive anomaly. Red represents locations of *K. brevis* blooms based on the criteria listed in Stumpf *et al.* (2009, table 1). The yellow areas did not match the criteria and are thus not considered to be due to *K. brevis*. (Bottom) Forecasted bloom components and percentage of assessable forecasts for the period October 2004–April 2006. In this context, accuracy is defined to be the sum of true positives and true negatives divided by the total number of forecasts. *Source*: From Stumpf *et al.* (2009), with permission of Elsevier.

associated impacts exist at spatial scales finer than that. An analogous forecast system is emerging for cyanobacterial blooms in the Great Lakes of North America, in which short-term forecasts of bloom transport are based on satellite imagery and a hydrodynamic model together with a particle-tracking algorithm (Wynne *et al.*, 2013).

Yet another approach to combining remote sensing with models is being used to predict T-HAB of diatoms of the genus *Pseudo-nitzschia* along the west coast of the United States. Logistic generalized linear models (GLMs) utilize time of year (month), remote-sensing reflectance at three wavelengths, and model-based temperature and salinity (Figure 3.3, top) to predict concentrations of *Pseudo-nitzschia* cells, as well as the particulate and cellular forms of the toxic domoic acid (particulate domoic acid [pDA] and cellular domoic



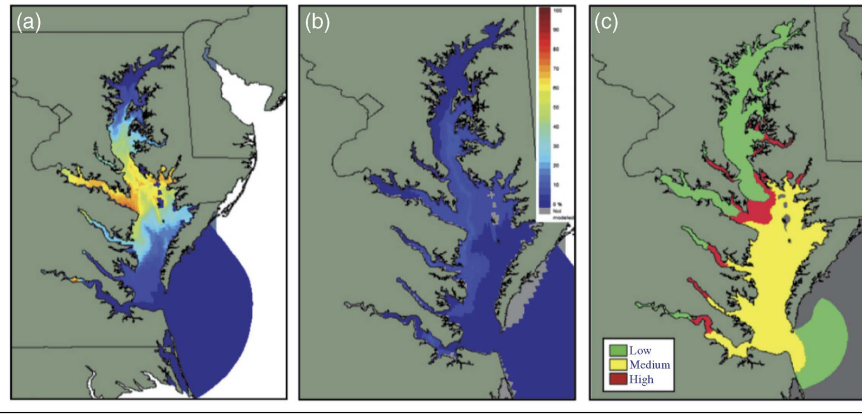
	CC	OT	POD	FAR	POFD
Pseudo-nitzschia (1)	0.33	10^4 cells L^{-1}	0.86	0.15	0.33
Pseudo-nitzschia (2)	0.28	10^4 cells L^{-1}	0.88	0.15	0.33
pDA	0.19	0.5 μg L^{-1}	0.83	0.48	0.35
cDA	0.15	10 pg $cell^{-1}$	0.90	0.55	0.52

Figure 3.3 (Top) Schematic of ROMS model and MODIS satellite products used to compute the "remote-sensing" T-HAB models for predicting the probability of elevated *Pseudo-nitzschia* abundance and toxin concentrations in the Santa Barbara Channel off the coast of central California. Numbers in the far-right map denote monthly "Plumes and Blooms" sampling stations 1–7, with station 1 nearest the mainland and station 7 off the shelf of Santa Rosa Island. The Santa Barbara Channel Islands from west to east are: San Miguel Island (SM), Santa Rosa Island (SR), Santa Cruz Island (SC), and Anacapa Island (A). (Bottom) Model skill assessment for two generalized linear models of *Pseudo-nitzschia* cell concentration, particulate domoic acid (pDA), and cellular domoic acid (cDA). The correlation coefficient (CC) is Nagelkerke's r^2. Probability of detection (POD), false alarm ratio (FAR), and probability of false detection (POFD) are calculated from optimized threshold values (OT). *Source*: From Anderson *et al.* (2011), with permission of the American Geophysical Union.

acid [cDA]) produced by these algae (Anderson *et al.*, 2011). These predictions have been evaluated using the 2004–2010 time series of data used to build the models (Figure 3.3, bottom). Although the correlation coefficients between the predicted and observed quantities are modest (Nagelkerke's r^2 ranging from 0.20 to 0.46), the probability of detection (POD; the ratio of true positives to the sum of true positives and false negatives) ranges between 83 and 90%. The false alarm ratio (FAR; false positives divided by the sum of true positives and false positives) is only 15% for *Pseudo-nitzschia* cell concentration, yet 48–55% for domoic acid constituents. An alternative metric for false positives normalizes them by the sum of true negatives and false positives, yielding the probability of false detection (POFD). POFD is about double the FAR for *Pseudo-nitzschia* cell concentration, and lower than the FAR for pDA and cDA. It is important to note that the skill assessment was performed using the same data used to calibrate the model (albeit with cross-validation). As longer time series become available, it will be possible to evaluate (validate) the model with independent observations.

Whereas the Anderson *et al.* (2011) approach uses remote sensing together with model-predicted temperature and salinity, Brown *et al.* (2013) utilize the output of a coupled physical-biogeochemical model to forecast the probabilities of HAB events and the presence of waterborne pathogens in Chesapeake Bay. These probabilities are derived from multivariate empirical habitat models (trained using *in situ* observations) that feed on model-based predictions of a suite of environmental variables. A summary of the target species, their habitat models, and model accuracy is provided in Figure 3.4, along with example forecasts to illustrate the high resolution of the predictions. Forecast accuracy, defined as the sum of true positives and true negatives divided by the total number of forecasts, ranges from 77 to 93%.

Coupled physical-biogeochemical models have shown prognostic utility themselves in circumstances where and when the algal biomass predicted by such models constitutes the bulk of the HAB of interest. Such is the case for cyanobacterial blooms in the Baltic Sea, for which the areal fraction of cyanobacterial accumulation is correlated with the concentration of chlorophyll-*a* during the bloom



Species	Model type	Input Variables	Forecast	Accuracy %
Chrysaora quinquecirrha	Logistic regression	SST, SSS	Probability of occurrence	87
Karlodinium veneficum	Artificial neural network	SST, SSS, month	Relative abundance	84
Microcystis aeruginosa	Hierarchical decision tree	SSS, DO, Chl*a*, DIN, TN, NH_4, TSS, K_d	Probability of bloom occurrence	90
Prorocentrum minimum	Logistic regression	Chl*a*, NH_4, TON, TSS, month	Probability of bloom occurrence	88
Vibrio cholerae	Logistic regression	SST, SSS	Probability of occurrence	77
Vibrio parahaemolyticus	Logistic regression	SST, Chl*a*	Probability of occurrence	82
Vibrio vulnificus	Logistic regression	SST, SSS	Probability of occurrence	93

Figure 3.4 (Top) Examples of species forecasts generated by the Chesapeake Bay Ecological Prediction System (CBEPS). (a) Likelihood of encountering sea nettles *Chrysaora quinquecirrha* on 17 August 2007. (b) Likelihood of *Vibrio vulnificus* on 20 April 2011. (c) Relative abundance of *Karlodinium veneficum* on 20 April 2005. Legend: low: 0–10 cells/ml; med: 11–2000 cells/ml; high: >2000 cells/ml. Color bar for likelihood is the same for both A and B. (Bottom) Synopsis of organism habitat models used in the CBEPS. Chl*a*, chlorophyll-*a* concentration; n, sample size; SST, sea-surface temperature; SSS, sea-surface salinity; TON, total organic nitrogen; TSS, inorganic suspended solids. Accuracy is expressed as the number of correct forecasts/n. *Source*: From Brown *et al.* (2013), with permission of Elsevier.

season (Kahru and Elmgren, 2014). Roiha *et al.* (2010) describe an ensemble forecasting system that provides quantitative predictions of cyanobacteria distributions in the Baltic, for which springtime phosphorus concentrations are a predictor of basin-scale spatial variations in the blooms. Likewise, Stumpf *et al.* (2012) linked springtime river discharge and total phosphorus load to interannual variability in cyanobacterial blooms in Lake Erie (North America), thereby providing the basis for seasonal forecasts.

In contrast to coupled physical-biogeochemical models that represent the bulk properties of an ecosystem, single-species population dynamics models offer an attempt to capture the life cycles of particular organisms. In some cases, ecological forecasts have been facilitated by specific characteristics of the population dynamics of HAB species. For example, interannual variations in the extent of T-HAB of the toxic dinoflagellate *Alexandrium fundyense* in the Gulf of Maine are influenced by the abundance of resting cysts (McGillicuddy *et al.*, 2011; Anderson *et al.*, 2014). Specifically, years with more abundant cysts are prone to more widespread blooms, as inferred from the along-coast extent of shellfish toxicity (Figures 3.5 and 3.6). In fact, the correlation coefficient for the time series of cyst abundance and the most southerly latitude of shellfish harvesting closures is −0.93 ($p = 0.02$) for the period 2005–2009. This relationship provides the basis for seasonal ensemble forecasts of T-HAB extent via a coupled physical–biological model that includes germination, growth, and mortality of *A. fundyense* cells, which are followed up with weekly nowcast and forecast simulations (McGillicuddy *et al.*, 2011). In years when conditions were

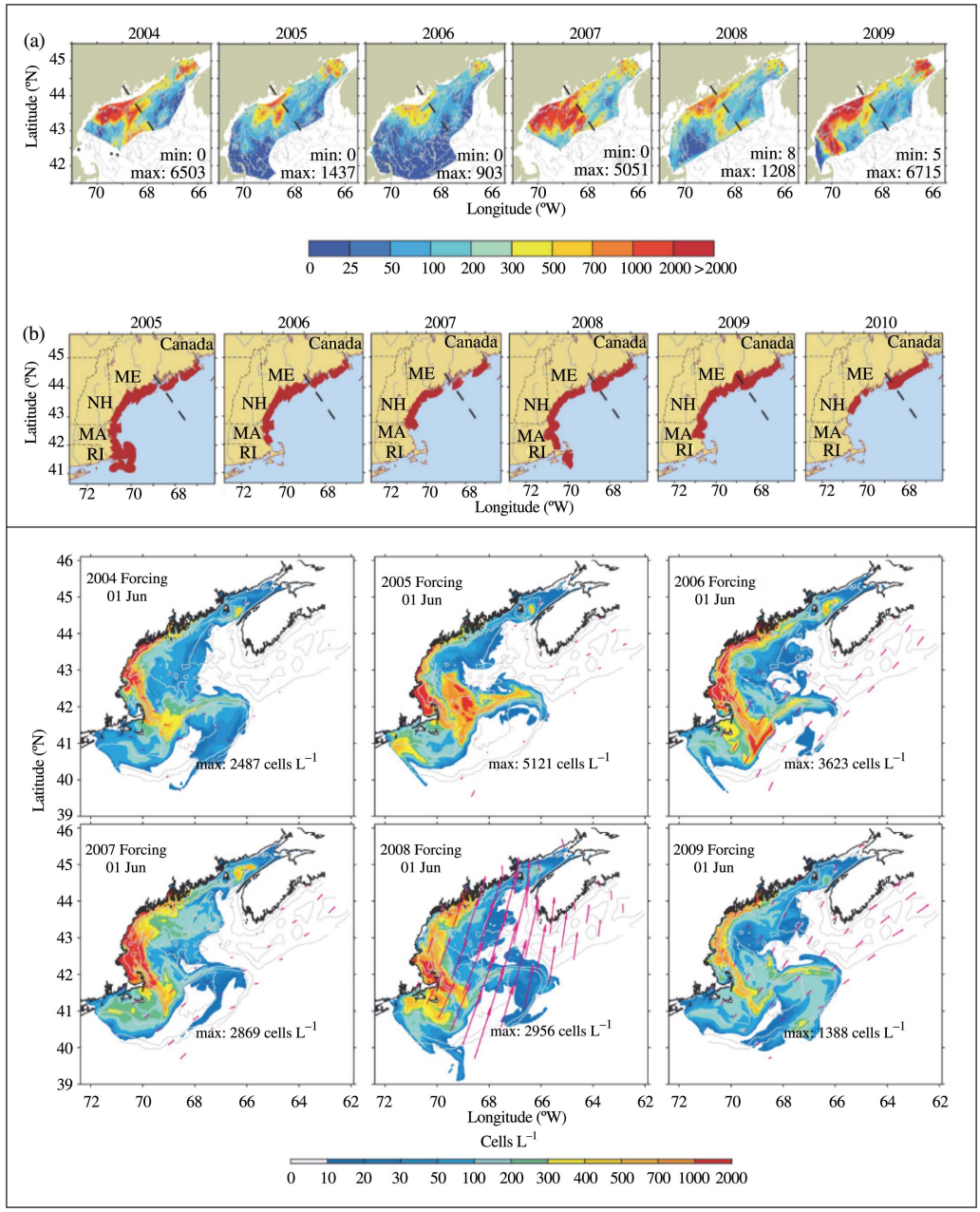


Figure 3.5 Top: (a) *Alexandrium fundyense* cyst abundance in the Gulf of Maine, 2004–2009. Minimum and maximum values are indicated in each panel. Open circles denote the locations of sediment samples used to construct the maps. (b) Spatial extent of PSP closures, 2005–2010. The calculations for the western Gulf of Maine and southern New England presented in Figure 3.6 pertain to the area south and west of the dashed line. Bottom: Ensemble *A. fundyense* forecast for 01 June, based on the autumn 2009 cyst map together with hydrodynamic and atmospheric forcing from 2004 to 2009. Pink arrows depict the instantaneous wind-forcing. Maximum (max) cell concentrations in each panel are indicated at the lower right. *Source*: From McGillicuddy *et al.* (2011), with permission of Association for the Sciences of Limnology and Oceanography, Inc.

"normal," this approach provided skillful hindcasts (He *et al.*, 2008) and forecasts (Li *et al.*, 2009); however, in 2010, the forecast system failed. Despite an unusually high abundance of resting cysts, a large-scale T-HAB event did not materialize (Figure 3.5, middle panel, Figure 3.6), thus putting the forecast in the category of a false positive. Observations from shipboard surveys and the coastal observing system

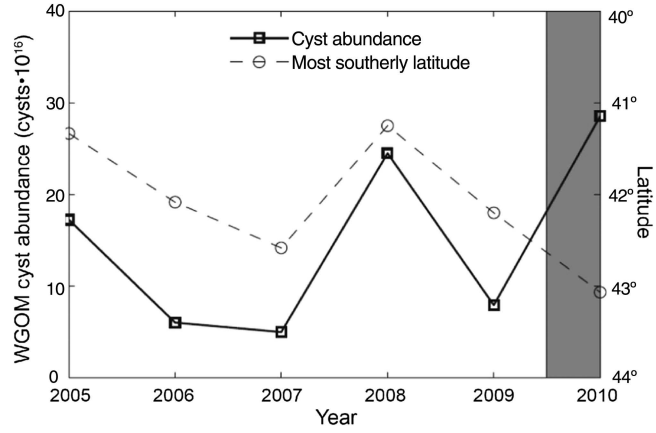


Figure 3.6 Time series of cyst abundance in the western Gulf of Maine (WGOM) and the most southerly latitude of coastal shellfish toxicity closures (note axis reversal). For visual compatibility and correlation analysis, the cyst abundance time series has been shifted by 1 year, such that the autumn of 2004 is reported as 2005, and so on. These calculations pertain to the area south and west of the line running southeast from Penobscot Bay (Figure 3.5, upper panel). *Source*: From McGillicuddy *et al.* (2011) with permission of Association for the Sciences of Limnology and Oceanography, Inc.

revealed water mass variations that had a direct impact on *A. fundyense*'s niche: near-surface waters were warmer, fresher, and lower in nutrients than prior years, leading to unfavorable growing conditions. Moreover, a weaker than normal coastal current lessened the along-coast transport of the *A. fundyense* that were present. Thus, the potential for a large bloom set by the high abundance of resting cysts was not realized because of anomalous environmental conditions.

This last example highlights the challenge of making ecological forecasts in a changing ocean environment. In essence, the forecast system for *A. fundyense* in the Gulf of Maine is predicated on the hypothesis that, all else being equal, a higher abundance of resting cysts will lead to a more widespread bloom. However, in 2010, all else was not equal: failure of the forecast was a direct consequence of the fact that conditions were outside the envelope of prior observations used to construct the model. In particular, nutrient concentrations were quite different from the climatological values used in the ensemble forecast and weekly real-time predictions. In the future, augmentation of the coastal observing system with nutrient sensors should help avoid this mode of false positive in the forecast model.

Looking toward the future, it is likely that a changing climate will lead to variations in oceanic conditions that are outside the ranges experienced in the recent past; certainly, that is so with respect to ocean acidification with potential changes in phytoplankton succession (Flynn *et al.*, 2015a). Such changes would influence the severity and extent of different types of HAB events (e.g., Meier *et al.*, 2011). Moreover, anthropogenic perturbations to coastal ecosystems continue to increase, yielding demonstrable impacts on T-HAB and associated toxin production (Glibert and Burkholder, 2011). Given the highly nonlinear nature of ecological systems, these changing conditions may have unexpected consequences for HAB species. As such, predictive modeling efforts will need to be designed in a manner that makes them adaptable to regime shifts that are almost certain to occur as earth's climate varies (Dippner and Kröncke, 2015).

3.6 Future Modeling of T-HAB and ED-HAB: Managing Expectations

Although a generalized framework for predicting HAB may be a long way off, good progress is being made with site-specific models in various regional applications. Enhancements may be expected to come from generalized conceptual studies of plankton dynamics relating the potential for development of sustained high-biomass ED-HAB under certain conditions of nutrient loading (concentrations of nutrient N, P, and Si), light (and hence interacting with mixing layer depth and absorbance), temperature, and pH. Studies of physical systems may then enable some level of proactive identification of water bodies becoming more or less susceptible to ED-HAB under developing climate change scenarios, with reinforcement of such identifications

from placement of suitable configured biological models within the physics framework. Identifying low-risk environments should be possible; certain conditions are clearly more or less conducive to high-biomass events.

Managing expectations from models for cryptic T-HAB is important; however, if toxicity can be aligned with specific physiological states such as P-stress in the presence of adequate ammonium or nitrate (Flynn, 2002; John and Flynn, 2002), then modeling should again be able to perform a useful role in supporting a traffic-light approach to risk management. It should be noted that simply considering nutrient concentrations and ratios (i.e., N:P) need not support an understanding of the likelihood of a toxic event. This is because of the importance of nutrient and light fluxes into the system (Flynn, 2010a), and the consequences of different levels of biological and physiological interactions (competition, mixotrophy, self-shading, predator–prey interactions, etc.).

3.7 Improving Our Capabilities

3.7.1 Changes in the Biological–Modeling Interface

The fundamental challenge to future progress rests in improving our basic understanding of physiology and ecology, and of how these interrelate when set within a given physical system. In essence, the linkages shown in Figure 3.1 need to become more active. More of the same types of studies that have been conducted over the past decades are now needed. At least four things need to change from a biological model perspective.

1) The types of data collected in especially laboratory experiments need to be broadened. Thus, there is a need for data in terms of C, N,P biomass and so on, and not just with respect to organism numbers, or chlorophyll; the problem is that organism size and pigment content (as applicable) vary with growth status, and for trophic dynamics both biomass and stoichiometric quality are important.
2) More attention needs to be paid to the types of abiotic drivers applied in experiments, and the combination in which they are applied. The most obvious drivers in question are light, temperature, and pH. With respect to the latter, linked to the subject of ocean acidification, it is notable that changes in pH during bloom growth rather than growth at any particular (fixed) pH have been indicated by modeling to be important (Flynn *et al.*, 2015a).
3) An enhanced understanding is required for realistic organism–organism interactions horizontally (competitors), upward (predators), and downward (prey) from the HAB species of interest. The absence of data for encystment and excystment is another shortcoming in some regimes. A better holistic understanding of what is going on between organisms is needed when they grow under the types of conditions (including biomass densities and hence nutrient loading) likely in nature under climate change and land use change scenarios.
4) There is a need to understand the implications of mixotrophy for plankton ecology. Emphasis has been hitherto placed on abiotic photoautotrophic drivers for growth of HAB (inorganic nutrients, light), ignoring the potential role of DOM and of prey fields. The impact of this paradigm change for the understanding of protist ecophysiology (Mitra *et al.*, 2014b, 2016) will take some time to work through.

In essence, while modeling could be criticized for being for the most part not mechanistic enough to enable predictive simulations, in part this simply reflects inadequacies within the wider science to understand the underlying ecological interactions and measure the appropriate parameters. This is not a new observation, and it applies to plankton research in general, but it is one that needs acting upon through coordinated field and laboratory experimental work together with modeling. It also requires that modeling (as systems modeling, with time as a variable) becomes more fully embedded in the ecology and physiological science.

None of this is going to occur quickly or cheaply. Phenomenological understanding (born of what many may dismiss as observational "natural history") always develops before sufficient data are gathered to support empirical, let alone mechanistic, modeling; however, this phenomenological understanding, viewed as non-numeric data, is actually of great potential value and often overlooked in modeling. During recent workshops on enhancing models of mixotrophic protists (leading to Mitra *et al.*, 2014b, 2016), there was a specific attempt made to engage in "expert witness validation." Expert witness validation requires that modelers work with experts in physiology and ecology to build conceptual understanding and then models that conform to the essence of what is seen in nature

Table 3.2 Suggested realized and potential scope for modeling in T-HAB and ED-HAB natural and management science.

	Uses for models in HAB science	Now	Future
i	Provide a focus for investigations and discussions by providing a rigorous framework for testing knowledge	✓	✓
ii	Drive closer links between scientists at all levels, for fully integrated programs	✓	✓
iii	Provide a platform for testing generic "What-if?" questions	✓	✓
iv	Provide a platform for testing organism-specific "What-if?" questions	✗	✓
v	Provide a generalized predictive geographic capacity for algal blooms	✓	✓
vi	Provide a detailed predictive geographic capacity	✓	✓
vii	Provide a detailed predictive temporal geographic capacity	✗	?

and understood from experimental manipulations. This approach also recognizes the importance of generalities in ecology rather than specifics to a strain or particular experimental setup.

Careful consideration is required on what modeling may provide us with respect to T-HAB and ED-HAB; a general summary is attempted in Table 3.2. The history of applied plankton modeling is rooted in the support of biogeochemical science and in algal blooms in drinking water lakes, where the description of biotic details took (and still largely takes) a backseat to describing the abiotic features. T-HAB and ED-HAB are functions equally of abiotic and biotic features. Some combination of using mechanistic, physiology-based models and complex abiotic descriptions (third-generation, "3G ecosystem" models in Figure 3.1) played out in different physicochemical scenarios should be able to provide an enhanced management tool for mitigating against the occurrence of T-HAB and especially ED-HAB. When linked with weather and coastal physics projections, there should be reasonable scope for site-specific capabilities as well. Moving to the detail of what species and what toxins in particular, when, and where represents a far greater challenge. While waiting for that advance, there is good reason to draw some comfort from the developing coupled remote-sensing and abiotic modeling platforms for near-future forecasting.

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4

Harmful Algal Blooms and Shellfish

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4.1 Introduction

Coastal marine ecosystems are subjected to environmental changes related to human activities and climate change. Phytoplankton, responsible for most of the primary production on Earth, is a central component of these ecosystems. Due to its dynamics, the phytoplankton compartment is very quickly subjected to environmental disturbances resulting in successive cascades of effects throughout the food web (pelagic and benthic). These disturbances include an increase in phytoplankton biomass usually caused by eutrophication of coastal ecosystems (Diaz and Rosenberg, 2008), changes in the structure of plankton communities (Philippart *et al.*, 2000), and increased occurrence of harmful algal blooms (HAB) (Hallegraeff, 2010).

HAB are considered a major threat to marine coastal areas due to the wide range of impacts they have on the ecology of coastal marine ecosystems, either through a sheer increase of their biomass or through the production of potent toxins and bioactive compounds (Burkholder, 1998). Recent decades have witnessed the emergence and spread of HAB paralleled with associated major effects on marine ecosystems and related human activities, such as fishing and aquaculture, but also tourism (Smayda, 1990; Hallegraeff, 1993; Van Dolah, 2000; Zingone and Oksfeldt Enevoldsen, 2000; Allen *et al.*, 2006; Matsuyama and Shumway, 2009). In addition, HAB produce several types of toxins that are bioaccumulated and bioamplified throughout the food web to affect humans and other organisms, either following the consumption of contaminated seafood or via direct exposure to bloom waters and aerosols (Van Dolah, 2000). Shellfish, mainly filter-feeding bivalve molluscs of commercial interest that are most exposed to these HAB (e.g., mussels, oysters, scallops, clams, and cockles), are the major vectors of human intoxications associated with the toxins of HAB at a global scale (Van Dolah, 2000). Some other shellfish species that are not routinely monitored by conventional regulatory programs, however, can also accumulate these toxins, and include gastropods, crustaceans, cephalopods, and echinoderms.

There are several shellfish-mediated intoxications involved in human poisoning worldwide caused by HAB. Shellfish poisonings are classified with respect to their bioactivity or to the symptoms caused in humans, such as diarrheic, paralytic, neurotoxic, and amnesic poisonings (see Chapter 9). North America is particularly concerned with:

1) The paralytic shellfish toxins (PST), saxitoxin (STX) and its derivatives, produced by dinoflagellates of the genera *Alexandrium* (previously named *Gonyaulax* or *Protogonyaulax*), *Gymnodinium*, and *Pyrodinium*, and responsible for paralytic shellfish poisoning (PSP) (Sommer and Meyer, 1937; Luckas, 1992; Shimizu, 2000; Weise *et al.*, 2010);

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2) The diarrheic toxins (DST), okadaic acid (OA) and its analogs, produced mainly by dinoflagellates of the genera *Dinophysis* and *Phalacroma* and to a lesser extent *Prorocentrum* spp., and responsible for diarrheic shellfish poisoning (DSP) (Yasumoto, 1985; Aune and Yndestad, 1993; Vale and Sampayo, 2002; Campbell *et al.*, 2010; Reguera *et al.*, 2014);
3) The neurotoxic toxins (NST), brevetoxins (BTX or PbTX) and their analogs, produced mainly by the dinoflagellate *Karenia brevis* and responsible for neurotoxic shellfish poisoning (NSP) (Davis, 1948; Shimizu *et al.*, 1974; Magaña *et al.*, 2003; Brand *et al.*, 2012);
4) The amnesic toxins (AST), domoic acid (DA) and its isomers, produced by diatoms of the genus *Pseudo-nitzschia* and responsible for amnesic shellfish poisoning (ASP) (Bates *et al.*, 1989; Shimizu *et al.*, 1989; Wright *et al.*, 1989; Martin *et al.*, 1990a; Perl *et al.*, 1990; Garrison *et al.*, 1992);
5) The azaspiracid toxins (AZA) produced by the small dinoflagellate *Azadinium* spp. and *Amphidoma* spp., and responsible for the most recently characterized shellfish poisoning, azaspiracid shellfish poisoning (AZP) (McMahon and Silke, 1996; Ito *et al.*, 1997; Satake *et al.*, 1997b, 1998a, 1998b; Ofuji *et al.*, 1999, James *et al.*, 2000; Tillman *et al.*, 2009, 2012; Krock *et al.*, 2012; Trainer *et al.*, 2013);
6) Other toxins, the yessotoxins (YTX) produced by the dinoflagellates *Prorocentrum reticulatum*, *Lingulodinium polyedrum*, and *Gonyaulax sinifera* (Draisci *et al.*, 1999a, 1999b; Satake *et al.*, 1999; Paz *et al.*, 2004) and pectenotoxins (PTX) produced by *Dinophysis* spp. (Camacho *et al.*, 2007), that exhibit several toxicological and pharmacological bioactivities but do not clearly fall within the conventional groups of clinical symptoms in humans (Terao *et al.*, 1986; Yoon and Kim, 1997; Satake *et al.*, 1999; Ito *et al.*, 2008); and
7) The nonregulated emerging toxins, particularly the macrocyclic imines, which include the highly potent and fast-acting toxins (FAT) such as gymnodimines and spirolides (Torigoe *et al.*, 1988; Hu *et al.*, 1995, 1996b; Seki *et al.*, 1995; Uemura *et al.*, 1995; Lu *et al.*, 2001; Takada *et al.*, 2001; Cembella and Krock, 2008).

The development, propagation, and distribution of blooms of the toxic phytoplankton species and genera mentioned above are related to complex environmental conditions. Their ability to produce shellfish poisoning is species-specific and controlled by several environmental factors. Contamination of shellfish by toxins could represent a major health risk for consumers without the intervention of several monitoring networks developed all along the coast of North America (see Chapter 10). In return, the active monitoring may result in banned sales of shellfish due to contamination by phycotoxins with direct economic impediments to shellfish commercial activity, and in terms of environmental quality for the impacted region and consequently tourism (Wessels *et al.*, 1995). In addition to these effects, phycotoxins also affect the health of marine organisms, killing marine life and damaging habitats and ecosystems.

4.2 Major Shellfish Poisonings

4.2.1 Paralytic Shellfish Poisoning (PSP)

Paralytic shellfish toxins (PST) responsible for PSP are reported almost worldwide, especially in North America, Europe, Asia, Oceania, and also South America and South Africa, with approximately 2000 cases of human PSP reported each year and a 15% mortality rate through fish or shellfish consumption (Hallegraeff, 1993). Moreover, several authors suggest a gradual expansion of the global distribution of PSP in recent years (Hallegraeff, 1993; Anderson, 2008).

In humans, PSP is caused by the ingestion of shellfish containing PST. These PST are accumulated by shellfish grazing on algae producing PST. Symptoms of human PSP intoxication vary from a slight tingling or numbness, first around the lips and mouth and then involving the face and neck, evolving toward muscular weakness, sensation of floating, ataxia, motor incoordination, drowsiness, incoherence, progressive loss of ventilatory efficiency, and (at high doses) complete respiratory paralysis and death (Catterall, 1985; Kao, 1993).

The first chemically characterized PST was saxitoxin (STX). Since its first description from the Alaskan butter clam, *Saxidomus giganteus*, in 1957 (Schantz *et al.*, 1957), at least 57 naturally occurring analogs of STX have been described (Weise *et al.*, 2010). The various PST significantly differ in their toxicity, with STX being the most toxic. STX and its analogs target the voltage-gated sodium channel, causing a reversible blockage of the channels and thus a blockage of membrane depolarization preventing transmission of action

potential in excitable cells (Jost *et al.*, 2008; Huang *et al.*, 2010). Recent studies have also shown that STX additionally targets voltage-gated potassium and calcium channels, and even copper transporters (Cusick *et al.*, 2012). It partially blocks the voltage-gated calcium channels, resulting in an influx of calcium ions that initiates contraction, secretion, neurotransmission, and other intracellular regulatory events (Catterall, 2000; Zakon, 2012). In addition, it binds to voltage-gated potassium channels, altering the control of electrical signaling in excitable cells and the regulation of ion flux and calcium transients in non-excitable cells (Wang *et al.*, 2003; Catterall *et al.*, 2007).

The PST are produced mainly by dinoflagellates belonging to the genus *Alexandrium*, which may occur in both tropical and temperate climate zones. They are also produced by the marine genera *Gymnodinium* and *Pyrodinium*, and by the brackish or freshwater cyanobacteria of the genera *Lyngbya*, *Aphanizomenon*, *Cylindrospermopsis*, *Anabeana*, *Planktothrix*, and *Rivularia* (Deeds *et al.*, 2008; Weise *et al.*, 2010). During the last 20 years, there seems to have been an increase in intoxications caused by PSP (FAO/IOC/WHO, 2004); however, it is yet unclear whether the increase is real; whether it could be a consequence of improved identification, detection, and medical registration; or whether it is due to expanded shellfish culture and consumption. A few dozen countries have regulations for PSP toxins.

PSP events on the U.S. and Canadian coasts are attributable to *Alexandrium* spp., whereas, in Mexico, this syndrome is mainly associated with outbreaks of *Gymnodinium catenatum* and *Pyrodinium bahamense* var. *compressum* (Lewitus *et al.*, 2012).

4.2.2 Diarrheic Shellfish Poisoning (DSP)

Diarrheic shellfish poisoning is associated with the consumption of bivalve molluscs contaminated with lipophilic, polyether diarrheic shellfish toxins (DST) produced by marine microalgae of the genus *Dinophysis* (Hallegraeff, 1993; Van Dolah, 2000; Reguera *et al.*, 2012), and to a lesser extent by a few species of *Phalacroma* and the epibenthic dinoflagellates of the genus *Prorocentrum* (Gayoso *et al.*, 2002; Maranda *et al.*, 2007; Reguera *et al.*, 2014). The DST complex originally comprised three groups of lipophilic toxins: Okadaic acid (OA) and its analogs the dinophysistoxins (DTX), yessotoxins (YTX), and pectenotoxins (PTX) (Yasumoto *et al.*, 1985; Yasumoto and Murata, 1993). These toxins are detected all together by the conventional mouse bioassay (MBA) because the causative agents often co-occur in blooming waters and co-excrete the DST in the same lipophilic fraction (European Commission, 2002). Nowadays, thanks to advancement in the analytical methods of lipophilic toxins, the three DST are being detected separately and are shown to have different biological effects (Reguera and Pizzaro, 2008). Subsequently, both PTX and YTX are no longer considered part of the DST complex as no human intoxication associated with these toxins has ever been reported (Lawrence *et al.*, 2011), in spite of several toxicological and pharmacological effects (Terao *et al.*, 1990; Jun *et al.*, 1997; Ogino *et al.*, 1997; Yoon and Kim, 1997; Satake *et al.*, 1999; Ito *et al.*, 2008; Vilariño and Espiña, 2008). The most important diarrheic toxins are OA and its analogs, especially DTX1 and DTX2 (Terao *et al.*, 1986). In mammals, these toxins are specific inhibitors of the serine/threonine protein phosphates 1 (PP1) and 2A (PP2A) and cause inflammation of the intestinal tracts and diarrhea (Bialojan and Takai, 1998; Fernández *et al.*, 2002). In addition, OA and its analogs, notably DTX3, have tumor-promoting activity and exhibit several cellular effects both *in vitro* and *in vivo* (Fujiki *et al.*, 1999; Tubaro *et al.*, 2008; Valdiglesias *et al.*, 2013).

The first cases of gastrointestinal sickness associated with shellfish consumption, for which a definitive causative agent was not identified at first, were recorded in the Netherlands (Korringa and Roskam, 1961) and in Chile (Guzmán and Campodonico, 1975). It was not until the end of the 1970s that the first DSP intoxication associated with toxins from *Dinophysis fortii* in Japanese scallops, *Patinopecten yessoensis*, was reported from the northeastern regions of the Tohoku district in Japan (Yasumoto *et al.*, 1978, 1980; Tachibana *et al.*, 1981; Murata *et al.*, 1982). Subsequently, DSP outbreaks were recorded in the early 1980s in Western Europe with human intoxications following the consumption of contaminated Mediterranean mussels, *Mytilus galloprovincialis,* from northwest Spain (Campos *et al.*, 1982), and blue mussels, *Mytilus edulis,* in Brittany and Normandy in France (Alzieu *et al.*, 1983; Lassus *et al.*, 1985). The implication of *Dinophysis acuminata* in the human illness reported at first from the Netherlands was confirmed after the advances in causative agent identification in Japan (Kat, 1983, 1985). Between 1976 and 1982, some 1300 DSP cases were reported in Japan; in 1981,

more than 5000 cases were reported in Spain; some 3300 cases were reported in France in 1983; and a shutdown of the mussel industry in Sweden lasted almost a year in 1984 (Eberhart *et al.*, 2013). Incidence of outbreak and high toxicity of DSP have since been reported globally in East and Southeast Asia, Western Europe, New Zealand and Australia, North America, Latin America, South America, and South Africa (Van Dolah, 2000; Reguera *et al.*, 2012). The widespread occurrence of DSP incidence reflects the cosmopolitan geographical distribution of the main causative genus *Dinophysis* that comprises more than 120 species (Hasrup-Jensen and Daugbjerg, 2009; Gómez *et al.*, 2011) with at least 10 species confirmed to produce lipophilic toxins, and six species unambiguously associated with DSP events worldwide, namely, *D. acuminata*, *D. caudata*, *D. fortii*, *D. miles*, *D. ovum*, and *D. sacculus* (Reguera *et al.*, 2012).

The extent to which *Prorocentrum* species actually cause DSP has not been resolved. This genus includes at least 56 species from marine and estuarine waters (Gómez, 2005), 15 of which are considered harmful. Of these, six planktonic species form high-biomass blooms, including the potentially toxic *P. minimum*, and nine predominately benthic and toxigenic species (Glibert *et al.*, 2012). Some strains produce DSP toxins including water-soluble FAT, and/or OA, methyl-OA, and/or DTX1, other OA derivatives, and prorocentrolides (Vale, 2007; Cembella and Krock, 2008; Glibert *et al.*, 2012). The contribution of these species to DSP events remains unsolved, and the fact that the toxigenic species are benthic makes them unavailable for filter feeders except during periods of turbulence when they are re-suspended in the water column (Glibert *et al.*, 2012). Therefore, this chapter focuses mainly on *Dinophysis* species as the major producers of OA and its analogs, with some mention of the benthic *Prorocentrum* species, as they may be a source of intoxication to benthic shellfish grazers such as gastropod species.

4.2.3 Neurotoxic Shellfish Poisoning (NSP)

Neurotoxic shellfish poisoning is one of the oldest reported HAB and is caused by the ingestion of filter-feeding shellfish contaminated with brevetoxins (BTX), a family of polycyclic ether toxins produced mainly by the dinoflagellate *Karenia brevis* (formerly *Gymnodinium breve*) (Ingersoll, 1882; Steidinger, 1973; Baden, 1983). Although BTX can also be found in non-filter-feeder molluscs and in fish, ingestion of this seafood rarely causes NSP as BTX accumulate in the fatty organs, which are not edible (Poli *et al.*, 2000; Naar *et al.*, 2007; Brand *et al.*, 2012). Several BTX-like compounds have also been found in other *Karenia* species and in some ichthyotoxic raphidophytes such as *Fibrocapsa japonica*, *Chattonella antiqua*, *C. marina*, and *Heterosigma akashiwo*, but no cases of NSP incidence have ever been associated with blooms of raphidophytes (Landsberg, 2000; Furey *et al.*, 2007; Ramsdell, 2008; Brand *et al.*, 2012).

Depending on the backbone, two types of congeners of BTX are produced by *K. brevis*, among which PbTx-2T is the most common (Baden *et al.*, 2005). Contrary to the action of PST, BTX act mainly by depolarizing the open voltage-gated sodium ion channels of cell walls, resulting in an increased influx of Na^+ into the cell (Baden, 1983). The BTX were also shown to increase cytosolic calcium ion concentration (LePage *et al.*, 2003). In culture, *K. brevis* was found to produce smaller cyclic ether ring structures of reduced biological activity with affinity to the site of action of BTX, the hemi-brevetoxins. In addition, brevenals, the antagonists of BTX, were also shown to be produced by *K. brevis* (Ramsdell, 2008).

The NSP is a non-life-threatening syndrome, with symptoms appearing within 30 minutes to 3 hours following consumption of shellfish and resolving within a few days. Symptoms of NSP include abdominal pain, nausea, vomiting, diarrhea, headache and vertigo, tingling and numbness of the perioral area, severe muscular pain, and loss of motor control with respiratory distress and temperature sensation reversal (Morris *et al.*, 1991; Baden and Adams, 2000; Kirkpatrick *et al.*, 2004; Wang, 2008; Watkins *et al.*, 2008; Fleming *et al.*, 2011). When blooms of *K. brevis* are carried into coastal areas, ruptured cells under the action of wind and waves can aerosolize the toxins leading to respiratory distress in coastal populations (Cheng *et al.*, 2005, 2010). No human fatalities have ever been associated with NSP as humans are probably exposed to low doses of BTX (Landsberg *et al.*, 2009). The causative agent of NSP, *K. brevis*, is a delicate and unarmored dinoflagellate whose cell can rupture easily by turbulence, facilitating the release of the BTX into aerosols, subsequently causing irritation of the respiratory tracts in humans (Fleming *et al.*, 2005; Abraham and Baden, 2006). Cell lysis of *K. brevis* releases BTX into the surrounding water

causing skin irritation (Kusek *et al.*, 1999; Ramsdell, 2008). Thanks to the rigorous monitoring program of *K. brevis* blooms and associated banning of shellfish harvest, NSP cases are now extremely rare, although recent data suggest that fish could also be a vector of NSP toxins (Naar *et al.*, 2007; Brand *et al.*, 2012). In addition to its effects on human health, blooms of *K. brevis* and other species of the genus *Karenia* have caused recurrent mass mortalities of invertebrates, fish, marine birds, turtles, and marine and terrestrial mammals (Gunter *et al.*, 1948; Forrester *et al.*, 1977; Landsberg, 2002; Landsberg *et al.*, 2009).

4.2.4 Amnesic Shellfish Poisoning (ASP)

Domoic acid (DA) is a neurotoxin responsible for ASP and is produced by many species of the diatom genus *Pseudo-nitzschia*. During the past three decades, DA has been recurrently detected in fish and shellfish and has been associated with frequent closures of fisheries worldwide. Both DA and its isomers can accumulate in trophic chains within tissues of primary and secondary consumers, including numerous commercial species. Most of the food chain is thus affected by its toxin up to the higher levels, including sea lions, whales, and seabirds. Contamination with DA has also been associated with mass mortalities of birds and mammals (Work *et al.*, 1993; Ochoa *et al.*, 1996; Lefebvre *et al.*, 1999; Scholin *et al.*, 2000).

Between 14 and 37 species of *Pseudo-nitzschia* are currently described as capable of producing DA, which is deadly to humans but also to birds and marine mammals (Scholin *et al.*, 2000). The production of DA varies among not only species but also strains and their environment (reviewed in Lelong *et al.*, 2012). This natural amino acid and its toxic properties were discovered in 1987 (Bates *et al.*, 1989; Wright *et al.*, 1989) after intoxication of a hundred persons, among which three died after consuming contaminated mussels (Todd, 1993). Consumption of DA-contaminated shellfish resulted in diarrheic symptoms within 24 hours and neurological symptoms within 48 hours. In the most dramatic cases, loss of memory, alterations in consciousness, and coma were also observed (Todd, 1993). The bioactivity of DA is explained by its similarity with glutamic acid, a neurotransmitter, and its fixation to kainate and AMPA receptors of the central nervous system (Todd, 1993; Berman and Murray, 1997). As mentioned above, DA is more and more frequently detected in shellfish and often for extended periods of time.

4.2.5 Azaspiracid Shellfish Poisoning (AZP)

Azaspiracids (AZA) are a group of polyether marine toxic congeners produced by a small dinoflagellate, *Azadinium spinosum*, originally isolated from the North Sea in 2009 (Tillman *et al.*, 2009; Jauffrais *et al.*, 2012). Initially, AZA were attributed to the dinoflagellate *Protoperidinium crassipes* (James *et al.*, 2003); however, it is thought that *P. crassipes* accumulates AZA by preying upon the source dinoflagellate(s) (Tillmann *et al.*, 2009; Furey *et al.*, 2010). Recently, AZA were found to be produced by the genus *Amphidoma*, which is closely related to *Azadinium*, and other species of *Azadinium* (Krock *et al.*, 2012; Tillman *et al.*, 2012). In spite of inducing symptoms very similar to those of DSP, AZA do not inhibit protein phosphates (Twiner *et al.*, 2005), but inhibit endocytosis of plasma membrane proteins, although the molecular target(s) of AZA remain unknown (Bellocci *et al.*, 2010).

The first event of human intoxication with AZP occurred in the Netherlands in 1995 and was associated with the ingestion of blue mussels from Ireland contaminated with a new toxin similar in its effects to DSP, but later isolated and characterized as a new shellfish toxin (Satake *et al.*, 1998a, 1998b). Since then, several AZP events have been reported in Europe from the consumption of blue mussels, *M. edulis*, and scallops, *Pecten maximus*, and the AZA have been detected in several other bivalves including oysters, *C. gigas* and *O. edulis*; clams, *Tapes philippinarum*; cockles, *Cerastoderma edule*; and razor clams, *Ensis siliqua*; and in the brown crab, *Cancer pagurus* (Hess *et al.*, 2001, 2003; Furey *et al.*, 2003; Torgersen *et al.*, 2008). To date, at least 24 AZA analogs have been described, including several thought to be the products of bioconversion in bivalve molluscs (Rehmann *et al.*, 2008; Furey *et al.*, 2010). Cases of AZP and/or toxin in phytoplankton and contaminated shellfish have been reported in many locations in Western Europe, Northwest Africa, Japan, Chile, and North America (Furey *et al.*, 2003; Taleb *et al.*, 2006; Torgersen *et al.*, 2008; Ueoka *et al.*, 2009; López-Rivera *et al.*, 2010; Trainer *et al.*, 2013). The EU regulates the levels of AZP in shellfish at a level of 16 μg AZA equiv/100 g of shellfish meat; however, such regulations are still lacking in North America in spite of

the presence of AZA and the threat posed to consumers of imported shellfish (Trainer *et al.*, 2013).

4.3 Other Toxins: Pectenotoxins (PTX) and Yessotoxins (YTX)

Pectenotoxins and yessotoxins are lipophilic toxins produced by *Dinophysis* spp., and *Lingulodinium reticulatum*, *L. polyedrum*, *Protoceritium reticulatum*, and *Gonyaulax spinifera*, respectively (Satake *et al.*, 1997a, 1999; Draici *et al.*, 1999; Miles *et al.*, 2003, 2006; Paz *et al.*, 2004; Rhodes *et al.*, 2006; Vilariño and Espiña, 2008).

The PTX are a group of polyether-lactones (Munday, 2008) with similar structure to okadaic acid (OA) showing hepatotoxicity to mice following intraperitoneal (i.p.) injection (Terao *et al.*, 1986; Yoon and Kim, 1997; Ito *et al.*, 2008), and cytotoxicity in several mammalian cells (Jun *et al.*, 1995) with tumorigenic properties (reviewed in Vilariño and Espiña, 2008), but they have low toxicity via oral administration (Miles *et al.*, 2004, 2006; Ito *et al.*, 2008). They have been isolated from *Dinophysis acuta*, *D. acuminata*, *D. caudata*, *D. fortii*, *D. infundibulus*, *D. norvegica*, and *Phalacroma rotundata* (Holmes *et al.*, 2014), and therefore often co-occur with OA and its derivatives. Pectenotoxins, PTX1 and PTX2, were first isolated from the Japanese scallop *P. yessoensis* in Japan (Yasumoto *et al.*, 1984, 1985). Since then, at least 22 PTX have been isolated from both *Dinophysis* and shellfish (Munday, 2008). PTX1, PTX2, PTX3, and PTX11 are the most toxic analogs when injected i.p. (Munday, 2008). Recently, a novel PTX, PTX15, was identified (Suzuki *et al.*, 2012); however, the structures of PTX5 and PTX10 have not yet been determined, and information about PTX12 and PTX13 is lacking (Munday, 2008; Suzuki, 2012).

In shellfish, PTX are metabolized via two routes that transform the parental compounds from *Dinophysis* spp., PTX2, to less toxic analogs (James *et al.*, 1999; Pavela-Vrančič *et al.*, 2000, 2002). A cascade of stepwise oxidations of PTX2 yields less toxic PXT1 (alcohol), PTX3 (aldehyde), PTX4 (7-epimer of PTX1), PTX6 (carboxyl acid), and PTX7 (7-epimer of PTX6) (Draisci *et al.*, 1999b; Munday, 2008). In addition, PTX2 is hydrolyzed in many shellfish species to generate PTX2-seco acid (PTX2-SA) and its epimer 7-epi-PTX-2SA during detoxification process (Yasumoto *et al.*, 1989; James *et al.*, 1999; MacKenzie *et al.*, 2004). Enzymatic conversion of PTX2 has been reported in several shellfish species, including the blue mussel *M. edulis*; the Mediterranean mussel *M. galloprovincialis*; the greenshell mussel *Perna caniculus*; the oyster *C. japonica*; the New Zealand scallop *Pecten novaezelandiae*; clams of the genera *Donax*, *Ruditapes*, *Venerupis*, and *Solen*; the common cockle *Cerastoderma edule;* and the green crab *Carcinus maenas* (reviewed in Munday, 2008). PTX11, PTX12, and PTX13 are considered parental, with PTX11 having similar structure to PTX2; however, PTX11 and PTX12 might be hydrolyzed detoxifying compounds of PTX2 (Munday, 2008). Other PTX that are considered parental compounds for being detected in microalgae and shellfish are PTX11, PTX12, and PTX13 (Suzuki and Yasumoto, 2000; Suzuki *et al.*, 2001; MacKenzie *et al.*, 2002; Miles *et al.*, 2004). PTX11 has a similar toxicity to PTX2 and PTX1. Unfortunately, there is no information about PTX12 and PTX13; however, it is noteworthy that some structural features of PTX11 and PTX12 are relevant to detoxification of these compounds by hydrolysis that completely hydrolyzes PTX2.

The YTX are a group of polycyclic ether toxins with similar structures to BTX and ciguatoxins (Ciminiello and Fattorusso, 2008; Paz *et al.*, 2008), but having one to three sulfates in their ladder-shaped structure (Munday *et al.*, 2008). They were first isolated from Japanese scallops *P. yessoensis* (Murata *et al.*, 1987) and, like PTX, they co-occur with DST. They do not induce diarrhea and are not lethal via oral route (Tubaro *et al.*, 2003). Because of the widespread distribution of YTX, their toxicity both *in vitro* and *in vivo* has been extensively studied. The YTX are cytotoxic and cause adverse effects on cellular calcium regulation and phosphodiesterase coordination, but exhibit lower potency for the inhibition of PP2A than OA and its analogs via oral route (Terao *et al.*, 1990; Ogino *et al.*, 1997; Satake *et al.*, 1999). Nonetheless, the primary mechanism of action of YTX is still not known (Franchini *et al.*, 2010; Tubaro *et al.*, 2010).

More than 30 YTX analogs have been identified in contaminated shellfish and/or in cultures of dinoflagellates. The main structural feature of (YTX,1) was first isolated from the Japanese scallop *P. yessoensis* (Murata *et al.*, 1987). Subsequently, YTX and their analogs have been isolated from shellfish from different countries, including Norway, Italy, the United Kingdom, Russia, New Zealand, Chile, and Canada (Lee *et al.*, 1989; Ciminiello *et al.*, 1997; Yasumoto and Takizawa, 1997; Draisci *et al.*, 1999a; Quilliam,

1999; Stobo *et al.*, 2005; Vershinin *et al.*, 2006). In addition, YTX have also been isolated from causative dinoflagellates from Spain and the United States (Paz *et al.*, 2004).

Many YTX analogs have been isolated from the dinoflagellates that also produce YTX, and the same analogs have been isolated from shellfish following ingestion of the algae (reviewed in Ciminiello and Fattorusso, 2008). Either YTX or homoYTX have been found in *P. reticulatum*, *G. spinifera*, and *L. polyedrum* and are absorbed by bivalve tissue where they are the most or second-most dominant toxins in addition to other YTX (Ciminiello *et al.*, 1998, 2000a, 2000b, 2001, 2002; Aasen *et al.*, 2005a; Finch *et al.*, 2005). There is, however, evidence that hydroxylated and carboxylated derivatives largely result from metabolism of YTX in shellfish after ingestion (Munday *et al.*, 2008).

Originally, both PTX and YTX were detected all together with DST by the conventional MBA; however, both have been removed from the DSP toxin group as they have different chemical structures from DST, are unable to cause diarrhea, and have not been linked to human intoxications (Lawrence *et al.*, 2011).

4.4 Emerging Shellfish Poisonings

Monitoring of phycotoxins and shellfish poisonings is conventionally evaluated through phytoplankton counting and testing for the presence of accumulated biotoxins in bivalves (see Chapter 10). Nonetheless, there can be a lack of data on new and emerging marine toxins produced by both benthic and pelagic dinoflagellates, and on other routes of contamination via the consumption of seafood not monitored by conventional programs, including gastropods and crustaceans. Emerging biotoxins comprise gamberiol, palytoxin, osterocin, ovatoxin, *Pfisteria* toxins, and several cyclic imines. In particular, cyclic imines are a group of heterogenous toxins and include prorocentrolides and spiro-prorocentrimine, pteriatoxins, pinnatoxins, spirolides, and gymnodimines (Torigoe *et al.*, 1988; Seki *et al.*, 1995; Hu *et al.*, 1995, 1996a; Uemura *et al.*, 1995; Lu *et al.*, 2001; Takada *et al.*, 2001). These are FAT that trigger an acute threshold response in mammalian bioassays but are still not regulated as they are assumed to be safe for human consumption (Cembella and Krock, 2008).

Prorocentrolides and spiro-prorocentrimines have so far been isolated only from benthic species of the genus *Prorocentrum* with no reported contamination of shellfish (Torigoe *et al.*, 1988; Hu *et al.*, 1996b; Lu *et al.*, 2001; Sleno *et al.*, 2004). The origins of pinnatoxins and pteriatoxins, which were first found only in the bivalve shellfish *Pinna muricata* and *Pteria penguin* from Okinawa, Japan (Uemura *et al.*, 1995; Chou *et al.*, 1996; Takada *et al.*, 2001a, 2001b), have remained uncertain; however, dinoflagellates have been strongly suspected due to similarities with dinophysistoxins and spirolides (MacPherson *et al.*, 2003; MacKinnon *et al.*, 2006). In 2011, pinnatoxin was reported to be produced by a new species of benthic dinoflagellate isolated from a French Mediterranean lagoon, *Vulcanodinium rugosum* (Nézan and Chomerat, 2011; Rhodes *et al.*, 2011; Smith *et al.*, 2011).

Gymnodimines were first described from New Zealand as neurotoxic shellfish incidents that were first ascribed to *Gymnodinium* sp. (MacKenzie *et al.*, 1993; Seki *et al.*, 1995) now identified as *Karenia selliformis* (Miles *et al.*, 2003). Gymnodimines have since been detected in shellfish from Australia, New Zealand, Tunisia, and the Netherlands (Stirling, 2001; Biré *et al.*, 2002; Ben Naila *et al.*, 2012; Van de Waal *et al.*, 2015). Recently, gymnodimines were also isolated from *A. ostenfeldii* in estuaries in the east coast of the United States and from *A. peruvianum* (Van Wagoner *et al.*, 2011; Borkman *et al.*, 2012).

Spirolides were first isolated from the scallops *Placopecten magellanicus*, the mussels *M. edulis*, and phytoplankton from aquaculture sites in Nova Scotia, Canada (Hu *et al.*, 1995). The dinoflagellate *A. ostenfeldii* was identified as the primary causative organism of spirolide shellfish toxins that induce rapid death upon i.p. injection in mice, with neurotoxic symptoms and high oral potency, although other species of *Alexandrium* also produce spirolides, especially *A. peruvianum* (Cembella *et al.*, 1999, 2000; Richard *et al.*, 2001; Cembella and Krock, 2008). In addition to producing spirolides, *A. ostenfeldii* and *A. peruvianum* produce PST, and the latter produces gymnodimines (Hansen *et al.*, 1992; Lim *et al.*, 2005; Van Wagoner *et al.*, 2011). No adverse effects have so far been reported in humans, and their toxicity is still under investigation with antagonist effect at the muscarinic acetylcholine receptor already reported (Richard *et al.*, 2000; Gribble *et al.*, 2005). Since first reported from Nova Scotia, spirolides have been detected in plankton fractions and shellfish around the globe in several locations

in Europe and the Mediterranean Sea, Chile, New Zealand, and the Baltic Sea (MacKenzie, 1996; Aasen *et al.*, 2005b; Graham *et al.*, 2007; Mallat *et al.*, 2007; Harju *et al.*, 2016), as well as Atlantic Canada and the Gulf of Maine in the United States (Gribble *et al.*, 2005).

4.5 Toxin Uptake, Accumulation, and Depuration

There are very few studies on the kinetics of uptake, accumulation, and detoxification of HAB toxins, which are, when available, focused on bivalve molluscs. In addition, the long-term effects of bioaccumulated phycotoxins on marine organisms have not been thoroughly investigated, and the available information is generally limited to the most commercially exploited species.

Toxin content in shellfish is the result of the equilibrium between the rates of uptake, metabolism, and elimination of the toxins. Such a balance depends on several factors related to the toxic microalga on the one hand, to the shellfish species on the other hand, but also to other environmental factors that may influence both the HAB species and the shellfish species (reviewed in Bricelj and Shumway, 1998; Landsberg, 2002; Lassus *et al.*, 2014). Toxin uptake depends on the microalgal cell density, the nature of the toxin, the specific toxicity of the microalga, the duration of the bloom, the ratio of the toxic cells in the total plankton and the structure of the plankton community during the bloom, and the bioavailability of the toxins in its dissolved fractions, as biodeposits or as contaminants of preys transferred to higher trophic levels. Some species might experience feeding inhibition in the presence of highly toxic dinoflagellate cells, as demonstrated by Lee *et al.* (1993), who highlighted an inhibition of clearance rate in *M. edulis*, *C. gigas*, and *Mya arenaria* above a threshold of cell toxicity using several *Alexandrium* strains. Uptake and accumulation of the available toxins depend also on the ability of the shellfish to ingest and/or absorb the toxic fractions and on the physiological state of the shellfish, such as the reproductive state and the ability of the shellfish species to depurate the toxins either directly or indirectly via extracellular and intracellular digestive and/or enzymatic bioconversions of the toxins.

For bivalve molluscs, even though filtration of planktonic, vegetative cells represents the most direct means of direct exposure, toxic algal cells can also be found on the benthos or into the sediment, either as resting or temporary cysts or as dying cells, thus representing a food source for benthic organisms and thereby exposing the deposit feeders to biotoxins. Toxin uptake can also occur indirectly (i.e., concentrated through the food chain), as is mostly the case in gastropods and crabs feeding on bivalves or other organisms, thus indirectly exposed to toxins from prey that have already accumulated phycotoxins. As filter feeders, bivalve shellfish ingest cells through the process of selective feeding (Ward and Shumway, 2004), which pass through their stomach and digestive gland where they are assimilated or rejected as intact cells. As some of the harmful algae are digested, their toxins can be differentially accumulated within the tissues of the bivalves (Bricelj and Shumway, 1998). The ability of shellfish to retain and accumulate toxins varies greatly with the shellfish species (Bricelj and Shumway, 1998; Fernández *et al.*, 2004), thus clearly highlighting the importance of considering both shellfish species and the type of toxins when trying to assess toxin uptake, accumulation, and depuration.

Toxins accumulated in bivalve tissues can undergo metabolic processes by which they are chemically modified, sometimes in forms more or less toxic. These biotransformations can result from either digestive or active detoxification processes, or may simply result from the involvement of toxins in the general intra- or extracellular metabolic processes. These bioaccumulated toxins, which are in many cases bioconverted and biomagnified, are now available to secondary consumers. Humans are examples of predators only exposed to biotoxins following indirect exposures, as they may eat shellfish previously exposed to microalgal toxins. For instance, several BTX metabolites have been described in shellfish. The taurine conjugate of the BTX backbone B was first described from the New Zealand cockle *Austrovenus stutchburyi*, and three new BTX were later isolated from the green mussel *Perna caniculus* (Ishida *et al.*, 1995; Morohoni *et al.*, 1995, 1999; Murata *et al.*, 1998). Since then, several cysteine and desoxy cysteine conjugates of both BTX A and B have been described from shellfish in the Gulf of Mexico, including eastern oysters and whelks (Dickey *et al.*, 1999; Poli *et al.*, 2000; Plakas *et al.*, 2004; Wang *et al.*, 2004). For DSP toxins, differential uptake of *Dinophysis* cells is thought to explain the differential accumulation of DST between mussels and oysters, the latter showing relatively low toxin content. The okadaate diol-esters are hydrolyzed into the main free DST (OA, DTX1, and DTX2), whereas DTX4 and DTX5 are

first hydrolyzed to diol-esters and then to the main free DST, and then are esterified with fatty acids inside the cells of the digestive gland of several species of bivalve shellfish, including mussels and clams (Suzuki *et al.*, 1999; Rossignoli, 2011; Rossignoli *et al.*, 2011; Konoki *et al.*, 2013), to finally undergo enzymatic acylation before being excreted into free fatty acids mainly in the feces (reviewed in Reguera *et al.*, 2014). In the case of PSP toxins, scallops *P. magellanicus* convert neoSTX and GTX into the more toxic STX; and several species of shellfish, including clams *Mactra chinensis* and *Peronidia venulosa*, hydrolyze carbamate toxins into the more toxic dcGTX (Shimizu and Yoshioka, 1981; Sullivan *et al.*, 1983a, 1983b; Doucette *et al.*, 2006). Nonetheless, several toxins from the dinoflagellates and intermediate conjugates from the shellfish remain unknown, showing that the bioconversions of STX and its derivatives differ among species of shellfish and among individuals from the same species, but are also influenced by several environmental factors (Kodama and Sato, 2008).

4.6 Shellfish Contamination in North America

4.6.1 Bivalves

4.6.1.1 Paralytic Shellfish Contamination

The first report of PSP syndrome dates back to 1920 in California, United States, when at least six people died (Anderson *et al.*, 1990). Until the 1970s, PSP was detected in Europe, North America, and Japan. In the United States, the first major PSP event occurred in September 1972 from southern Maine to Cape Ann, Massachusetts, with several species of bivalves testing positive for PST (Twarog *et al.*, 1975).

The State of Maine is regularly subjected to blooms of *A. fundyense*, causing massive shellfishery closure due to PST (Shumway *et al.*, 1988; Bean *et al.*, 2005; Anderson *et al.*, 2014). Neighboring states such as Massachusetts and New Hampshire as well as the eastern Canadian coastline are also victims of these *A. fundyense* blooms, which can lead to closure of harvests of several bivalve species, starting with blue mussels, which are often considered "indicator" species as *M. edulis* takes up and depurates the PSP toxins more quickly than other organisms such as softshelled clams, surfclams, or ocean quahogs that can retain toxins for a very long period of time (Shumway *et al.*, 1988; Bricelj and Shumway, 1998). Indeed, blue mussels tend to depurate much faster than other bivalves, whereas surfclams for example are characterized by accumulation of high levels of PST, slow toxin elimination, and an extremely high capacity for toxin bioconversion (Shumway *et al.*, 1994; Bricelj and Shumway, 1998; Bricelj *et al.*, 2012). Another mussel species, the northern horse mussel *Modiolus modiolus*, was found to contain up to 9000 μg STX eq 100 g^{-1} whole tissue in the Bay of Fundy, United States, and represents the best potential candidate for use as a sentinel species (Jamieson and Chandler, 1983).

The maximum concentrations of PST in Atlantic surfclams (*Spisula solidissima*), ocean quahogs (*Arctica islandica*), and northern quahogs or hard clams (*Mercenaria mercenaria*) were monitored in Maine and reached 7934, 1895, and 1113 μg STX eq 100 g^{-1} whole tissue, respectively (Shumway *et al.*, 1988; 1994; Bricelj and Shumway, 1998; Table 4.1). The highest recorded levels of PST in other clam species, such as softshell clams, *Mya arenaria,* and Atlantic razor clams, *Siliqua costata*, were recorded respectively in Massachusetts and New Hampshire with 9600 and 1727 μg STX eq 100 g^{-1} whole tissue (Twarog, 1974; Sasner, 1975). The maximum levels of PST in sea scallops, *P. magellanicus*, and bay scallops, *Argopecten irradians*, were also recorded on the east coast of North America, at 10,864 and 2040 μg STX eq 100 g^{-1} whole tissue, respectively (Twarog, 1974; Bricelj and Shumway, 1998). Two oyster species, also mainly found on the east coast of North America, are often subjected to blooms of *A. fundyense*; however, they tend to accumulate much less toxins, as their maximum levels only reached 214 μg STX eq 100 g^{-1} whole tissue for eastern oysters, *C. virginica* (Worms *et al.*, 1993), and 1300 μg STX eq 100 g^{-1} whole tissue for edible or European oysters, *O. edulis* (Shumway *et al.*, 1990).

As described in this chapter, mussels are the bivalves that accumulate the highest concentrations of PSP, which is why they are most often utilized as sentinel species. The most commonly used are mytilids (e.g., blue mussels, *M. edulis*), which are distributed worldwide and all along most of the eastern and western coasts of North America; however, California mussels (*M. californiensis*) are also in some places considered as the sentinel species (Bretz *et al.*, 2002). Blue mussels are able to accumulate much higher concentrations of PST, often reaching levels around 20,000 to 30,000 μg STX eq 100 g^{-1} whole tissue, with a record high of 30,360 μg STX eq 100 g^{-1}

Table 4.1 Maximum levels of paralytic shellfish toxins recorded in shellfish from North America.

Bivalve species	Common name	Toxin source	Toxin level	Notes	Location	Reference
Arctica islandica	Ocean quahog		1895 µg STX-eq 100 g^{-1} whole body		Machias Bay, Maine, USA	Shumway *et al.* (1988)
Argopecten irradians	Bay scallop		2040 µg STX-eq 100 g^{-1} whole body		Eastham, Massachusetts, USA	Twarog (1974)
Chlamys hastata	Spiny scallop	*Alexandrium catenella*	5900 µg STX-eq 100 g^{-1}		Alaska to southern California	DFO (1989) in Beitler (1992) in Bricelj and Shumway (1998)
			11,945 µg STX-eq 100 g^{-1} whole body			Ralonde (1996)
Chlamys rubida	Pink scallop	*Alexandrium catenella*	5900 µg STX-eq 100 g^{-1}		Alaska to southern California	DFO (1989) in Beitler (1992) in Bricelj and Shumway (1998)
			11,945 µg STX-eq 100 g^{-1} whole body			Ralonde (1996)
Crassadoma gigantea (Hinnites multirugosus)	Giant rock-scallop	*Alexandrium catenella*.	13,593 µg STX-eq 100 g^{-1} whole body	26,000 µg STXeq 100 g^{-1} Visceral mass	Timber Cove, California, USA	Sharpe (1981), Beitler (1991)
Crassostrea gigas	Pacific oyster	*Alexandrium* spp.	48 µg STX-eq 100 g^{-1} whole body		Mystery Bay, Washington, USA	Wekell *et al.* (1996)
		Protogonyaulax tamarensis	9929 µg STX-eq 100 g^{-1} whole body		Okeover Inlet, British Columbia, Canada	Bricelj and Shumway (1998)
Crassostrea virginica	Eastern oyster		214 µg STX-eq 100 g^{-1} whole body		Acadian peninsula, SE Gulf, St. Lawrence, Canada	Worms *et al.* (1993)
Mercenaria mercenaria	Northern quahog		1113 µg STX-eq 100 g^{-1} whole tissue		Mohegan Island, Maine, USA	Bricelj and Shumway (1998)
Modiolus modiolus	Northern horse mussels	*Alexandrium* spp.	9000 µg STX-eq 100 g^{-1} whole tissue		Bay of Fundy, USA	Jamieson and Chandler (1983)
Mya arenaria	Softshell clam	*Protogonyaulax tamarensis*	9600 µg STX-eq 100 g^{-1} whole tissue		Merrimack Estuary, Massachusetts, USA (1972)	Twarog (1974) in Bricelj and Shumway (1998)
Mytilus edulis	Blue mussel	*Alexandrium* spp.	652 µg STX-eq 100 g^{-1} whole body		Mystery Bay, Washington, USA	Wekell *et al.* (1996)
			20,606 µg STX-eq 100 g^{-1} whole body		Kodiak Island, Alaska, USA	Lewitus *et al.* (2012)

Table 4.1 (*Continued*)

Bivalve species	Common name	Toxin source	Toxin level	Notes	Location	Reference
		Alexandrium spp.	30,000 µg STX-eq 100 g^{-1} whole body		Work Channel, British Columbia, USA	Chiang (1988) in Lewitus *et al.* (2012)
		Alexandrium spp.	30,360 µg STX-eq 100 g^{-1} whole body		Orcas Island, Washington, USA	Lewitus *et al.* (2012)
Mytilus californianus	California mussel	*Alexandrium catenella.*	770 µg STX-eq 100 g^{-1} whole body		Santa Cruz Wharf, California, USA	Jester *et al.* (2009)
Ostrea edulis	Edible oyster	*Protogonyaulax tamarensis*	1300 µg STX-eq 100 g^{-1} whole body		Long Point Creek, Maine, USA	Shumway *et al.* (1990)
Panopea abrupta (=*generosa*)	Pacific geoduck		2200 µg STX-eq 100 g^{-1} visceral mass		British Columbia, Canada	DFO (1989) in Beitler (1992) in Bricelj and Shumway (1998)
			1818 µg STXeq 100 g^{-1} whole body		Ketchikan, Alaska, USA	Lewitus *et al.* (2012)
Patinopecten caurinus	Weathervane scallop	*Alexandrium catenella*	Adductor (58), viscera (4945), gills (504), gonads (1361), mantle (243) µg STX-eq 100 g^{-1}		Kodiak Bay, Alaska, USA	Ralonde (1996)
		Alexandrium catenella		Toxic?	Pacific coast, USA	Nishitani and Chew (1988)
Placopecten magellanicus	Sea scallop	*Protogonyaulax tamarensis*	10,864 µg STX-eq 100 g^{-1} whole tissue		Georges Bank, USA	Bricelj and Shumway (1998; calc. from White *et al.*, 1993)
Protothaca staminea	Pacific littleneck clam	*Alexandrium catenella*	5053 µg STX-eq 100 g^{-1} whole tissue		Water Bay, British Columbia, Canada	Bricelj and Shumway (1998)
Saxidomus giganteus	Butter clam	*Alexandrium* spp.	94 µg STX-eq 100 g^{-1} whole tissue		Puget Sound, Washington, USA	Wekell *et al.* (1996)
		Alexandrium catenella	9600 µg STX-eq 100 g^{-1} whole tissue		Gilford Island, northern British Columbia, Canada	Chiang (1988), in Bricelj and Shumway (1998)
Saxidomus nuttalii	Washington clam		14,000 µg STX-eq 100 g^{-1} whole tissue		Campbell Cove, California, USA	Price *et al.* (1991)
Siliqua costata	Atlantic razor clam		1727 µg STX-eq 100 g^{-1} whole tissue		Hampton, New Hampshire, USA	Sasner *et al.* (1975)

(*continued*)

Table 4.1 (*Continued*)

Bivalve species	Common name	Toxin source	Toxin level	Notes	Location	Reference
Siliqua patula	Pacific razor clam		720 µg STX-eq 100 g^{-1} whole tissue		Long Beach, Washington, USA	Bricelj and Shumway (1998)
Spisula solidissima	Atlantic surfclam	*Alexandrium fundyense?*	7934 µg STX-eq 100 g^{-1} whole tissue		Head Beach, Maine, USA	Shumway *et al.* (1994)
Tapes philippinarum	Japanese littleneck clam		6086 µg STX-eq 100 g^{-1} whole tissue		Okeover Inlet, British Columbia, Canada (1986)	Bricelj and Shumway (1998)
Tresus capax	Fat gaper		3520 µg STX-eq 100 g^{-1} whole tissue		Theodosia Inlet, British Columbia, Canada	Quayle (1969)
Tresus nuttalli	Horse clam		342 µg STX-eq 100 g^{-1} whole tissue		Bering Sea, Alaska, USA	Lewitus *et al.* (2012)
			3880 µg STXeq 100 g^{-1} whole tissue		Orcas Island, Washington, USA	Lewitus *et al.* (2012)

Crustacean species	Common name	Toxin source	Toxin level	Notes	Location	Reference
Cancer magister	Dungeness crab		1094 µg STX-eq 100 g^{-1} whole tissue		Kodiak Island, Alaska, USA	Lewitus *et al.* (2012)
Cancer antennarius	Brown rock crabs	*Alexandrium catenella*	49.3 µg STX-eq 100 g^{-1} hepatopancreas		Santa Cruz Wharf, California, USA	Jester *et al.* (2009)
Cancer borealis	Jonah crab	*Alexandrium tamarense*	56 µg STX-eq 100 g^{-1} tissues		Maine, USA	Goggins (1961) in Shumway (1985)
Cancer productus	Red rock crabs	*Alexandrium catenella*	23.8 µg STX-eq 100 g^{-1} hepatopancreas		Santa Cruz Wharf, California, USA	Jester *et al.* (2009)
		Alexandrium catenella	285 µg STX-eq 100 g^{-1} hepatopancreas and 27 µg STX-eq 100 g^{-1} muscle tissue		Washington State, USA	Jonas-Davis and Liston (1985)
Chionoecetes bairdi	Taner crab				Alaska, USA	ADHHS-ES database in Lewitus *et al.* (2012)
Chionoecetes opilio	Snow crab				Alaska, USA	ADHHS-ES database in Lewitus *et al.* (2012)
Emerita analogua	Sand crab	*Alexandrium* sp.	468 µg STX-eq 100 g^{-1} whole tissue		Pescadero Beach, California, USA	Bretz *et al.* (2002)
Erimacrus isenbeckii	Hair crab				Alaska, USA	ADHHS-ES database in Lewitus *et al.* (2012)

Table 4.1 (*Continued*)

Crustacean species	Common name	Toxin source	Toxin level	Notes	Location	Reference
Fabia subquadrata	Grooved mussel crab	*Alexandrium catenella*	32 µg STX-eq 100 g^{-1} whole tissue		Washington State, USA	MacDonald (1970) in Shumway (1985)
Pagurus sp.	Hermit crab	*Alexandrium catenella*	35 µg STX-eq 100 g^{-1} whole tissue		Washington State, USA	MacDonald (1970) in Shumway (1985)
Paralithodes camtschaticus	Red king crab				Alaska, USA	ADHHS-ES database in Lewitus *et al.* (2012)
Pugettia producta	Northern kelp crab	*Alexandrium catenella*	1710 µg STX-eq 100 g^{-1} hepatopancreas and 146 µg STX-eq 100 g^{-1} eggs		Maine, USA	Goggins (1961) in Shumway (1985)
Hemigraspus oregonensis	Bay Shore Crab	*Alexandrium* sp.	32 µg STXeq 100 g^{-1} whole tissue		Washington State, USA	Jonas-Davis and Liston, 1985
Homarus americanus	American lobster	*Alexandrium tamarense*	1654 µg STX-eq 100 g^{-1} hepatopancreas		Cutler, Maine, USA	Shumway (1985)

*) MU: Mouse units (1 MU: 0.18 µg STX).
**) Presumed; genus and species name not given by author.

whole tissue in Orcas Island, Washington, United States (Lewitus *et al.*, 2012). These levels are much higher than those of California mussels, in which the maximum concentration of 770 µg STX eq 100 g^{-1} whole tissue was measured in Santa Cruz Wharf, California (Shumway *et al.*, 1990).

Several species of scallops commercialized in North America are grown on the west coast, where high levels of PST have also been recorded along the entire shoreline, from Alaska to California (Sharpe, 1981; Beitler, 1991, 1992; Ralonde, 1996; Bricelj and Shumway, 1998). The highest concentrations for both spiny scallops (*Chlamys hastata*) and pink scallops (*Chlamys rubida*) were recorded in Alaska as high as 11,945 STX eq 100 g^{-1} whole tissue. Meanwhile, concentrations of PST as high as 4945 STX eq 100 g^{-1} viscera were recorded in the weathervane scallop *Patinopecten caurinus* (Ralonde, 1996). The giant rock scallops, *Crassadoma gigantea*, also accumulate high levels of PST in their tissues. They have been shown to accumulate as much as 13,593 STX eq 100 g^{-1} whole tissue following a bloom of *A. catenella* in Timber Cove, California (Sharpe, 1981; Beitler, 1991).

Several clam species exploited in North America are also recurrently exposed to PST, with the highest concentrations recorded in the two clams of the genus *Saxidomus*, Washington clams *S. nuttalii* and butter clams *S. giganteus*, with as much as 14,000 and 9600 µg STX eq 100 g^{-1} whole tissue in California and British Columbia, respectively (Chiang, 1988; Price *et al.*, 1991). Japanese littleneck clams, *Tapes philippinarum*, were also shown to accumulate high levels of PST, albeit less than the other two species with up to 6086 µg STX eq 100 g^{-1} whole tissue recorded in 1986 in Okeover Inlet, BC, Canada. Relatively similar amounts of PST (up to 5053 µg STX eq 100 g^{-1} whole tissue) were detected in the Pacific littleneck clam, *Protothaca staminea*, in Water Bay, BC, Canada (Bricelj and Shumway, 1998). Finally, large clams such as the fat gaper, *Tresus capax*, or the horse clam, *Tresus nuttalli*, can accumulate up to 3520 and 3880 µg STX eq 100 g^{-1} whole tissue as observed respectively in Theodosia Inlet, BC, Canada (Quayle, 1969), and Orcas Island, Washington, United States (Lewitus *et al.*, 2012).

Pacific oysters, *C. gigas*, are mainly cultivated on the northwest coast of the United States and

Canada. Even though oysters are known to accumulate lower amounts of PST than most other bivalves, they have still been reported with as high as 9929 µg STX eq 100 g^{-1} whole tissue in British Columbia, Canada (Bricelj and Shumway, 1998). In Long Beach, Washington, Pacific razor clams, *S. patula*, were sampled with up to 720 µg STX eq 100 g^{-1} whole tissue (Bricelj and Shumway, 1998). Pacific geoducks, *Panopea abrupta* (=*generosa*), were sampled with up to 1818 µg STX eq 100 g^{-1} whole body in Ketchikan, Alaska, United States, and the horse clams *T. nuttalli* (Lewitus *et al.*, 2012).

Very large interspecific differences can be observed in toxin accumulation (Bricelj and Shumway, 1998), as reported in Table 4.1, with large differences in the highest concentrations reached by the different species. Species such as *M. edulis*, rapidly accumulating large amounts of toxins, are more often used as sentinel species than other sympatric bivalve species such as *M. arenaria* or *C. gigas*. Ocean and northern quahogs, *A. islandica* and *M. mercenaria*, are however, species that tend to accumulate fewer concentrations of PST.

Differences in PST accumulation have been associated with the sensitivity of specific bivalve species to the toxin (Twarog *et al.*, 1972; Bricelj and Shumway, 1998). For example, mussels, *M. edulis*, show greater nerve insensitivity compared to other species, and tend to feed readily on toxic cells thus reaching high toxin contents (Bricelj *et al.*, 1990). Conversely, oysters, *C. virginica*, which are highly sensitive to PST, tend to accumulate less toxins. Bricelj *et al.* (2005) also showed differences in toxin accumulation in several populations of softshell clams, *M. arenaria*, according to their origin and previous history of exposure to PST. Softshell clams regularly exposed to PSP were indeed able to accumulate much higher toxin content, and showed a much larger nerve insensitivity that could be associated with a mutation resulting in a single amino acid substitution in the sodium channel pore region, target of PST. Shumway and Cucci (1987) also demonstrated that *M. edulis* sampled in areas regularly exposed to PSP showed a higher feeding rate on toxic *Alexandrium* cells.

Conversely, the high toxin resistance in the butterclam, *S. giganteus*, seems to be innate and not related to previous exposure to PST (Kvitek and Beitler, 1991). The species accumulates high concentrations of toxins in its siphon, which remains toxic for very long times. This toxicity has been hypothesized to serve as a feeding deterrent against potential predators (Kvitek, 1991); however, the extent of the possible deterrence is not clear.

In bivalves, PST are not evenly distributed in all tissues (i.e., different tissues may accumulate different levels of toxin). Cembella *et al.* (1993), for example, demonstrated the difference in the distribution of toxins within the tissues of two bivalve species: sea scallops *P. magellanicus* and surfclams *S. solidissima*. The greatest proportion of toxin is always contained in the digestive gland, after ingestion of toxic cells and absorption of their toxins, especially during the toxification phase, and can range from 80 to 98% of the toxin body burden (Bricelj and Shumway, 1998). Locomotory tissues, such as adductor muscle, pallial muscle, or muscular foot, representing a large portion of the body weight, accumulate very low concentrations of toxins (Cembella *et al.*, 1994; Bricelj and Shumway, 1998). For scallop species, where organs can be separated, this still allows commercialization of these tissues during PSP outbreaks. Thus, a good understanding of the anatomical partitioning of toxins within each tissue is of particular interest.

Toxin composition in bivalves often differs from the PST profile of the toxic cells ingested, due to biotransformation of toxins, which differs according to the bivalve species (reviewed in Bricelj and Shumway, 1998). Some bivalve species, such as *M. arenaria*, *C. gigas*, or *M. mercenaria* (Martin *et al.*, 1990b; Bricelj *et al.*, 1991, 1996; Oshima *et al.*, 1987), show a lower capacity to biotransform PST than do *S. giganteus*, *P. staminea*, or *S. solidissima* (Beitler, 1992; Sullivan *et al.*, 1983a, 1983b; Bricelj and Shumway, 1998). These changes in toxin profiles are the results of potential selective retention, elimination, or biotransformation of PST, such as reduction, hydrolysis, or enzymatic conversion (see Bricelj and Shumway, 1998). Additionally, the types of PST are different according to the bivalve tissues. Viscera contain PST, usually with the closest profile to the phytoplankton cells, whereas differences in toxin profiles among the non-visceral tissues are hypothesized to be associated with a tissue-specific flux of toxin and binding capabilities (Cembella *et al.*, 1994; Cembella and Shumway, 1995).

The ability of bivalves to detoxify is also very species-dependent. The species can be classified into two main groups: species with rapid to moderate detoxication rates, such as blue or California mussels *M. edulis* and *M. californiensis*; and species that can take up to several months or years to depurate such as *S. giganteus*, *S. nuttalli*, and *S. solidissima*. Sea scallops *P. magellanicus* are also considered slow detoxifiers. The mechanisms associated with detoxification are not well understood and require further elucidation.

4.6.1.2 Diarrheic Shellfish Contamination

Historically, *Dinophysis* spp. have been found in North America with rare closures of shellfish beds related to DSP contamination associated with accumulation of DST in shellfish in the United States, with only one event reported in Canada (Quilliam *et al.*, 1991; Subba *et al.*, 1993; Todd, 1997; Tango *et al.*, 2004). Since 2008, however, expansion of *Dinophysis* blooms and associated contamination of shellfish exceeding the U.S. Food and Drug Administration (FDA) limit level of 0.16 µg g^{-1} of shellfish tissue (Table 4.2) have been reported from the west coast of the United States and along the Gulf Coast of Texas (Campbell *et al.*, 2010; Deeds *et al.*, 2010; Swanson *et al.*, 2010), from Washington State (Trainer *et al.*, 2013), and from the east coast of the United States in New York (Hattenrath-Lehmann *et al.*, 2013).

High levels of OA in scallops, *P. magellanicus*, and evidence of DSP toxicity associated with *Dinophysis* spp. were reported along the eastern coasts of North America in the 1980s (Freudenthal and Jijina, 1985; Maranda and Shimizu, 1987; Subba *et al.*, 1993). Trace amounts of OA and DTX1 were found in plankton tows from the northwest Atlantic (Cembella, 1989; Morton

Table 4.2 Maximal levels of okadaic acid and its analogs (DST) in shellfish recorded from North America.

Scientific name	Common name	Toxin source	Toxin level	Location	Notes	References
Crassostrea gigas	Pacific oyster	*Dinophysis acuminata*	26.20 µg OA-eq. · 100 g shellfish^{-1}	Sequim Bay, WA, USA		Trainer *et al.* (2013)
			<60 µg OA-eq. 100 g shellfish^{-1}	Blyn beach, WA, USA		
Crassostrea virginica	Eastern oyster	*Dinophysis* cf. *ovum*	47 µg OA-eq · 100 g tissue^{-1}	East coast of Texas, USA		Deeds *et al.* (2010)
Mytilus edulis	Blue mussel	*Dinophysis acuminata*	92.69 µg OA-eq · 100 g shellfish^{-1}	Sequim Bay, WA, USA	DTX-1 primary isomer	Trainer *et al.* (2013)
			<65 µg OA-eq. · 100 g shellfish^{-1} <100 µg OA-eq. · 100 g shellfish^{-1}	Blyn beach, WA, USA		
			0.037–1.245 µg OA-eq. · 100 g shellfish^{-1}	State Park, WA, USA		
		Dinophysis acuminata	0.23 DTX1	Northport Bay, NY, USA	Co-occurring with *Alexandrium fundeyense*	Hatternath-Lehmann *et al.* (2013)
		Unspecified	0.72 g DTX3 g^{-1} whole meat	Strait of Georgia, BC, Canada		Taylor *et al.* (2013)
Mya arenaria	Soft shell clam	*Dinophysis acuminata*	0.957–1.089 µg OA-eq. · 100 g shellfish^{-1}	Northport Bay, NY, USA	Co-occurring with *Alexandrium fundeyense*	Hatternath-Lehmann *et al.* (2013)
Geukensia demissa	Ribbed mussels	*Dinophysis acuminata*	1.137 µg OA-eq. · 100 g shellfish^{-1}	Northport Bay, NY, USA	Co-occurring with *Alexandrium fundeyense*	Hatternath-Lehmann *et al.* (2013)
Leukoma staminae	Littleneck clam	*Dinophysis acuminata*	7.79 µg OA-eq. · 100 g shellfish^{-1}	Sequim Bay, WA, USA		Trainer *et al.* (2013)
			<5 µg OA-eq. · 100 g shellfish^{-1}	Blyn Beach, WA, USA		
			<10 µg OA-eq. · 100 g shellfish^{-1}	State Park Beach, WA, USA		

*) MU: Mouse units (1 MU: 0.18 µg STX).
**) Presumed; genus and species name not given by author.

et al., 1999; Tango *et al.*, 2002) and in oysters, *C. gigas* (Marshall *et al.*, 2002). Nonetheless, during the dense blooms of *D. acuminata* in New York waters in 2010, high levels of DST in blue mussels, *M. edulis*, exceeding the regulatory limits were detected (Deeds *et al.*, 2010).

Along the coast of western North America, shellfish have occasionally tested positive for the presence of DST in Washington State. Manila clams tested positive for the presence of OA at low levels for the first time in British Columbia in 2003 (Canadian Food Inspection Agency data), and OA and DTX1 levels reaching more than threefold the regulatory limit were reported in British Columbia between 2003 and 2005 (Trainer and Trick, 2006; Trainer *et al.*, 2010). In 2003–2004, California mussels in Monterey Bay, California, tested positive for DSP toxins (Southerland, 2008). While Freudenthal (personal communication) suggested the presence of DSP toxins in New York waters, the first documented reported human intoxications with DSP in Canada and the United States were reported in 2011, following dense blooms of *D. acuminata*, with three cases of human DSP illness in Sequim Bay, Washington, and 62 illnesses from George Harbor, BC; in both areas, the intoxications were associated with the consumption of blue mussels (Lloyd *et al.*, 2011; Esenkulova and Haigh, 2012; Lewitus *et al.*, 2012; Taylor *et al.*, 2013; Trainer *et al.*, 2013). Blue mussels collected from Sequim Bay State Park contained levels of DST 2–10 times the regulatory limits. In the summer of 2012, DST above the regulatory limit were detected in shellfish from the Puget Sound, resulting in harvest closure of California mussels, *M. californianus*; varnish clams, *N. obscurata*; manila clams, *V. philippinarum*; and Pacific oysters, *C. gigas* (Trainer *et al.*, 2013). In the same year, a six-month monitoring study of *Dinophysis* spp. and DST in nine shellfish species (*M. edulis*, *C. gigas*, *V. philippinarum*, *N. obscurata*, *M. californianus*, *Leukoma stamineae*, *P. generosa*, *S. giganteus*, and *S. patula*) in Washington State, from June to November, showed the presence of at least four species of *Dinophysis* and DST in five shellfish species with at least one sample above the regulatory limit, namely in *M. edulis*, *C. gigas*, *V. philippinarum*, *N. obscurata*, and *M. californianus*. Blue mussels showed the highest concentration of toxins, with Manila clams showing the lowest concentration and Pacific oysters intermediate concentrations (Eberhart *et al.*, 2013).

In the Gulf of Mexico, contamination of *C. virginica* with OA just above the regulatory limit occurred in 1991 in the Alabama Gulf Coast associated with blooms of *D. caudata* (Dickey *et al.*, 1992). In 2008, the levels of DST in eastern oysters reached threefold the regulatory limit in Texas, and were associated with a dense bloom of *D. ovum* resulting in closure of shellfish harvest (Swanson *et al.*, 2008; Campbell *et al.*, 2010; Deeds *et al.*, 2010).

In Mexico, no cases of DSP have ever been officially recognized (Hernández-Becerril *et al.*, 2007; Cortés-Altamirano and Sierra-Beltrán, 2008). Measurement of DSP toxins started in 2009, and regulation of DSP toxins started only recently, in 2011. Contamination of shellfish with DST associated with *D. caudata* was reported from Bahaí de Manzanillo, Colima, 2008, and in Pacific oysters from Baja California in 2010 resulting in closure of harvest on two occasions (Ochoa *et al.*, 1998; García-Mendoza *et al.*, 2011; Lewitus *et al.*, 2012). In 2012, blooms of *D. fortii* and *D. acuminata* resulted in contamination and closure of shellfish harvest in Baja California (García-Mendoza *et al.*, 2013).

4.6.1.3 Neurotoxic Shellfish Contamination

Historically, the Gulf of Mexico, notably the west coast of Florida, has witnessed the most NSP events; hence, the name *Florida red tide* is used to describe blooms of *K. brevis* in the Gulf of Mexico. Blooms of *K. brevis* have been reported almost annually along the Florida southwest coasts (Steidinger and Vargo, 1988), less frequently on the coastal regions of the Gulf of Alabama to Texas and the eastern coast of Mexico (Magaña *et al.*, 2003), as well as along the southeast Atlantic coast of North Carolina (Tester and Steidinger, 1997). The incidence of NSP in North Carolina has occurred due to an unusual transport of *K. brevis* bloom along the Gulf Stream from the west coast of Florida (Tester *et al.*, 1989; Tester and Stumpf, 1991). In New Zealand and South Africa, more recent cases of NSP-like symptoms with respiratory distress occurred and were attributed to *K. concordia* and *K. brevisulcata*, respectively (Botes *et al.*, 2003; Chang, 2011).

Shellfish are able to accumulate varying concentrations of BTX and are a significant vector of the toxins to higher trophic levels including humans, although mortalities of shellfish, including bivalves, have been recorded (Macfarren *et al.*, 1965; Ishida *et al.*, 1996, 2004; Steidinger *et al.*, 1998; Poli *et al.*, 2000; Landsberg, 2002; Naar *et al.*, 2004; Pierce *et al.*, 2004; Wang *et al.*, 2004). Lipophilic BTX accumulate in fatty

Table 4.3 Maximal levels of brevetoxins in shellfish recorded from North America.

Scientific name	Common name	Toxin source	Toxin levels	Location	Notes	References
Crassostrea gigas	Pacific oyster	*K. brevis*	146 MU 100 g^{-1}			Pierce *et al.* (2004)
Crassostrea virginica	Eastern oyster		180 MU 100 g^{-1}			Tester and Fowler (1990)
Donax variabilis	Coquinas		550 MU 100 g^{-1}	Midnight Pass, FL		Cummins *et al.* (1971)
Mercenaria campechiensis	Hard clams		270 MU 100 g^{-1}	Venice Inlet, FL		Cummins *et al.* (1971)
Macrocallista nimbosa	Sunray Venus clam		140 MU 100 g^{-1}	Venice Inlet, FL		Cummins *et al.* (1971)
Mercenaria mercenaria	Hard clams		69 MU 100 g^{-1}			Pierce *et al.* (2004)
Chione cancellata	Cross-barred Venus		95 MU 100 g^{-1}			Steidinger *et al.* (1998)
Perna viridis			73.59 ng PbTx-3 equiv. g^{-1}			McFarland *et al.* (2015)
Busycon sp.	Whelk		22 MU 100 g^{-1}	Venice Inlet, FL		Pierce *et al.* (2004)
Busycon contrarium	Lightning whelk		22.5 µg PbTx3 equiv. g^{-1}	Sarasota Bay, FL		Poli *et al.* (2000)

*) MU: Mouse units (1 MU: 0.18 µg STX).
**) Presumed; genus and species name not given by author.

organs of shellfish, and naturally exposed bivalves such as Pacific oysters, *C. gigas*, and hard clams, *M. mercenaria*, can accumulate high levels of the toxins, more than a hundredfold the regulatory limit of 20 MU 100 g^{-1} shellfish meat (Table 4.3). Although under laboratory conditions, shellfish, typically bivalves, can depurate BTX within a few days, they generally can remain toxic over an extended period of time, ranging from a few weeks up to months in the field (Morton and Burklew, 1969; Pierce *et al.*, 2004). The prolonged shellfish bed closure in 2005–2007 associated with a multispecific, 13-month-long bloom of *Karenia* along the coasts of four Gulf states is an example of the devastating effects of *Karenia* blooms not only on the shellfish industry but also on the marine ecosystems. Indeed, mass mortalities of benthic communities, fish kills, and negative effects on seabirds, turtles, and marine mammals occurred (Steidinger *et al.*, 2008; Heil and Steidinger, 2009). The bloom resulted in 24 cases of NSP linked to the consumption of clams recreationally harvested in or in close proximity to regulated shellfish-harvesting areas (Reich *et al.*, 2015).

4.6.1.4 Amnesic Shellfish Contamination

The first shellfish poisoning associated with DA and the diatom *Nitzschia pungens* occurred in 1987, when three people died and 105 became sick after eating contaminated blue mussels *M. edulis* from Eastern Prince Edward Island, Canada (Bates *et al.*, 1989). Blue mussels were reported with a maximum of 790 µg DA g^{-1} whole tissue. Since this intoxication event, most of the reported DA contamination events in North America have occurred on the west coast of the United States, including the Washington, Oregon, and California Pacific coast line (Lewitus *et al.*, 2012). Events of DA, however, have also been recorded on the east coast of the United States and Canada, especially in eastern Prince Edward Island, in the George River, in the German and Brown Banks, and in the Bay of Fundy, New Brunswick, Canada. Bivalves are the most exploited shellfish globally and in North America; however, only a few species in North America have been recorded to accumulate DA.

The highest concentrations of DA measured in North America were recorded on the west coast for most of the bivalve species present on the North American coast line (Table 4.4): California

Table 4.4 Maximum levels of domoic acid in shellfish from North America.

Bivalve species	Common name	Toxin source	Toxin level	Notes	Location	Reference
Crassostrea gigas	Pacific oysters	*Pseudo-nitzschia pseudo-delicatissima*	30 μg DA g^{-1} whole tissue		Sequim Bay, WA, USA	Trainer *et al.* (2007)
Crassostrea virginica	Eastern oysters		0.9 μg DA g^{-1} whole tissue		Neguac Bay, NB, Canada	Data from Canadian Food Inspection Agency (CFIA) in Mafra *et al.* (2010)
Mya arenaria	Softshell clam	*Pseudo-nitzschia multiseries*	38 μg DA g^{-1} whole tissue		Eastern Prince Edward Island, Canada	Bates *et al.* (1989)
Mytilus edulis	Blue mussel	*Pseudo-nitzschia multiseries*	790 μg DA g^{-1} whole tissue		Eastern Prince Edward Island, Canada	Bates *et al.* (1989)
Mytilus californianus	California mussel	*Pseudo-nitzschia multiseries*	5.8 μg DA g^{-1} whole tissue		San Diego, CA, USA	Busse *et al.* (2006)
Placopecten magellanicus	Sea scallop	*Pseudo-nitzschia* sp.	3400 μg DA g^{-1} in digestive gland		George, German, and Brown Banks, Canada	Bates (1997)
		Pseudo-nitzschia pseudo-delicatissima (= *P. calliantha*)	460 μg DA g^{-1} whole tissue	Max. 4180 μg DA g^{-1} DG	Bay of Fundy, New Brunswick, Canada	Haya *et al.* (1991)
Panopea abrupta	Geoduck clams	*Pseudo-nitzschia australis*	Below the regulatory limit		Puget Sound, WA, USA,	Bill *et al.* (2006)
Protothaca staminea	Littleneck clams	*Pseudo-nitzschia australis*	Below the regulatory limit		Puget Sound, WA, USA,	Bill *et al.* (2006)
Siliqua patula	Pacific razor clam	*Pseudo-nitzschia australis*	230 ppm DA in foot		Monterey Bay, California, USA	Wekell *et al.* (1994)
		Pseudo-nitzschia australis	154 μg DA g^{-1} in edible tissue		Monterey Bay, California, USA	Horner and Postel (1993)
		Pseudo-nitzschia pseudodelicatissima	308 μg DA g^{-1} whole tissue		Clatsop Spit, Oregon, USA	Trainer *et al.* (2000)
Tapes philippinarum	Manila clams	*Pseudo-nitzschia australis*	68 μg DA g^{-1} whole tissue		Penn Cove, WA, USA Oct 2005	Bill *et al.* (2006), Trainer *et al.* (2007)

Crustacean species	Common name	Toxin source	Toxin level	Notes	Location	Reference
Callianassa (=*Neotrypaea*) *californiensis*	Ghost shrimp	*Pseudo-nitzschia australis*	145 ppm DA whole tissue		Monterey Bay, CA, USA	Kvitek *et al.* (2008), Goldberg (2003)
Callinectes sapidus	Blue crab	*Pseudo-nitzschia australis*	1.1 ppm DA whole tissue		Washington and Oregon Pacific Coast Line	Altwein *et al.* (1995)
Cancer magister	Dungeness crab	*Pseudo-nitzschia* sp.	495 ppm DA g^{-1} hepatopancreas		Pacific Coast, USA	Villac *et al.* (1993)
		Pseudo-nitzschia australis	90 ppm DA whole tissues		Monterey Bay, California, USA	Wekell *et al.* (1994)

Table 4.4 (*Continued*)

Crustacean species	Common name	Toxin source	Toxin level	Notes	Location	Reference
Cancer pagurus	Rock crab	*Pseudo-nitzschia australis*	>30 ppm DA whole tissue		Washington and Oregon Pacific Coast Line	Altwein *et al.* (1995)
Emerita analoga	Sand crab	*Pseudo-nitzschia australis*	277.9 ppm DA whole tissue		Monterey Bay, CA, USA	Kvitek *et al.* (2008), Goldberg (2003)
		Pseudo-nitzschia sp. (*australis* and *multiseries*)	13.4±3.11 µg DA g^{-1}		Monterey Bay, CA, USA	Ferdin *et al.* (2002)
Euphausia pacifica	Krill	*Pseudo-nitzschia* sp.	44 µg DA g^{-1} tissue (3 µg DA equiv. $krill^{-1}$)	Digestive tract contents dominated by *Pseudo-nitzschia australis*	Offshore of Monterey Bay, CA, USA	Bargu *et al.* (2002)
Menippe adina	Stone crab	*Pseudo-nitzschia australis*	>30 ppm DA whole tissue		Washington and Oregon Pacific Coast Line	Altwein *et al.* (1995)
Palinurus elephas	Spiny lobster	*Pseudo-nitzschia australis*	24 ppm DA whole tissue		Washington and Oregon Pacific Coast Line	Altwein *et al.* (1995)
				1170 ppm DA in viscera	Channel Isalnds, California, USA	Lewitus *et al.* (2012)
Pagurus samuelis	Hermit crab	*Pseudo-nitzschia australis*	55.9 ppm DA whole tissue		Monterey Bay, CA, USA	Kvitek *et al.* (2008), Goldberg (2003)
Cephalopod species	**Common name**	**Toxin source**	**Toxin level**	**Notes**	**Location**	**Reference**
Loligo opalescens	Squid	*Pseudo-nitzschia* sp.	<0.5 µg DA g^{-1} tissue		Monterey Bay, CA, USA	Bargu *et al.* (2008)
Echinoderm species	**Common name**	**Toxin source**	**Toxin level**	**Notes**	**Location**	**Reference**
Dendraster excentricus	Sand dollar	*Pseudo-nitzschia australis*	15 ppm DA whole tissue		Monterey Bay, CA, USA	Kvitek *et al.* (2008), Goldberg (2003)

*) MU: Mouse units (1 MU: 0.18 µg STX).
**) Presumed; genus and species name not given by author.

mussels, *M. californiensis*, in California; Pacific razor clams, *S. patula*, in Oregon; and Pacific oysters, *C. gigas*, geoducks (clams) *P. abrupta*, littleneck clams *P. staminea*, and Manila clams *T. philippinarum* in Washington (Horner and Postel, 1993; Wekell *et al.*, 1994; Adams *et al.*, 2000; Trainer *et al.*, 2000, 2007; Bill *et al.*, 2006; Busse *et al.*, 2006). Most of the highest concentrations of DA observed in bivalves on the west coast can be attributed to *P. australis* and *P. multiseries*.

Indeed, Lewitus *et al.* (2012) reported that of the 12 species of *Pseudo-nitzschia* known to produce DA, ten of them have already been reported from west coast waters of the United States (Horner *et al.*, 1997; Anderson *et al.*, 2008). The subspecies *P. australis* Frenguelli and *P. multiseries* (Hasle) Hasle are most commonly associated with toxic events throughout this region, with *P. pseudodelicatissima* (Hasle) Hasle and *P. cuspidata* (Hasle) Hasle also implicated in toxic events in Washington waters (Adams *et al.*, 2000; Trainer *et al.*, 2009). Except for Pacific razor clams, *S. patula*, with recorded levels up to 308 μg DA g^{-1} whole tissue in Oregon (Trainer *et al.*, 2000), most of the other species tend to accumulate relatively lower levels of DA, often close to or even below the regulatory limit.

On the east coast, however, record levels of DA reached much higher concentrations, as high as 790 and 38 μg DA g^{-1} whole tissue for blue mussels, *M. edulis*, and softshell clams, *M. arenaria*, in Prince Edward Island, and 460 μg DA g^{-1} whole tissue corresponding to a maximum of 4180 μg DA g^{-1} in the digestive gland of sea scallops, *P. magellanicus*, in the Bay of Fundy (Bates *et al.*, 1989; Haya *et al.*, 1991; Table 4.4).

Blue mussels accumulated the highest levels of DA because they have a great DA accumulation efficiency, which is a balance between uptake and depuration processes. Their accumulation efficiency can be compared to species such as *P. magellanicus*, which is much higher than *C. virginica* (Mafra *et al.*, 2010). The latter authors also demonstrated that blue mussels tend to depurate relatively quickly compared to *C. virginica*, even though oysters accumulate less DA. The authors attributed this fast depuration partly to the dilution of toxin content due to body mass gain, as suggested previously in the literature, but mostly demonstrated a link with the species itself. Conversely, Pacific razor clams, *S. patula*, seem to retain DA for longer periods in their digestive gland (Horner *et al.*, 1993).

Moreover, the distribution of DA varies among bivalve tissues during both uptake and depuration phases. Most of the toxin burden is, however, located in the digestive gland, especially during the first hours of toxication, which even though representing only a small part of the whole body (up to 35%) can accumulate 70 to 80% of the total DA burden in *C. virginica*, around 80–90% in *M. edulis*, and up to 99% in *P. maximus* (Grimmelt *et al.*, 1990; Blanco *et al.*, 2002; Campbell *et al.*, 2003; Mafra *et al.*, 2010). The distribution of DA throughout the tissues can vary between species as the toxification goes on and with depuration, with a much larger distribution of the DA in all tissues in oysters than in mussels (Mafra *et al.*, 2010). The transfer of DA from visceral to nonvisceral mass has clearly been observed in *C. virginica* (Mafra *et al.*, 2010), but also in *C. gigas* (Guéguen *et al.*, 2008).

These large interspecific differences in DA uptake and depuration suggest that species-specific management of bivalve species contaminated by DA could be a valuable practice during HAB events.

4.6.2 Gastropods

While bivalve molluscs, both wild and cultured, are the most commonly monitored and studied with respect to harmful and toxic algae, marine gastropods are gaining in popularity as food items globally and are thus a serious threat to humans as vectors of algal toxins. Numerous snail species (see Hwang *et al.*, 2003, for summary) are known to contain tetrodotoxin, and, as these are not of algal origin, they are not covered in the present summary. There have been a multitude of studies on accumulation of algal toxins by commercially exploited suspension-feeding bivalve molluscs (see Bricelj and Shumway, 1998), but far fewer investigations have addressed toxin accumulation or trophic transfer to other organisms in the food web, especially gastropods. There have also been several laboratory studies in which investigators have intoxicated bivalve molluscs by feeding them toxic algae and then feeding the filter feeders to carnivorous gastropods, or have fed herbivorous snail species on benthic cysts (see Table 4.5). All of these studies demonstrated the ability of the gastropods to attain, concentrate, and in some instances biotransform algal toxins. Many species of gastropod show differential sequestration of toxins in different tissues, most especially in the digestive gland and the muscular foot. Gastropods, especially abalone, also tend to depurate slowly if at all, biotransform toxins, and remain toxic for extended periods of time (discussed further in this chapter; and see Table 4.5).

Bivalve molluscs concentrate the toxins as a result of filter feeding, and the algal species of concern are predominantly present in the water column. Gastropods, however, include filter feeders, carnivores, scavengers, and detritivores, and thus the range of potential algal species that

Table 4.5 Maximum STX concentrations, microalgal sources, and global PSP reports in gastropods.

Gastropod species	Common name	Toxin source	Toxin level	Notes	Location	Reference
Adelomelon ancilla	Maidservant volute	*Alexandrium catenella*	Toxic		Chile	Shumway (1995)
Adelomelon beckii	Beck's volute	*Alexandrium* spp.	2049 μg STX-eq/kg foot		Argentina	Turner *et al.* (2014)
Adelomedon brasiliana	Volute whelk	*Alexandrium tamarense*	28 MU g^{-1} whole		Argentina	Carreto *et al.* (1996)
Argobuccinum ranelliformes	Caracol del sur	*Alexandrium catenella*	1850 μg STX-eq. · 100 g^{-1}		Aysen, Chile	Zamorano *et al.* (2013)
			~140 μg STX-eq. · 100 g dig. $gl.^{-1}$	Most foot tissue had no toxin	Darwin Channel, southern Chile	Compagnon *et al.* (1998)
		Dinophysis sp.	DTX-1 10 μg/kg wet wt., YTX 5.6 μg/kg wet wt., PTX-2 3.6 μg/kg wet wt.		Aysen, Chile	Zamorano *et al.* (2013)
Argobuccinum ranelliformes	Whelk	*Dinophysis* sp.	66 μg OA-eq. kg^{-1} foot		Huichas Island, Chile	Garcia *et al.* (2015)
		Alexandrium catenella	3657 μg STX-eq. · 100 g		Huichas Island, Chile	Garcia *et al.* (2015)
Argobuccinum sp.	Whelk	*Alexandrium catenella*	Stomach 5629 μg STX-eq. · 100 g $tissue^{-1}$; muscle 92 μg STX-eq. · 100 g $tissue^{-1}$		Chile	Uribe (1995)
Babylonia areolata	Maculated ivory shell	*Alexandrium minutum*		Demonstrated direct transfer of toxins from bivalve, *Hiatula diphos*	Laboratory study	Chen and Chou (1998)
Babylonia japonica	Japanese Babylon	Tetrodotoxin from starfish and pufferfish	180 MU g^{-1} dig. gl.	Illness and death	Japan	Shiomi *et al.* (1984), Noguchi *et al.* (1981a, 1992), Narita *et al.* (1981)
Buccinum undatum	Waved whelk	*Alexandrium tamarense* (= *Gonyaulax tamarensis*)	608 μg STX-eq. · 100 g $tissue^{-1}$ whole body; 1600 μg STX-eq. · 100 g $tissue^{-1}$ dig. gl.	12 cases PSP, 4 fatalities	Quebec, Canada	Medcof (1972), Shumway (1995), Prakash *et al.* (1971)
			1096 MU g^{-1}	Fed toxic mussels in laboratory	Great Britain	Ingham *et al.* (1968)
		Gonyaulax excavata	Not given	Snail mortalities	Faroe Islands	Mortensen (1985)
Busycon spp.	Whelk	*Alexandrium tamarense*	50-500 MU 100 g^{-1}		Quebec, Canada	Shumway (1995)
Charonia lampas	Trumpet shell	*Alexandrium* spp.	3.1–17.5 MU g. dig. $gl.^{-1}$	Muscular tissues nontoxic	Galicia, Spain	Nagashima *et al.* (1996)

(*continued*)

Table 4.5 (*Continued*)

Gastropod species	Common name	Toxin source	Toxin level	Notes	Location	Reference
Charonia sauliae	Saul's trumpet	Tetrodotoxin from starfish and pufferfish	1950 MU g. dig. $gl.^{-1}$	Illness and death	Japan	Shiomi *et al.* (1984), Noguchi *et al.* (1981a, 1992), Narita *et al.* (1981)
Colus stimpsoni	Stimpson's colus	*Alexandrium tamarense*	Toxic			Shumway (1995)
Concholepas concholepas	Barnacle rock shell; Loco	*Alexandrium catenella*	Toxic		Chile	Shumway (1995)
			9,164 μg STX-eq. · 100 g dig. $gl.^{-1}$; 737 μg STX-eq. · 100 g^{-1} foot		Darwin Channel, southern Chile	Compagnon *et al.* (1998)
		Dinophysis sp.	DTX−1 18.67 μg/kg wet wt., YTX 12.8 μg/kg wet wt., AZA−1 6.7 μg/kg wet wt.		Aysen, Chile	Zamorano *et al.* (2013)
			81 μg STX-eq. · 100 g $tissue^{-1}$ foot		Huichas Island, Chile	Garcia *et al.* (2015)
			400 μg OA-eq. · kg^{-1} foot; 10.7 μg OA-eq. · kg^{-1} dig. gl.		Huichas Island, Chile	Garcia *et al.* (2015)
Crepidula fornicata	Slipper limpet	*Alexandrium tamarense*	46-58 μg STX-eq. · 100 g $tissue^{-1}$			Shumway (1995)
Euspira heros (=*Lunatia heros, Polinices heros*)	Northern moon snail	*Alexandrium tamarense*	2922 μg STX-eq. · 100 g $tissue^{-1}$			Shumway (1995)
			247 μg STX-eq. · 100 g $tissue^{-1}$		Gulf of St. Lawrence, Canada	Worms *et al.* (1993)
			1450 μg STX-eq. · 100 g $tissue^{-1}$	2 cases of PSP; victims also ate *Littorina littorea*	Massachusetts, USA	Tufts *et al.* (1975), Tufts (1979)
Haliotis tuberculata	Green abalone	*Gymnodinium catenatum*	467 μg STX-eq. · 100 g $muscle^{-1}$		Spain	Bravo *et al.* (1996)
		Gymnodinium catenatum (?)	0.78 ng/g meat	All browsers; tests for DSP negative	Spain	Martinez *et al.* (1993)
Lambis lambis	Spider conch	*Pyrodinium bahamense*	ND – 175 MU 100 g^{-1} whole	Several PSP cases	Sabah, Malaysia	Sang and Ming (1994), Ming *et al.* (1989)
Littorina littorea	Common periwinkle	*Alexandrium minutum*	5.3 ng STX g dig. gl^{-1} wet wt; 1.3 ng STX g dig. $gl.^{-1}$ wet wt		Laboratory study Natural bloom	Neves *et al.* (2015)

Table 4.5 (*Continued*)

Gastropod species	Common name	Toxin source	Toxin level	Notes	Location	Reference
		Probably *Alexandrium tamarense*	72 µg STX-eq. · 100 g tissue^{-1}	2 cases of PSP; victims also ate *Polinices heros*	Massachusetts, USA	Tufts *et al.* (1975), Tufts (1979)
		Alexandrium tamarense	37 µg STX-eq. · 100 g tissue^{-1}	Whole snails	New Brunswick, Canada	Matter (1993)
Littorina sitkana	Sitka periwinkle	*Alexandrium tamarense*	Trace whole animal		Washington, USA	Shumway (1995)
		Gonyaulax tamarensis	Trace	Whole snails	Washington, USA	MacDonald (1970)
Littorina sp.		*Gymnodinium catenatum* (?)	None detected	All browsers; tests for DSP negative	Spain	Martinez *et al.* (1993)
			3337 µg STX-eq. · 100 g tissue^{-1}	Illnesses and deaths	Gulf of Maine, USA	Shumway (1995), White *et al.* (1993)
Nassarius festivus		*Alexandrium tamarense*	6.43 µg STX-eq. · 100 g tissue^{-1} wet wt		Laboratory Study	Choi *et al.* (2006)
Nassarius fossatus	Channeled basket snail	*Pseudo-nitzschia australis*	Domoic acid (DA) 673.9 ppm DA in whole tissue		Monterey Bay, California	Goldberg (2003)
Nassarius siguijorensis	Nassa	Unknown toxin source	370 MU 100 g^{-1}		Daya Bay, Guangdong Province	Li *et al.* (1999)
Nassarius succinctus	Nassa	Unknown toxin source		68 cases of PSP, March–Aug 1979; 1 fatality and 7 hospitalized	Zhejiang Province, China	Choi *et al.* (2006), Li and Chen (1981)
Nassarius sp.	Nassa mud snail (dog whelk)	*Alexandrium catenella*	9 µg STX-eq. · 100 g tissue^{-1}	Scavengers	Washington, USA	Shumway (1995), Beitler (1992)
		Prorocentrum minimum	1820–1890 MU g^{-1}	Many human fatalities	Zhengjiand and Fuzlang, China	Chen and Gu (1993)
Nassarius spp.		Unknown toxin source		50 PSP cases, 3 fatalities, April–May 2002	Fujian Province, China	Choi *et al.* (2006)
		Unknown toxin source		55 PSP cases, 1 fatality; summer 2004	Yin Chuan City, China	Choi *et al.* (2006)
		Unknown toxin source	107,413 MU 100 g^{-1}		Zhoushan Islands, China	Choi *et al.* (2006)
Natica sp.**	"Tekuyong"	*Pyrodinium bahamense*	71–876 MU 100 g^{-1}		Borneo	Jaafar and Subramaniam (1984), Jaafar *et al.* (1989)
Natica lineata	Lined moon shell	Unknown toxin source	PSP toxins		Taiwan	Liao and Hwang (2000)
		Tetrodotoxin	12 MU g dig. gl.$^{-1}$		Taiwan	Hwang *et al.* (1990)
		Anhydrotetrodotoxin	720 MU g^{-1} muscle		Taiwan	Hwang *et al.* (1990)

(*continued*)

Table 4.5 (*Continued*)

Gastropod species	Common name	Toxin source	Toxin level	Notes	Location	Reference
Natica vitellus	Calf moon shell	Unknown toxin source				
Neptunea arthritica	Arthritic Neptune	Unknown toxin source	GTX 1–4, neoSTX, STX		Sanriku coast, Japan	Sato *et al.* (1993)
Neptunea decemcostata	Ten-ridged whelk	*Alexandrium tamarense*	Raw ~3000–4000, steamed 1060 µg STX-eq. · 100 g tissue^{-1}			Shumway (1995)
Neptunea spp.		*Alexandrium catenella*	200–250 MU 100 g^{-1} whole individuals	Whole individuals	Alaska, USA	Shumway (1995)
		Dinophysis sp.	OA meat 0.8, dig. gl. 3.6 ng/g; DTX1 meat 3.2, dig. gl. 5 ng/g; YTX meat 0.9; dig. gl. 13.7 ng/g		Tongyeong market, Korea	Lee *et al.* (2012)
Niotha clathrata	Basket shell	Unknown toxin source	PSP, GTX-3			Liao and Hwang (2000), Hwang *et al.* (1994)
		Tetrodotoxin	35 MU g^{-1}	Edible parts	Japan	Jeon *et al.* (1984)
Olivella sp.		*Karenia brevis*	2.6 µg PbTx-3 eq/g wet wt.		Sarasota Bay, Florida, USA	Bricelj *et al.* (2012)
Olivella biplicata	Olive snail	*Pseudo-nitzschia australis*	Domoic acid 3.1 ppm DA in whole tissue		Monterey Bay, California	Goldberg (2003)
Oliva vidua fulminans	Olive	*Pyrodinium bahamense*	2525 MU 100 g^{-1} whole	5 human fatalities; 8 cases of PSP	Malaysia	Sang and Ming (1994), Ming *et al.* (1989), Kan *et al.* (1986)
Patella sp.		*Gymnodinium catenatum* (?)	None detected	All browsers; tests for DSP negative	Spain	Martinez *et al.* (1993)
Polinices lewisii	Lewis moon snail	*Alexandrium catenella*	176–600 µg STX-eq. · 100 g tissue^{-1}		British Columbia, Canada	Quayle (1971), Matter (1993)
Polinices duplicata	Moonsnail	*Gonyaulax monilata*	Not given	Snail mortalities	Texas, USA	Wardle *et al.* (1974)
Rapana rapiformis	Turnip shell; cantaloupe	Tetrodotoxin	140 MU g dig. gl.$^{-1}$		Taiwan	Hwang *et al.* (1991)
Rapana venosa	Veined rapa whelk	*Alexandrium tamarense*	11.4 MU g^{-1} viscera		Hiroshima Bay, Japan	Ito *et al.* (2004)
		Dinophysis sp.	OA meat 2.9, dig. gl. 21.5 ng/g; DTX1 meat 1.8; dig. gl. 8.4 ng/g; YTX meat 0.6, dig. gl. 0.2 ng/g		Tongyeong market, Korea	Lee *et al.* (2012)
Rapana venosa venosa		Tetrodotoxin	13 MU g dig. gl.$^{-1}$		Taiwan	Hwang *et al.* (1991)

Table 4.5 (*Continued*)

Gastropod species	Common name	Toxin source	Toxin level	Notes	Location	Reference
Searlesia dira	Snail	*Alexandrium* spp.	50 µg STX-eq/ 100 g		Mystery Bay, WA, USA	Wekell *et al.* (1996)
Tectus fenestratus	Fenestrate top shell	Unknown toxin source	18.7 µg STX-eq. · 100 g tissue^{-1}		Northwest Australia	Negri and Llewellyn (1998)
Tectus nilotica maxima	Commercial top shell	Unknown toxin source	5.0 MU g^{-1} whole		Ishigaki Island, Japan	Oshima *et al.* (1984)
		Jania sp.	5.0 MU g^{-1} whole		Japan	Yasumoto and Kotaki (1977, 1983), Kotaki *et al.* (1981, 1983), Kanno *et al.* (1976)
Tectus pyramis	Pryam top shell	Unknown toxin source	19 MU g^{-1} whole		Ishigaki Island, Japan	Oshima *et al.* (1984)
		Jania sp.	19 MU g^{-1} whole		Japan	Yasumoto and Kotaki (1977, 1983), Kotaki *et al.* (1981, 1983), Kanno *et al.* (1976)
Thais sp.	Oyster drill	*Alexandrium catenella*	23 µg STX-eq. · 100 g tissue^{-1} (GTX-2 and GTX-3 only)		Washington, USA	Shumway (1995)
Thais lamellosa	Frilled dog winkle	*Alexandrium catenella*	Whole animal positive		Washington, USA	MacDonald (1970)
		Alexandrium sp.	72 µg STX-eq/ 100 g		Puget Sound basin, USA	Wekell *et al.* (1996)
Thais lapillus	Purpura	*Alexandrium tamarense* (= *Gonyaulax tamarensis*)	34 µg STX-eq. · 100 g tissue^{-1}		Maine, USA	Goggins (1961)
Thais lima	Oyster drill	*Alexandrium catenella*	Whole animal 180 µg STX-eq. · 100 g tissue^{-1}		Washington, USA	Shumway (1995)
Thais haemastoma	Red-mouthed rock shell; Florida dog winkle	*Gonyaulax monilata*	Not given	Snail mortalities	Texas, USA	Wardle *et al.* (1974)
Trophon sp.	Trophon	*Alexandrium catenella*	Toxic		Chile	Shumway (1995)
Turbo argyrostoma	Green turbo	Unknown toxin source	20 MU g^{-1} whole		Ishigaki Island, Japan	Oshima *et al.* (1984)
		Jania sp.	20 MU g^{-1} whole	All grazers	Japan	Yasumoto and Kotaki (1977, 1983), Kotaki *et al.* (1981, 1983), Kanno *et al.* (1976)
Turbo (=*Batillus cornutus*) *cornutus*			OA meat 0.6, dig. gl. 0.1 ng/g; DTX1 meat 0.6, dig. gl. 1.0 ng/g; YTX meat 0.3, dig. gl. 0.7 ng/g		Tongyeong market, Korea	Lee *et al.* (2012)

(*continued*)

Table 4.5 (*Continued*)

Gastropod species	Common name	Toxin source	Toxin level	Notes	Location	Reference
Turbo marmoratus	Great green turban	*Jania* sp.	4.2 MU g^{-1} whole	All grazers	Japan	Yasumoto and Kotaki (1977, 1983), Kotaki *et al.* (1981, 1983) Kanno *et al.* (1976)
Tutufa lissostoma	Frog shell		700 MU g dig. $gl.^{-1}$	Toxic year-round	Japan	Yasumoto *et al.* (1981), Noguchi *et al.* (1984)
Zeuxis siquijorensis	Burned nassa	Tetrodotoxin	3.4 MU g^{-1}	Edible parts	Japan	Narita *et al.* (1984)
*Zidona angulata**	Volute	*Alexandrium tamarense*	210 MU g^{-1} viscera; 25 MU g^{-1} foot; 17 MU g^{-1} mucus	Mild case of PSP		Carreto *et al.* (1996)
		Alexandrium excavatum	Not given	1 mild case of PSP	Argentina	Elbusto *et al.* (1991)
Zidona dufresnei	Angular volute	*Alexandrium* spp.	22,468 µg STX-eq/kg viscera; 3365 µg STX-eq/kg foot	Several illnesses, 1 death	Argentina	Turner *et al.* (2014)

*) MU: Mouse units (1 MU: 0.18 µg STX).
**) Presumed; genus and species name not given by author.
Source: Updated from Shumway *et al.* (1995) and Deeds *et al.* (2008).

these animals may accumulate is expanded to include the benthic species in addition to toxins that have already been accumulated by their filter-feeding prey (e.g., bivalve molluscs). The increased use of nontraditional species, including gastropods, as a human food source means that many species not previously included in algal toxin monitoring programs need to be considered. The following summary (see also Table 4.5) is not meant to be an exhaustive examination of algal toxins in gastropods, but rather to provide background information, and to demonstrate the clear need to include gastropods in routine monitoring programs. Much new information regarding gastropods has been published post-2000, again demonstrating the new focus on this group.

The highly variable nature of toxicity in gastropods makes them a threat to human health and difficult to monitor. While toxins tend to be concentrated in the digestive glands and viscera of most species of gastropods, this is not always the case (Shumway, 1995 – *Lunatia* and abalone – Natashima *et al.*, 1995; Turner *et al.*, 2014; and others). Indeed, in a most recent study, Turner *et al.* (2014) reported that 85% of more than 40,000 samples of gastropod muscular foot tested were toxic. As with bivalve molluscs, cooking does not eliminate the toxins or the risks associated with consuming carnivorous gastropods.

Persson *et al.* (2008) demonstrated that the eastern mud snail, *Ilyanassa obsoleta*, was attracted to cysts of the dinoflagellate *Scrippsiella lachrymose*, and, while no toxin transfer was demonstrated in this study, their finding has implications for potential toxicity of this scavenging snail and others in areas where toxic dinoflagellates produce cysts (see also Table 4.5 for prior studies including Nassarid species).

Wang and coworkers (Wang *et al.*, 2004; Wang and Wu, 2006) analyzed a number of species purchased at the local Shanghai fish markets. Only one of nine samples of *Neverita didyma* contained PSP toxin (42 µg/100 g), and nine species tested positive for DSP toxins; none were above the regulatory limit (*Ampullarum crossean*, *Babylonia lutosa*, *B. lutosa*, *Mactra veneriformis*, *Meretrix meretrix*, *Papana bezoar, Tegillarca granosa*, and *Sanguinolaria olivacea*).

Zamorano *et al.* (2013) evaluated *Concholepas concholepas* and *Argobuccinum ranelliformes,* but homogenized the entire animal, so it is not possible to determine whether or not the PSP toxin was

confined to the digestive gland or had moved into the muscle tissue. In *Buccinum undatum* from the Gulf of Maine (United States), PST were concentrated in the digestive gland and none were measured in the "other" tissue. It is highly likely that the same partitioning of toxins is present in *Argobuccinum* and that the PST levels are even higher than the levels reported by Zamorano *et al.* (see Shumway, 1995). The digestive glands were separated from the animals and analyzed for other toxins (see Table 4.5), and in the cases of both *C. concholepas* and *A. ranelliforme*, the toxin profiles were indicative of the prey items available and also demonstrated biotransformation of accumulated toxins by both species of snail.

Lee *et al.* (2012) investigated the presence of lipophilic toxins in three species of Korean gastropods, two carnivores (*Neptunea cumingii* and *Rapana venosa*) and one herbivore (*Batillus cornutus*), and found OA, dinophysistoxin-1, and YTX in the meat and digestive glands in all species. No PTX2 was found. Toxin levels were below the regulatory level and showed a seasonal pattern of occurrence, being most prevalent in the spring and summer. While present, levels of toxin in the herbivorous species, *B. cornutus*, were negligible.

Abalone, herbivorous grazers, are among the most expensive shellfish on the world seafood market and are cultured globally, and have thus received considerable attention with regard to their interactions with harmful and toxic algal species and potential for trophic transfer. Of particular concern to consumers, growers, industry, and management is that the toxins in this important species are concentrated in the primary edible portion, the foot. The first reported toxin in abalone (ormers), *Haliotis tuberculata*, was in animals collected in 1991 from the Galician coast of Spain. PST were detected in the foot muscle at levels reaching 0.78 µg STX eq/100 g of meat (Martinez *et al.*, 1993). While no definitive evidence could be ascertained, it was surmised that the toxins originated from prior blooms of *G. catenatum* as toxin profiles (measured by high-performance liquid chromatography [HPLC]) were similar in mussels collected during an actual bloom and in the abalone. In 1995, tissues from abalone imported from Vigo, Spain, to Japan in 1994 were analyzed (Nagashima *et al.*, 1995); again, toxins were shown to be concentrated in the foot tissue at levels, and there was high variability between individuals (range: 33–106 MU/g; see Table 4.5). Interestingly, there was little toxin in the viscera (4–16 MU/g). In two later studies (Bravo *et al.*, 1996, 1999), PST in *H. tuberculata* were again found (maximum: 443 µg STX eq 100 g meat); however, the source still was not confirmed. The geographic distribution of *G. catenatum* was not consistent with the distribution of the toxic ormers, and it was suggested that *A. minutum* might play a role in the toxicity. Other possible sources of toxin were investigated (macroalgae), but there was no direct evidence for toxin transfer. Interestingly (Bravo *et al.*, 1999), it was found that the highest values for toxin accumulation were in the epithelium of the foot (maximum: 10,500 µg toxin/100 g) versus only 26 µg toxin/100 g of muscle. It was also shown in these studies that toxins were retained for >30 months with little evidence of depuration.

In South Africa, the abalone *Haliotis midae* was found to contain PSP toxins (maximum: 1609 443 µg STX eq 100 g whole animal; Pitcher *et al.*, 2001) coincident with blooms of *Alexandrium catenella*, although direct transfer was not demonstrated. Interestingly, it was also found that toxins were disproportionately concentrated in the epipodal fringe. Their study also demonstrated deleterious impacts of toxicity on spawning and larval survival, the inability of the abalone to detoxify or depurate accumulated toxins (> 7 months), high individual variability (77–383 µg STX eq 100 g in animals collected from the same basket), and paralyzed abalone that could no longer attach to the substrata. The vast differences between the toxin profiles of the suspect algae and the abalone tissue suggest, as with other abalone species, that they probably have a high capacity for toxin transformation. In a laboratory study, Etheridge *et al.* (2004) fed abalone with artificial diets or with kelp (their *in situ* food source) to assess depuration and toxin transformation. Abalone fed artificial feed depurated at a low rate of 6.3 mg STX eq 100 g tissue day, while animals fed kelp or starved did not depurate at all.

The most recent reports on PST in abalone, *Haliotis laevigata*, are from Australia (Dowsett *et al.*, 2011; Harwood *et al.*, 2014). Dowsett *et al.* (2011) fed abalone with commercial seaweed-based food pellets supplemented with toxic *A. minutum*. Behavior was not affected, and very low toxin accumulation of up to 1.6 ug STX eq $100\,g^{-1}$ in the foot tissue was noted. Scrubbing reduced the toxin levels in the food to 0.48 ug STX eq $100\,g^{-1}$, and the authors suggest that there may be a low risk of PSP poisoning to humans from the species when exposed to a bloom of *A. minutum*. It is likely that, since the animals did not accumulate a significant amount of toxin during these experiments and were also naturally "depurating" during the entire 50 days of "uptake," the reported

depuration rates are inaccurate and the results should be treated as preliminary, especially given results from other species of abalone that show no depuration over extended time periods. The natural source of toxin was not identified. In a subsequent study in Tasmania, Harwood *et al.* (2014) confirmed the presence of PST in wild-caught *Haliotis rubra* following a bloom of *G. catenatum*.

Silva *et al.* (2013) carried out a survey in Portugal to identify new invertebrate vectors for PST, spirolides, and OA. They collected numerous species of edible gastropods (six grazers and one carnivore) and demonstrated varying presence and levels of toxins among the species. They identified PST in *Monodonta* sp., *Gibbula umbilicalis*, *Nucella lapillus*, and *Aplysia depilans*; spirolide toxins in *G. umbilicalis*, *N. lapillus*, and *Monodonta* sp.; and OA in *N. lapillus*. Most of their paper deals with methods of detection, and, in most instances, it was difficult to ascertain actual toxin levels measured. The carnivorous gastropod *N. lapillus* contained OA at a level of 429.4 ng/g fresh weight, and it was suggested that the toxins were obtained after consuming toxic mussels (*M. galloprovincialis*). The only record of OA and YTX in abalone is the work by Kim *et al.* (2012). They identified the lipophilic toxins in the digestive gland but no toxins in the foot muscle. The highest levels recorded were 4.7 ng/g OA and 1.3 ng/g YTX, mainly during spring and winter. The toxin levels were never above the European regulatory limit of 0.16 mg OA equivalents per kilogram of meat, and while they suggested that a species of *Dinophysis* known to occur in the area might be responsible, no direct link was demonstrated. Removal of the contaminated epithelium and gut might render abalone safe for human consumption and thus marketable, but this would be a most laborious and time-consuming process, and commercial application of any such process would be driven by market value. The increased incidence of algal toxins in gastropods coupled with gastropods' increased use as a human food source, prior records of toxicity in an array of gastropod species, and the renewed awareness and study of algal toxins and gastropods clearly demonstrate that non-filter-feeding molluscs need to be included in routine monitoring programs.

4.6.3 Crustaceans

Relatively limited data are available on HAB toxin contamination in crustaceans. Concentrations of up to 468 µg STX eq 100 g^{-1} tissue were measured in the sand crab, *Emerita analoga*, in Pescadero Beach, California (Bretz *et al.*, 2002; Table 4.1). Following blooms of *Alexandrium* sp., PST were also measured in some other crab species on the west coast of the United States, such as the brown rock crab, *Cancer antennarius*, and the red rock crab, *C. productus*, with respectively up to 49.3 and 23.8 µg STX eq 100 g^{-1} hepatopancreas (Jester *et al.*, 2009). The latter species was also reported with a maximum concentration of 285 µg STX eq 100 g^{-1} viscera in Washington State, United States (Jonas-Davis and Liston, 1985). Lewitus *et al.* (2012) also listed several crab species reported to accumulate PST in Alaska, such as the tanner crab, *Chionoecetes bairdi*, the snow crab, *C. opilio*, the hair crab, *Erimacrus isenbeckii*, the red king crab, *Paralithodes camtschaticus*, and the Dungeness crab, *Cancer magister*, the latter with up to 1094 µg STX eq 100 g^{-1} tissue in Kodiak Island, based on the Alaska Department of Health and Human Services–Epidemiology Section (ADHHS-ES) database, 1973–2008. A few other crabs such as the grooved mussel crab, *Fabia subquadrata*, the hermit crab, *Pagurus* sp., and the bay shore crab, *Hemigraspus oregonensis*, were also recorded with very low levels of PST of ~30 µg STX eq 100 g^{-1} whole tissue on the west coast of the United States (Jonas-Davies and Liston, 1985; reviewed in Shumway, 1985). The Jonah crab, *Cancer borealis*, was also reported in Maine with up to 56 µg STX eq 100 g^{-1} whole tissue (Shumway, 1985). PST were also reported in hepatopancreas of two other crustacean species, the American lobster, *Homarus americanus*, and the kelp crab, *Pugettia producta*, with 1654 and 1710 µg STX eq 100 g^{-1} hepatopancreas, respectively (Shumway, 1985). Up to 146 µg STX eq 100 g^{-1} was reported in the eggs of the latter species, suggesting a potential transfer to the offspring.

Crustaceans, mainly on the west coast of North America, have also been observed to accumulate DA above the regulatory limit. This is especially the case of Dungeness crabs, which were shown to accumulate 485 µg DA g^{-1} in the hepatopancreas (Villac *et al.*, 1993; Table 4.4), and sand crabs were also recorded to accumulate up to 277.9 ppm DA in whole tissue (Goldberg, 2003; Kvitek *et al.*, 2008) in Monterey Bay. Goldberg (2003) and Kvitek *et al.* (2008) also reported record concentrations of DA in the hermit crab (55 ppm) and the ghost shrimp, *Callianassa* (=*Neotrypaea*) *californiensis*, (145 ppm) in Monterey Bay, following a bloom of *P. australis*.

Between 1991 and 1993, a large monitoring on the Washington and Oregon Pacific coast line also

recorded the presence of DA in some other crustacean species (Altwein *et al.*, 1995). Rock crabs, *C. pagurus*, and stone crabs, *Menippe adina*, were sampled with more than 30 ppm DA in whole tissue following a bloom of *P. australis* as well. In the meantime, spiny lobsters, *Palinurus elephas*, were measured with up to 24 ppm DA in whole tissue, and blue crabs, *Callinectes sapidus*, with up to 1.1 ppm DA in whole tissue. More recently, *P. australis* was observed in the digestive tract content of krill, *Euphausia pacifica*, which was shown to accumulate up to 44 µg DA g^{-1} tissue, corresponding to 3 µg DA eq $krill^{-1}$ offshore of Monterey Bay (Bargu *et al.*, 2002).

Crustaceans, such as the intertidal sand crab, *E. analoga*, have been identified as new potential indicators of the presence of DA in the surrounding water (Ferdin *et al.*, 2002), as their toxicity coincided exactly with a *Pseudo-nitzschia* bloom, whereas mussels also simultaneously present did not show detectable levels of DA. The authors also noted the easy accessibility of this intertidal species. Another study (Bretz *et al.*, 2002) further assessed the potential for this same species to be also considered an indicator of the presence of PST.

4.7 Impacts on Shellfish

The toxins that affect human health can also have an impact on marine organisms (reviewed in Shumway, 1990; Landsberg, 2002). The first organisms affected by HAB are filter-feeding shellfish, mainly bivalves, which form the bulk of the primary consumers of microalgae. Exploited species, such as clams, cockles, mussels, oysters, and scallops, are most at risk. These blooms clearly represent a stress for species-consuming toxic phytoplankton, with a potential impact on fishery and aquaculture industries and related activities. Organisms that survive ingestion of such toxins can be consumed by higher trophic levels of the food chain. Toxins can thus be transferred from one species to another along food chains (biomagnification). Each trophic level may potentially be affected by the toxin, causing biological disorders that may in some cases be lethal (e.g., mammals and birds contaminated with DA). In addition, other bioactive compounds and allelochemicals produced by HAB species can be toxic to some marine animals and plants, without presenting any known or demonstrated threat to humans (reviewed in Shumway, 1990; Landsberg, 2002).

HAB have acute, chronic, and sublethal effects on marine organisms (reviewed in Shumway, 1990; Landsberg, 2002). Sessile organisms are the most affected by these toxic microalgae. The acute nature of the exposure is linked to the rapid mode of action of the toxins and the high concentration of toxin-producing cells. These acute exposures induce several physiological and cellular responses, as well as injuries and diseases that can cause the death of organisms. There is very little information about the long-term effects of HAB toxins bioaccumulated in marine organisms. Moreover, the available information is limited to the most commonly exploited species.

HAB can alter the physiology of certain species or important communities and the food chain they support, resulting in changes in marine ecosystems (Harvell *et al.*, 1999). It is also interesting to note that, faced with these toxic episodes, some bivalves have developed strategies and defense mechanisms to survive (Shumway and Cucci, 1987; Gainey and Shumway, 1988; Ward and Shumway, 2004), or modification of their genetic material (resistance to phycotoxins; Bricelj *et al.*, 2005). Behavioral changes, such as burrowing, reduction of filtration and respiration, valve closure, shell gap modulation, escape response, and production of pseudofeces and mucus, have been reported mainly in bivalves but also in some gastropod species in the presence of HAB (Shumway and Cucci, 1987; Gainey and Shumway, 1988; Bricelj *et al.*, 1996; Lassus *et al.*, 1999; Basti *et al.*, 2009, 2011a; Haberkorn *et al.*, 2010a, 2010b; Hégaret *et al.*, 2012; Neves *et al.*, 2015a). HAB can have deleterious effects at the tissue, cellular, and molecular levels. Indeed, toxic microalgae can cause inflammation, necrosis, and atrophy of most tissues, including gills, labial palps, muscles, mantle, stomach, intestines, and digestive gland of bivalves and gastropods (Wikfors and Smolovitz, 1995; Basti *et al.*, 2011a, 2015a; Neves *et al.*, 2015b). Histological observations also highlight heavy hemocytic infiltration in most tissues of shellfish exposed to toxic microalgae (Hégaret *et al.*, 2009, 2010; Basti *et al.*, 2011a, 2015b). These hemocytic infiltrations are often associated with modulations of the immune system. In particular, the cellular immune system shows modification of the concentration and morphology of the circulating hemocytes as well as the percentage of viable cells, and in general a decrease in phagocytosis capacity and an increase in apoptosis (Hégaret and Wikfors, 2005a, 2005b; Hégaret *et al.*, 2007a, 2007b, 2011; Galimany *et al.*, 2008a, 2008b; Basti *et al.*, 2011a). Changes in hemocytes clearly reflect the deleterious effects

of toxic microalgae on shellfish physiology. Modifications of immune or antioxidant systems can also be observed at the molecular level (Manfrin *et al.*, 2012; Mello *et al.*, 2012; Nuñez-Acuña *et al.*, 2013; Lassudrie *et al.*, 2014; Astuya *et al.*, 2015; Fabioux *et al.*, 2015). Other physiological alterations may be caused by exposure to HAB, including modifications of the physiological energetics and modulation of the neuroenzymatic activity in bivalves (Basti *et al.*, 2016a).

HAB can also impact the reproduction and recruitment of shellfish. Indeed, several recruitment failures were observed in the field following HAB, and even if it is not possible at present to prove that these blooms are directly responsible for these recruitment failures, this hypothesis was often formulated (Erard-Le Denn *et al.*, 1990; Summerson and Peterson, 1991; Matsuyama, 2012). Other studies also show an effect of toxic microalgae on gametes and the embryonic and larval development of several bivalve species (Springer *et al.*, 2002; Yan *et al.*, 2003; Leverone *et al.*, 2006; Padilla *et al.*, 2006; Shumway *et al.*, 2006; Basti *et al.*, 2011b, 2013, 2015b; Rolton *et al.*, 2014, 2015, 2016), such as a reduction in the hatching rate of fertilized eggs or larval survivorship of the scallop *Chlamys farreri* during exposure to *A. tamarense* (Yan *et al.*, 2001), inhibition of the first and second cleavage in Japanese pearl oysters during exposure to *A. catenella* and *A. affine* (Basti *et al.*, 2015b), and decreased motility and increased mortality of larvae during exposure to ichthyotoxic raphidophytes (Basti *et al.*, 2016b). In addition, experimental exposure of adult *C. gigas* to *A. minutum* caused a reduction in motility and ATP content, and a change in the DNA content of the spermatozoa of Pacific oyster, *C. gigas* (Haberkorn *et al.*, 2010b). Finally, more recent works have highlighted the deleterious effects of toxic microalgae, including *H. circularisquama* and species of genus *Alexandrium* on the quality of gametes, both sperm and oocytes, and on the fertilization success; results that show that a HAB can have major impacts on the reproduction and recruitment of shellfish (Basti *et al.*, 2013; Le Goïc *et al.*, 2013, 2014).

The physiological state of shellfish can, in return, modify their responses to HAB exposure. For instance, recent studies showed that the state of ploidy of Pacific oyster affects the accumulation of PST, and that it is related to the reproductive stage of the oysters (Haberkorn *et al.*, 2010a; Guéguen *et al.*, 2012). Although the accumulation and depuration of PSP toxins have been studied in diploid oysters, few studies have considered triploid oysters, which are commonly produced for their enhanced growth enabling their consumption throughout the year. Differential detoxification of PSP toxins in diploid and triploid oysters could be an interesting line of research for aquaculture farms and related businesses.

Several studies have investigated the effects of HAB exposures on the susceptibility of bivalves to diseases and predators. Both shellfish diseases and HAB have been expanding at a global scale. Their interactive effect can cause changes in marine ecosystems, and the deleterious effects of HAB on the physiology of shellfish can facilitate the spread of infectious diseases in commercially important species of shellfish (Harvell *et al.*, 1999). Recent studies have shown that exposure of many bivalve species to harmful algal blooms can change the host–pathogen interaction (Hégaret *et al.*, 2007a, 2009, 2012; da Silva *et al.*, 2008; Lassudrie *et al.*, 2014, 2015a, 2015b, 2016) or host–predator interaction (Hégaret *et al.*, 2012; Borcier *et al.*, 2017). The study of the influence of toxic microalgae blooms on the physiological response of bivalves therefore seems essential to understand how the toxicity of microalgae can (1) affect the response of bivalves to infection by pathogens and (2) cause physiological distress reducing the defense mechanisms of bivalves and eventually leading to reduced fitness or death of the animal when associated with an infectious disease.

4.8 Conclusions and Perspectives

Relationships between the toxicity of marine bivalves for human consumption and microalgal bloom-producing toxins are sometimes particularly difficult to establish. Recent years have witnessed an increase in the number of cases where bivalves tested positive for conventional shellfish poisonings with the mouse bioassay without identification of a clear causative HAB species, thus making it difficult to pinpoint the toxin related to the positive MBA results and highlighting the need to use more sensitive technologies of toxin detection. For instance, oyster farmers in the Arcachon Basin, France, are regularly confronted with this situation. Scallops in the Bay of Brest are also often banned from marketing because of ASP toxicity; however, no blooms of *Pseudo-nitzschia* spp. have been linked to the toxicity. Moreover, these animals tend to remain contaminated for long periods

of time, during which no blooms of *Pseudo-nitzschia* spp. can be detected (James *et al.*, 2003; Bogan *et al.*, 2006, 2007). In contrast, some intense episodes of HAB, notably *Pseudo-nitzschia* spp. blooms, do not result in toxicity of bivalves. Therefore, prediction of shellfish toxicity based on the prediction of HAB events relying on conventional oceanographic and ecological approaches seems insufficient. Beyond understanding the initiation and dynamics of HAB, it is essential to better address: (1) the abiotic factors but also the biotic factors (e.g., bacteria, competition with other species of unicellular photosynthetic eukaryotes) conditioning toxin production by the harmful algae; (2) the impacts of other biological entities such as the primary consumers (micro-zooplankton and other benthic filter feeders) that can alter the trophic availability of harmful algae and divert and/or transfer their toxic content to higher levels of the food web; and (3) the relationship between the effects of the HAB on the physiology of the shellfish and the processes of assimilation and detoxification of the toxins in shellfish.

In addition to the discrepancy between shellfish toxicity and HAB events, a distinction should be made between direct and indirect exposures on the one hand and acute and chronic exposure on the other hand. Shellfish are exposed to HAB endotoxins during the ingestion/digestion process either directly from the seawater in the case of filter feeders or indirectly through predation. Nonetheless, the endotoxins can also be released directly into the seawater, either following cell lysis under the action of waves, winds, and/or turbulence or during bloom die-offs, and be readily available in dissolved fractions. In addition, HAB produce exotoxins, such as allelopathic compounds that may impact the sympatric phytoplankton community and grazers, but also hemolytic and cytotoxic compounds that can be more deleterious to shellfish than the known toxins associated with human poisonings (Arzul *et al.*, 1999; Weissbach *et al.*, 2010; Leão *et al.*, 2012). Research on the effects of dissolved toxins and of secondary exotoxic metabolites from HAB should be developed to better address the physiological state of shellfish during HAB events, and consequently to better predict the implications for the toxication/detoxication process.

To the established list of HAB species, new species of harmful and toxic microalgae are being described annually, both benthic and pelagic. Their toxin content in shellfish and/or their potential effects on shellfish have not been investigated. This is particularly the case of emerging toxins and AZA. In addition, the occurrence of multiple toxins, reported for example in the United States where shellfish tested positive for both DST and AZA, raises concerns about the impacts on both human and shellfish health (Trainer *et al.*, 2013). Although most HAB events are dominated by one species, in many instances blooms are composed of several species, which is the case of *K. brevis* blooms in the Gulf of Mexico, where *K. mikimotoi*, a well-known ichthyotoxic HAB also affecting shellfish, co-occurs (Brand *et al.*, 2012). The effects of multispecific blooms on the fitness of shellfish and on the accumulation/depuration of the associated toxins responsible for shellfish poisonings have never been investigated and deserve attention.

Harmful events associated with benthic harmful algal blooms (BHAB) have been reported more frequently over the last decade, including areas where BHAB were not known. The genus of benthic microalga *Gambierdiscus* produces ciguatoxins (CTX) that are bioaccumulated in shellfish and herbivorous and carnivorous fishes via the food chain (Yasumoto *et al.*, 1977) and are responsible for the most common algal-toxin-related seafood poisoning, ciguatera fish poisoning (CFP), which globally affects the greatest number of victims with long-term health effects. In addition, the genus *Gambierdiscus* may also produce other toxins such as maitotoxins (MTX), gambierol, and gambieric acid (Fraga *et al.*, 2011). Originally, CFP was a tropical sickness; however, it is now being reported from subtropical waters and in the Mediterranean Sea. In addition, new species of *Gambierdiscus* have been identified in these areas. For example, in Japan the incidence of CFP was rare, and until the 1980s, cases of CFP were restricted to the subtropical area of Okinawa (Oshiro *et al.*, 2010); however, in recent years, CFP incidents have increasingly been reported from the temperate areas of the main islands of Honshu, Shikoku, and Kyushu (Taniya, 2008; Ishikawa *et al.*, 2010; Oshiro *et al.*, 2011). Recent studies have revealed a surprising diversity of Japanese *Gambierdiscus* with clear geographical patterns of species/phylotypes in coastal areas (Clade I to Clade V). On the other hand, outbreaks of the toxic benthic dinoflagellate *Ostreopsis* are on a similar trend of expansion. The blooms of *Ostreopsis* were first recorded in the Mediterranean Sea in 1972, where they have become more frequent and intense, encompassing the whole area. Very recently, *Ostreopsis* blooms have already been reported in Russia, France, the Atlantic coast

of Spain, New Zealand, and Japan. They are associated with human illnesses and faunal damage. This genus is known as a potential producer of palytoxin and its derivatives (PTX). PTX is the most potent non-proteinaceous compound reported so far that causes fatal illness to humans (known as *clupeotoxism*) following consumption of shellfish and bottom-feeding fish that have accumulated the toxins (Wiles *et al.*, 1974; Yasumoto, 1998; Taniyama *et al.*, 2003; Aligizaki *et al.*, 2008; Sato *et al.*, 2011). There have been a growing number of reports of PTX-like poisoning in southern Japan. In addition, damage to marine fauna has been reported in association with *Ostreopsis* blooms. Recent phylogeographic study of *Ostreopsis* along the West Pacific coast unveiled diverse and widespread distribution of Japanese *Ostreopsis*, particularly for the widely distributed and highly toxic clade *Ostreopsis* sp. 1 (Onuma *et al.*, 1999). The scenario of more frequent BHAB events with range extensions to higher latitudes has become troubling because the knowledge of BHAB is nascent. Therefore, world experts have identified BHAB study as a priority research topic in the field, and research on the diversity, biogeography, sampling techniques, ecophysiology, life cycle, modeling, detection, and monitoring technologies have just started in the past four years (Sato *et al.*, 2011). Nonetheless, the impacts of rising blooms of both *Gambierdiscus* spp. and *Ostreopsis* spp. on marine life have not been considered at this very early stage of research. Indeed, marine faunal damage, including mass mortalities during blooms of *Gambierdiscus* spp., have been observed (personal communication), and very recently *O. siamensis* caused mass mortalities of sea urchins *Evechinus chloroticus* in New Zealand (Berdalet *et al.*, 2012). The toxication of benthic shellfish with CTX and PTX, and the potential associated effects on the organisms, should be studied. In addition, the recent increase of sea water temperature that has favored the expansion of these tropical BHAB, and the projected trends of increase of sea water temperature that would further favor BHAB expansion to higher latitudes, compel the study of the toxication and impacts on shellfish under future warming scenarios.

Shellfish–HAB interactions should be also placed within the wider context of abiotic, biotic, and anthropogenic factors related to climate change and projected levels of warming, acidification, and deoxygenation of the global ocean concomitant to the increasing pressure from a growing world population located mostly in coastal zones. In a recent review, Wells and coauthors (2015) summarized the anticipated linkages between HAB and climate change, the effects of single versus multiple stressors on HAB, and the local human-introduced pressure and global change. Changes in temperature, irradiance, chemical composition of sweater driven by alteration of the partial pressure of carbon dioxide, nutrient composition and speciation, vertical stratification, grazing pressures, phytoplankton species, and strain interactions are expected to affect the phytoplankton community composition and the prevalence and toxin profiles and contents of HAB. The increase in anthropogenic transfer of HAB with shellfish and other grazers, ballast water, biofouling of vessels, and aquaculture coupled with the increase in nutrient load and eutrophication of some coastal regions will contribute to the introduction, seeding, and spreading of nonnative HAB species to new areas (Ruiz *et al.*, 1997; Laabir and Gentien, 1999; Bauder and Cembella, 2000; Harper *et al.*, 2002, Hallegraeff, 2007; Laabir *et al.*, 2007; Hégaret *et al.*, 2008). Thus, particular care should be taken for transport of shellfish livestock, as practiced in many countries and for many shellfish species, especially bivalves (Powell and Ashton-Alcox, 2004). Precautions during transplantation and transfer of shellfish coupled with good knowledge as well as good understanding of the HAB–shellfish interactions and the environmental conditions that influence such interactions may mitigate the risk of inadvertent introductions to new sites and limit the anthropogenic expansion of HAB.

Climate-driven modification in energy and material flows and the biogeochemical cycling is also causing alterations in species interactions, dispersal patterns, and physiological status, leading to population shifts, altered community structure and diversity, and altered ecosystem functioning. Tropical and polar communities are particularly vulnerable to warming and acidification (Kroeker *et al.*, 2009; Hofmann *et al.*, 2010; Doney *et al.*, 2012). Shellfish are no exception to this trend and are expected to respond to climate change by alterations in their physiology and metabolic rates. The interactive effects of climate change and HAB on both the fitness of shellfish and the toxication–detoxification mechanism of shellfish poisoning are an area of research that should be explored, based on multi-stressors experimental frames to better address the limits and extents of the physiological plasticity of shellfish to HAB and their toxins.

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5

Vulnerabilities of Marine Mammals to Harmful Algal Blooms

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5.1 Introduction

Harmful algal blooms (HAB) occur periodically in coastal waters worldwide and are increasing in frequency and duration, and expanding in geographic distribution (Van Dolah, 2000). These blooms adversely affect aquatic ecosystems with impacts on human and animal health, coastal ecosystem integrity, and economies that depend on coastal resources. The harmful effects of most HAB result from production of biotoxins that disrupt physiological function in exposed organisms (Landsberg, 2002). Many of these compounds are potent neurotoxins that target ion channels or components of the cell-signaling pathways that interact with these channels. The ecological and physiological reasons why such toxins are produced are not fully understood.

Biotoxins are the third leading cause, after disease and human perturbation, of mass mortality events of all wildlife, primarily resulting from toxin-producing cyanobacteria and dinoflagellates that dominate marine and freshwater HAB (Fey *et al.*, 2015). Toxic HAB are often temporally and spatially associated with acute morbidity or mortality of marine mammals. Marine mammals may be exposed to algal toxins directly through inhalation of aerosolized toxins or indirectly via food web transfer. Susceptibility of these animals depends on the occurrence of toxin-producing diatoms, dinoflagellates, or cyanobacteria within an animal's habitat and, in the case of food web transfer, on the co-occurrence of prey species that ingest toxic algae and serve as vectors to transfer the toxins to higher trophic levels. Fish, seabirds, and other wildlife dependent on the marine food web may also be affected (Van Dolah, 2005; Fire and Van Dolah, 2012; see also Chapters 4, 6, and 7). In many cases, toxic algal species are normally occurring members of the phytoplankton community and are present in low concentrations with no obvious environmental health impacts; thus, toxic effects are generally dependent on their presence in higher than normal cell concentrations. When animals that have grazed on toxin-producing algae are consumed by marine mammals, morbidity and mortality can occur, and in extreme cases there are large-scale die-offs affecting one or more populations or species (Scholin *et al.*, 2000; Flewelling *et al.*, 2005; Fire and Van Dolah, 2012). Sublethal effects including reproductive failure and chronic neurological disease can occur as a result of one or more sublethal exposures, particularly in regions where repeated seasonal exposure to HAB toxins occurs (Brodie *et al.*, 2006; Gulland and Hall, 2007; Bejarano *et al.*, 2008a, 2008b).

Marine mammals are important sentinel species, and as such provide early warnings of existing or emerging health hazards in oceanic and coastal environments (Bossart, 2011; Schwacke *et al.*, 2013). An observed increase in the mortality or morbidity of marine mammals may provide a signal that HAB toxins are present (Scholin *et al.*, 2000; Flewelling *et al.*, 2005). Bottlenose dolphins (*Tursiops truncatus*), California sea lions (*Zalophus californianus*), and southern sea otters

Harmful Algal Blooms: A Compendium Desk Reference, First Edition. Edited by Sandra E. Shumway, JoAnn M. Burkholder, and Steve L. Morton.

(*Enhydra lutris nereis*) are examples of sentinel species used to evaluate the presence of algal toxins and other environmental threats in oceans and human health research studies (Schwacke *et al*., 2013; Backer and Miller, 2016). Climate change scenarios predict with high confidence that certain areas of the world's oceans will continue to experience shifts in ranges and changes in abundance of algal species (Doucette *et al*., 2012). Thus, management of algal toxins and their effects on marine mammal populations is multifaceted and requires an understanding of the causes and effects of HAB, the reasons for the increase in HAB incidence, and the relationship between HAB and climate change.

5.2 Overview of Algal Toxins

Several classes of algal toxins are associated with morbidity and mortality in marine mammals (Table 5.1). The lipophilic polyethers, including brevetoxins, ciguatoxins, and the diarrhetic

Table 5.1 Marine biotoxins associated with morbidity or mortality in marine mammals.

Toxin class	Syndrome	Structure	Mechanism/ target	Causative organism	Exposure	Symptomology
Brevetoxins (PbTx or BTX)	Brevetoxicosis, Neurotoxic shellfish poisoning (NSP) in humans	Ladder-like polycyclic ether	Neurotoxicity; Voltage-gated sodium channel, site 5	*Karenia brevis*	Inhalation of aerosolized toxin; dietary vectors including fish, shellfish, and invertebrates	Nausea, tingling/ numbness, muscular aches, loss of motor control, seizure; exposure to aerosolized toxin results in coughing, gagging, and burning of upper respiratory tract
Ciguatoxins (CTX)	Ciguatera fish poisoning (CFP)	Ladder-like polycyclic ether	Neurotoxicity; voltage-gated sodium channel, site 5	*Gambierdiscus* spp., *Prorocentrum* spp., *Ostreopsis* spp., *Coolia* spp.	Dietary vectors including fish and invertebrates	Initial effects similar to brevetoxin, but more potent. Reversal of temperature sensation, tachycardia, hypertension, blurred vision, paralysis, and death. Symptoms may persist for weeks to years following acute intoxication
Domoic acid (DA)	DA poisoning, amnesic shellfish poisoning (ASP) in humans	Tricarboxylic amino acid	Neurotoxicity; glutamate receptor	*Pseudo-nitzschia* spp.	Dietary vectors including fish, shellfish, and invertebrates	Gastrointestinal distress (nausea, vomiting, diarrhea), neurological difficulties (dizziness, disorientation, lethargy, seizures, permanent loss of short-term memory), brain

Table 5.1 (*Continued*)

Toxin class	Syndrome	Structure	Mechanism/ target	Causative organism	Exposure	Symptomology
						lesions in areas where glutamate receptors are concentrated (e.g., hippocampus)
Paralytic shellfish toxins (PSTs)	Paralytic shellfish poisoning (PSP)	Heterocyclic guanidine	Neurotoxicity; voltage-gated sodium channel, site 1	*Alexandrium tamarense*, *A. fundyense*, *A. catenella*, *Gymnodinium catenatum*, *Pyrodinium bahamense*	Dietary vectors including fish, shellfish, and invertebrates	Tingling/ numbness, loss of motor control, drowsiness, death by respiratory paralysis (or drowning)
Okadaic acid	Diarrhetic shellfish poisoning (DSP)	Polyether	Inhibition of serine/ threonine protein phosphatases	*Dinophysis* spp., *Procentrum* spp.	Dietary vectors including fish, shellfish, and invertebrates	Gastrointestinal distress (mild) that resolves in 2–3 days in humans
Microcystins	Microcystin intoxication	Cyclic heptapeptide	Inhibition of serine/ threonine protein phosphatases	*Microcystis* spp., *Oscillatoria* spp., *Anabaena* spp., *Cilindrosp-ermopsis* spp., and *Planktothrix* spp.	Consumption of contaminated drinking water; dietary vectors including fish, shellfish, and invertebrates	Liver injury characterized by endothelial damage, apoptosis, intrahepatic hemorrhage, and necrosis of liver parenchyma

shellfish toxins; water-soluble paralytic shellfish toxins and domoic acid; and the cyclic heptapeptide microcystins have all been associated with marine mammals stranded during mass mortality events. In some cases, as for the diarrhetic shellfish toxin okadaic acid, the role of the toxin in marine mammal health is unknown. For well-studied toxins such as the brevetoxins and domoic acid, a strong body of evidence suggests that high levels of exposure to these toxins are the cause of death in repeated marine mammal mass mortality events in regions where these toxins are produced during HAB. Other algal toxins with unknown effects to marine mammals include azaspiracids, yessotoxin, and the spirolides. These novel toxins have adverse health effects in humans or animal models, and should be considered when investigating marine mammal mortality events with no known cause. A comprehensive list of marine mammal mortality events associated with HAB toxins is provided in Table 5.2. The following sections of this chapter provide a review of each toxin class and documented impacts on marine mammals (see also Chapter 9).

5.2.1 Brevetoxins

Brevetoxins are ladder-like polyether toxins produced by the dinoflagellate *Karenia brevis* (formerly *Gymnodinium breve* and *Ptychodiscus brevis*), the causative organism of Florida red tides. The distribution of *K. brevis* is limited to the Gulf of Mexico and southeast U.S. coast, but *K. brevis*–like species occur globally and have been reported in geographically disparate regions including Spain, Japan, Hong Kong, New Zealand, Australia, and the Gulf of Mexico (Hallegraeff, 2014). Raphidophytes (family *Raphidophyceae*) isolated from Japanese waters and Delaware, United States, coastal waters also produce brevetoxins or brevetoxin-like compounds (Ramsdell, 2008).

Table 5.2 Summary of marine mammal mass mortality events with suspected HAB toxin involvement.

Time period	Marine mammal species; number affected	Location	Suspected agent	References
Nov 1946–Aug 1947	Bottlenose dolphins (*Tursiops truncatus*); <5	Southwestern Florida, USA	Brevetoxin suspected	Gunter (1948), Landsberg (2002)
Mar–Apr 1963	Manatees (*Trichechus manatus latirostris*); 8	Southwestern Florida, USA	Brevetoxin suspected	Layne (1965)
1978–1980	Hawaiian monk seals (*Monachus schauinslandi*); 50	Northwest Hawaiian Islands, USA	Ciguatoxin suspected	Gilmartin *et al.* (1980), Gulland and Hall (2007)
Feb–Apr 1982	Manatees (*Trichechus manatus latirostris*); 39	Southwestern Florida, USA	Brevetoxin suspected	O'Shea *et al.* (1991), Landsberg (2002)
1987	Sea otters (*Enhydra lutris*); 34	Kodiak Island, Alaska, USA	Saxitoxin suspected	DeGange and Vacca (1988)
1987	Humpback whales (*Megaptera novaeangliae*); 14	Massachusetts, USA	Saxitoxin suspected	Geraci (1989)
Jun 1987–May 1988	Bottlenose dolphins (*Tursiops truncatus*); >740	Eastern USA (New Jersey to Florida)	Morbillivirus; brevetoxin involved?	Geraci *et al.* (1989), Lipscomb (1994), Landsberg (2002)
Mar–May 1996	Manatees (*Trichechus manatus latirostris*); 151	Southwestern Florida, USA	Brevetoxin	NOAA, Florida FWC, Landsberg and Steidinger (1998), Landsberg (2002)
Nov-96	Bottlenose dolphins (*Tursiops truncatus*); 30	Mississippi, USA	Brevetoxin suspected	NOAA, Gulland and Hall (2007)
1997-May	Mediterranean monk seals (*Monachus monachus*); 150	Mauritania, West Africa	Morbillivirus; paralytic shellfish toxins suspected	Osterhaus *et al.* (1997), Harwood (1998), Hernandez *et al.* (1998)
1998	California sea lions (*Zalophus californianus*); 70	California, USA	Domoic acid	Scholin *et al.* (2000), Gulland (2000)
Aug 1999–Feb 2000	Bottlenose dolphins (*Tursiops truncatus*); >120	Florida (Panhandle), USA	Brevetoxin	NOAA, Twiner *et al.* (2012)
2000	California sea lions (*Zalophus californianus*); 184	California, USA	Domoic acid	NOAA, Gulland (2002)
2001	Bottlenose dolphins (*Tursiops truncatus*); 35	Indian River Lagoon, FL	Saxitoxin suspected	NOAA, Lansberg *et al.* (2002)
2002	Multispecies: Common dolphins (*Delphinus* sp.), California sea lions (*Zalophus californianus*), sea otters (*Enhydra lutris*); 500	California, USA	Domoic acid	NOAA Fisheries (2016)
Mar–Apr 2002	Manatees (*Trichechus manatus latirostris*); 30	Southwestern Florida, USA	Brevetoxin	NOAA, Florida FWC, Flewelling *et al.* (2005)
2003	Humpback whales (*Megaptera novaeangliae*) and other large whales	Maine, USA	Domoic acid and/or paralytic shellfish toxins suspected	NOAA Fisheries (2016)
Mar–Apr 2003	Manatees (*Trichechus manatus latirostris*); 69	Southwestern Florida, USA	Brevetoxin	NOAA, Florida FWC
Mar–Apr 2004	Bottlenose dolphins (*Tursiops truncatus*); 107	Florida (Panhandle), USA	Brevetoxin	NOAA, Twiner *et al.* (2012)

Table 5.2 (*Continued*)

Time period	Marine mammal species; number affected	Location	Suspected agent	References
2005	Large whales	Eastern North Atlantic, USA	Domoic acid suspected	NOAA
2005	California sea lions (*Zalophus californianus*); >100	California, USA	Domoic acid	Goldstein *et al.* (2005)
Jul 2005–Jun 2006	Bottlenose dolphins (*Tursiops truncatus*) and manatees (*Trichechus manatus latirostris*); 136 dolphins, 45 manatees	Southwestern Florida, USA	Brevetoxin	NOAA, Florida FWC
Sept 2005–Apr 2006	Bottlenose dolphins (*Tursiops truncatus*); 90	Florida (Panhandle), USA	Brevetoxin	NOAA, Twiner *et al.* (2012)
Jul–Dec 2006	Manatees (*Trichechus manatus latirostris*); 64	Southwestern Florida, USA	Brevetoxin	NOAA, Florida FWC
Mar–May 2007	Manatees (*Trichechus manatus latirostris*); 52	Southwestern Florida, USA	Brevetoxin	NOAA, Florida FWC
Oct 2007–Jan 2008	Multispecies: Bottlenose dolphins (*Tursiops truncatus*) and manatees (*Trichechus manatus latirostris*); 12 dolphins, 33 manatees	Indian River Lagoon, Florida, USA	Brevetoxin	NOAA, Florida FWC, Fire *et al.* (2015)
2007	Sea otters (*Enhydra lutris*)	Monterey Bay, California, USA	Microcystins	Miller *et al.* (2010)
Feb–Apr 2008	Bottlenose dolphins (*Tursiops truncatus*); >100	Texas, USA	Multiple toxins detected (domoic acid, brevetoxins, okadaic acid) but involvement not clear	Fire *et al.* (2011)
2013	Manatees (*Trichechus manatus latirostris*); 277	Southwestern Florida, USA	Brevetoxin	NOAA Fisheries, Florida FWC
2015	Large whales	Western Gulf of Alaska, USA	Domoic acid and/or paralytic shellfish toxins suspected	NOAA
2015	Large whales	British Columbia, Canada	Domoic acid and/or paralytic shellfish toxins suspected	NOAA

Brevetoxins bind with high affinity to site 5 of the voltage-gated sodium channel, causing the channel to remain open under conditions in which it is normally closed and resulting in inappropriate neuronal transmission (Ramsdell, 2008). At least nine different brevetoxin congeners are produced by *K. brevis*, and brevetoxin metabolites with variable toxicity have been identified in vector organisms such as fish and shellfish (Wang *et al.*, 2004; Dechraoui *et al.*, 2007; Fire *et al.*, 2008).

Humans are exposed to brevetoxins through consumption of shellfish or inhalation of toxic aerosols when *K. brevis* cells are lysed by wind or surf action along affected beaches. Ingestion of brevetoxins causes neurotoxic shellfish poisoning (NSP); symptoms include nausea, tingling, and numbness around the mouth, severe muscular aches, loss of motor control, and seizures in severe cases (Poli *et al.*, 2000). Exposure to aerosolized brevetoxins can result in coughing, gagging, and a burning sensation in the upper respiratory tract (Backer *et al.*, 2003; Pierce *et al.*, 2003; Fleming *et al.*, 2005). Shellfish monitoring programs (by state agencies) and beach safety warnings (by NOAA) exist to protect public health. No human fatalities have been attributed to NSP. Accumulation and trophic transfer of brevetoxins in coastal and marine ecosystems can lead to brevetoxicosis, which has been documented since the 1800s and can be fatal in a wide variety of wildlife species

(Landsberg, 2002). Clinical signs of brevetoxicosis in mammals include seizure, disorientation, incoordination, hyperflexion, muscle fasciculations, flaccid paralysis, and dyspnea. Symptoms are treated with steroids and nonsteroidal anti-inflammatory drugs; supportive care may be provided by administering fluids and nutritional supplementation and preventing drowning in affected animals (Bossart, 2001).

The Florida west coast experiences toxic blooms of *K. brevis* almost annually that may persist for several months. These red tides typically initiate in the Gulf of Mexico and are carried into coastal waters, where significant ecosystem impacts may be observed. Less frequently, Florida red tides are carried in the Loop Current to the Atlantic Ocean and northward by the Gulf Stream as far as North Carolina (Tester and Steidinger, 1997). During large blooms, brevetoxins affect marine animals at all trophic levels, from invertebrates to fish, birds, turtles, and marine mammals. Florida manatees (*Trichechus manatus latirostris*) and bottlenose dolphins (*Tursiops truncatus*) are commonly affected by *K. brevis*.

The first reported association of Florida red tides with manatee deaths was near Fort Myers, Florida, in 1963. Reports of manatee, cormorant, gull, and raccoon mortalities coincided with a red tide slightly north of the Caloosahatchee River in Englewood (Layne, 1965). In 1982, 39 manatee deaths were observed concurrent with a persistent *K. brevis* bloom associated with fish kills and double-crested cormorant (*Phalacrocorax auritus*) deaths (O'Shea *et al.*, 1991). Deaths during this event persisted for weeks after the bloom subsided, likely due to ingestion of filter-feeding organisms attached to seagrasses (Flewelling *et al.*, 2005). Similar manatee mortality events occurred in 1996, 2002, 2003, 2005, 2007, and 2013, with most carcasses found from February to April along Florida's southwest coast between Pinellas and Monroe counties (Table 5.3; FWC, 2016). *K. brevis* blooms typically develop offshore, make landfall during fall and winter, and then dissipate (Tester and Steidinger, 1997). Blooms in embayments where large numbers of manatees over-winter in warm water and low-salinity areas in the Caloosahatchee River region result in exposure of the manatees to brevetoxins and corresponding increases in morbidity and mortalities from brevetoxicosis. The presence of brevetoxins was first determined to be a factor in manatee mortalities during the 1996 event, when 151 manatees died. Stomach contents from several animals tested positive for brevetoxins (RBA; Trainer and Baden, 1999), consisting of seagrasses, tunicates, and other epifauna that were also suspected in the 1982 event (Landsberg and Steidinger, 1998; Landsberg, 2002). Following the 1996 event, high-affinity binding of brevetoxins to manatee brain synaptosomes ($K_D = 7.5$ nM) was demonstrated (Trainer and Baden, 1999). The manatees were likely exposed to brevetoxins through both ingestion, corroborated by detection of brevetoxins in stomach contents, and inhalation, as evidenced by lung damage observed in stranded animals (Bossart *et al.*, 1998).

In 2002, manatee deaths began several weeks after termination of a *K. brevis* bloom. High levels of brevetoxins were detected in sea grasses collected during the manatee mortality event, several months after termination of the bloom. Sea grass blades and associated filter-feeding organisms contained brevetoxins, indicating that brevetoxins are stable in the environment in the absence of an ongoing *K. brevis* bloom. Investigators concluded that ingestion was the route of primary exposure; this was supported by the absence of pulmonary lesions observed in manatees in the 1996 mortality event (Flewelling *et al.*, 2003). Manatees stranded in Florida have been regularly tested for brevetoxin-like activity since 2001 (Table 5.3; FWC, 2016).

Bottlenose dolphin mortalities also have long been associated with *K. brevis* blooms. The earliest documentation of this circumstance was by Gunter *et al.* (1948), who reported the death of "a small number" of bottlenose dolphins near Fort Myers, Florida, concurrent with a mass mortality of wildlife including fish, invertebrates, and turtles along the Florida lower west coast between November 1946 and August 1947. They observed that "the mass death of marine organisms was associated with the flowering of dinoflagellate, *Gymnodinium brevis*." Records of similar wildlife mortality events during red tides in this region date back to 1844 (Gunter *et al.*, 1948), but *K. brevis* was not conclusively identified as the causative organism, and brevetoxins were not identified and characterized until the early 1980s (Ramsdell, 2008).

Brevetoxins were initially identified as the causative agent for the unprecedented die-off of over 740 bottlenose dolphins that occurred on the U.S. east coast from June 1987 through May 1988 (Geraci, 1989), although this unusual mortality event was later attributed to dolphin morbillivirus (Lipscomb *et al.*, 1994). An approximately tenfold increase in dolphin strandings began in New Jersey and moved southward down the Atlantic coast over the fall and winter months, eventually

reaching Florida. The dolphin strandings coincided with a rare bloom of *K. brevis* that originated in the Gulf of Mexico and was carried via the Gulf Stream into Atlantic coastal waters where toxic shellfish and human NSP cases were reported (Tester *et al*., 1991). Brevetoxins were reported in the stomach contents and liver of several dolphins (fish bioassay and HPLC; Baden, 1989), but adequate confirmatory analytical methods were not available at the time of sample analysis (Fire and Van Dolah, 2012). The determination of a co-occurring morbillivirus infection and discrepancies in the timeline with regard to observations of the *K. brevis* bloom, and strandings of brevetoxin-positive animals, prompted reclassification of the primary cause of this unusual mortality event to morbillivirus-induced disease; the role of brevetoxin exposure in these dolphin mortalities remains equivocal.

Bottlenose dolphins in the Florida panhandle region are particularly susceptible to large-scale unusual mortality events associated with exposure to brevetoxins, and three such events occurred in this region between 1999 and 2006 (Twiner *et al*., 2012). From August 1999 until February 2000, more than 120 dolphins stranded dead along the Florida panhandle coast spanning Okaloosa to Franklin counties concurrent with a *K. brevis* bloom (Mase *et al*., 2000). Sea birds, sea turtles, fish, and squid mortalities were also reported during the stranding period. Brevetoxins were detected in stomach contents (4/4), liver (7/22), and/or kidney (4/11) samples in 52% (13/25) of the dolphins tested; brevetoxins were not detected in spleen or lung samples (Twiner *et al*., 2012). Stomach contents, consisting primarily of finfish, had the highest toxin concentrations (500 nanograms PbTx-3 equivalents per gram sample, RBA), and most individuals that stranded were in good body condition, indicating acute poisoning via oral exposure to brevetoxins.

A mass mortality of 105 bottlenose dolphins occurred during a four-week period (March 10–April 15) in the spring of 2004 in the Florida panhandle around St. Joseph Bay in the absence of an identifiable *K. brevis* bloom. Brevetoxins were detected in 100% (39/39) of dolphins tested, with concentrations reaching 29,126 nanograms PbTx-3 equivalents per milliliter gastric fluid (RBA). Stomach contents of these animals contained brevetoxin-contaminated menhaden (*Brevoortia* spp.). Brevetoxins were detected in all fish collected in March 2004, during the unusual mortality event. Before this event, it was suspected that the ichthyotoxic effects of brevetoxins would kill finfish before they could accumulate sufficient toxin to transfer to higher trophic-level predators. Subsequent experiments and fieldwork showed that brevetoxins could accumulate in multiple species of finfish despite a wide variety of feeding habits, indicating multiple sources of brevetoxins in the Florida coastal ecosystem (Naar *et al*., 2007; Fire *et al*., 2008; Fire and Van Dolah, 2012). The domoic acid–producing diatom *Pseudo-nitzschia* was present when strandings occurred, and low levels of domoic acid were also detected in 89% (8/9) of dolphins tested, indicating concurrent exposure to both toxins. This event illustrates the need for understanding brevetoxins in the marine food web, as dolphins were exposed to lethal levels of brevetoxins by feeding on toxic prey fish in the absence of an observable *K. brevis* bloom (Landsberg *et al*., 2009). The lag between HAB events and animal die-offs can be explained in two ways. Either a toxic bloom occurred in the stranding area before the onset of the mortality event and fish remained toxic after bloom conditions subsided, or a toxic bloom occurred elsewhere and toxins were transported to the stranding area by prey fish. It is important to understand that toxin-vectoring by fish may result in delayed or remote biotoxin exposures and that biotoxins should be considered in investigations of marine mammal mortalities even in the absence of notable HAB.

Ninety bottlenose dolphins stranded in the Florida panhandle region from Choctawhatchee Bay to Apalachicola Bay between September 2005 and April 2006; strandings were initially coincident with high densities of *K. brevis*. Brevetoxins were detected in 93% (38/41) of dolphins tested, with concentrations reaching 2724 nanograms per milliliter gastric fluid (enzyme-linked immunosorbent assay [ELISA]). In spite of the co-occurrence of a domoic acid–producing *Pseudo-nitzschia* bloom, domoic acid was not detected in the dolphins. Multiple peaks in the rate of dolphin strandings were observed during this time, with the first peak during the *K. brevis* bloom and another several months following bloom dissipation (Twiner *et al*., 2012). Brevetoxins were also detected in multiple species of finfish known to be major prey items for the dolphins, showing exposure to brevetoxins from multiple sources in the food web. A two-year follow-up study found that brevetoxins persisted in the livers of live fish for over a year after the *K. brevis* bloom had ceased (Naar *et al*., 2007).

Along the Florida east coast, a multispecies mass mortality of bottlenose dolphins and manatees co-occurred with a severe *K. brevis* bloom between

October 2007 and January 2008 (Fire *et al*., 2015). High cell concentrations of *K. brevis* (exceeding 1 million cells per liter) were reported September 26, 2007, and exceeded 6 million cells per liter by mid-October. Manatee strandings ($n = 33$) were reported between October and January, while the stranded dolphins ($n = 12$) were all sampled between December 12 and 27. Brevetoxins were detected in 92% (11/12) of dolphins sampled, with gastrointestinal contents reaching 626 nanograms PbTx-3 equivalents per gram sample (ELISA). Brevetoxin-like activity was detected in dolphin stomach contents, feces, liver, kidney, muscle, lung, blood, blubber, spleen, and eye, but toxin was not detected in the two urine samples collected. The presence of brevetoxin congeners was confirmed by liquid chromatography–mass spectroscopy (LC-MS) in liver and feces from two animals. In the manatees, brevetoxicosis was concluded as a probable cause of death in 11 animals, with diagnosis based on observed vascular congestion with brevetoxin-like activity detected in multiple sample types. Brevetoxin-like activity was detected in 69% (20/29) of manatees sampled, with gastrointestinal contents reaching 1173 nanograms PbTx-3 equivalents per gram (ELISA). Brevetoxin-like activity was detected in manatee stomach contents, feces, liver, kidney, lung, and urine, and in milk from one animal. The primary toxin vector for dolphins was finfish (whole fish from stomach contents were PbTx-positive), while manatees had consumed seagrass with epifaunal tunicates that tested positive for brevetoxins. While background concentrations of *K. brevis* in seawater and low levels of brevetoxins have been detected in marine mammals along the Florida Atlantic coast, blooms in this region are infrequent, and this is the first confirmed association of marine mortalities with brevetoxins in this region (Fire *et al*., 2015).

Investigations of large-scale dolphin mortality events often indicate acute exposure to brevetoxins, but it is also necessary to have an understanding of baseline exposure levels during non-bloom conditions and during blooms that do not lead to mass mortalities. A retrospective study of bottlenose dolphins stranded in the Sarasota Bay, Florida, region from 1994 to 2003 aimed to provide these data (Fire *et al*., 2007). Tissue levels of brevetoxins were determined in dolphins stranded during *K. brevis* blooms and during the absence of detectable HAB to establish baseline exposure levels for Sarasota Bay dolphins. Brevetoxins were detected in at least one tissue, gastric, or excreta sample in 84% (16/19, 7–2896 nanograms PbTx-3 equivalents per gram sample; ELISA) of dolphins stranded during a notable red tide versus 56% (9/16, 6–44 nanograms PbTx-3 equivalents per gram sample) that stranded during background cell concentrations of *K. brevis*. Thus, dolphins in the Sarasota Bay region carry a baseline body burden from single or repeated exposures to brevetoxins. The highest toxin levels were observed in urine, feces, and gastric samples. These values were not confirmed with LC-MS (0/4 samples had detectable levels of PbTx-3 and PbTx-7, LOQ 300 nanograms per gram sample). Brevetoxin levels in dolphins stranded during blooms not associated with large-scale mortalities (max. ~80 nanograms per gram tissue, ~2900 nanograms per gram gastric) were comparable to levels observed in large-scale mortality events from other areas (2004 Florida panhandle UME mean values 51, 26, and 2100 nanograms per gram liver, kidney, and gastric, respectively). These data suggest that brevetoxins at these levels are biologically significant and may cause death in bottlenose dolphins. Dolphins in Sarasota Bay are exposed regularly to red tides with similar *K. brevis* cell densities and brevetoxin exposure levels (according to samples from dolphin carcasses), but have not experienced the large-scale mortalities observed in the Florida panhandle dolphin populations. It is notable that prey fish of dolphins in Sarasota Bay have detectable brevetoxins in the absence of bloom conditions and may play a role in post-bloom exposure (e.g., fish could consume plant material with adsorbed toxin or may be able to accumulate and store brevetoxins to serve as post-bloom vectors; Fire *et al*., 2008).

While biotoxin data obtained from animals stranded individually or *en masse* are useful to determine relative tissue distributions and to estimate exposure levels that may cause death, live capture–release animals sampled during health assessments provide more accurate data with regard to toxin levels, blood parameters, and demographics that can be used in conjunction with environmental parameters collected at the time of sampling (Twiner *et al*., 2011). Data from live-sampled animals provide insight into nonlethal exposures (acute and/or chronic) and the effects these exposures have on animal health. Live capture–release health assessments of the resident dolphin population in Sarasota Bay conducted during multiple *K. brevis* blooms occurring between 2003 and 2005 provided the first *in vivo* exposure data for these animals (Fire *et al*., 2008). Brevetoxins were detected in 63% (19/30) of dolphins sampled during red tides (urine

and gastric 2–9 PbTx-3 equivalents per gram, feces 45–231 nanograms PbTx-3 equivalents per gram; ELISA, LCMS). Brevetoxins were not detected in dolphins sampled during baseline *K. brevis* cell concentrations (≤1000 cells/L, 0/12 dolphins). These data demonstrated that live dolphins from the Sarasota Bay resident population accumulate brevetoxins when exposed to red tides, although brevetoxins were much lower in these animals than observed during brevetoxin-associated mortality events (Flewelling *et al*., 2005). As all brevetoxin-positive dolphins were still living at study conclusion, the levels observed were not toxic to these animals. A follow-up study determined brevetoxin and domoic acid exposure in Sarasota Bay dolphins evaluated during live capture–release health assessments from 2000 to 2009; 14% (12/83) of dolphins tested positive for both toxins in at least one tissue or fluid, indicating regular exposure to multiple toxins in these animals (Twiner *et al*., 2011).

In spite of controversy regarding causality (morbillivirus vs. brevetoxicosis) in the 1987–1988 mid-Atlantic bottlenose dolphin unusual mortality event, investigations of the persistent co-occurrence of toxin-producing *K. brevis* blooms and marine mammal mortalities has led to a preponderance of evidence for a direct link between inhalation and/or ingestion of brevetoxins and large-scale deaths of manatees and bottlenose dolphins in the Gulf of Mexico and on Florida's Atlantic coast (Landsberg *et al*., 2009; Fire *et al*., 2015). An improved understanding of gut and kidney toxin clearance times in manatees and dolphins would be helpful in extrapolating dose and effect data. Knowledge of the time required for digestion of prey and the rate at which toxins are released into the bloodstream from the digestive tract may be important to relate toxin values in gastric and blood samples (Fire *et al*., 2008). A clear limitation to current understanding of toxin exposure data in the wild is the lack of knowledge regarding dose and effect levels for individual species or populations. *K. brevis* bloom-related brevetoxin exposures appear similar between Florida panhandle and Sarasota Bay populations: the panhandle dolphins experience repeated mass mortality events, while strandings lower along the Gulf coast are more disparate in spite of more frequent blooms of similar magnitude. Thus, populations of bottlenose dolphins may differ in resistance to brevetoxin exposure due to differences in the frequency of exposure (Van Dolah, 2005; Twiner *et al*., 2012). A recent study provided evidence for genetic adaptations within local dolphin stocks that may account for increased resistance to frequent environmental threats such as red tides (Cammen *et al*., 2015). While brevetoxins are surely responsible for large-scale mortalities in marine mammals, animals exposed to sublethal doses of toxin can survive when provided with remedial treatment in captivity. Rehabilitation centers in Florida have successfully aided recovery in birds, turtles, and manatees affected by *K. brevis* blooms (Landsberg *et al*., 2009).

5.2.2 Ciguatoxins

Ciguatoxins are a suite of polyether toxins produced by dinoflagellates from the genus *Gambierdiscus*. Ingestion of ciguatoxins results in the clinical illness known as ciguatera fish poisoning (CFP). Ciguatoxins are similar to brevetoxins in chemical structure, pharmacological target, and clinical signs, but ciguatoxins are more potent and neurotoxic symptoms are often present for a longer duration (Fire and Van Dolah, 2012). Ciguatera fish poisoning affects thousands of people every year worldwide in the form of acute gastrointestinal and neurological illness with persistent symptoms resembling chronic fatigue syndrome. Symptoms can include reversal of temperature sensation, tachycardia, hypertension, paralysis, and death (Lewis, 2001; see Chapter 9). Benthic epiphytes such as *Gambierdiscus* spp. grow on filamentous algae associated with coral reefs and reef lagoons. Ciguatoxins enter the food web when toxin-producing algae are consumed by herbivorous fishes and invertebrates; the toxins can bioaccumulate in high trophic-level reef fishes including grouper and barracuda (Lehane and Lewis, 2000; Cruz-Rivera and Villareal, 2006). Ciguatoxin-producing *Gambierdiscus* populations occupy tropical and subtropical marine ecosystems around the world. ln the United States, CFP occurs in Hawaii, Puerto Rico, and southern Florida. Ciguatera is endemic at certain locations, but occurs in a sporadic and unpredictable manner in others. While the effects of ciguatoxins on humans are relatively well understood, our understanding of the role of ciguatoxins in mortality and morbidity of marine mammals is limited.

Ciguatoxins may be one of several factors involved in the decline of critically endangered Hawaiian monk seals (*Monachus schauislandi*) in the tropical Pacific. Hawaiian monk seals were first listed as "endangered" under the Endangered Species Act in 1976, and are also protected

by the Marine Mammal Protection Act and Hawaii state law. Two subpopulations exist: the smaller main Hawaiian Islands (est. 153 individual seals) population and the northwest Hawaiian Islands (est. 907 individual seals) population. The northwest population declined 3.3% annually from 2003 to 2012. In contrast, the main population has been steadily increasing in spite of high levels of human and fishing activity. The northwest Hawaiian island population spans eight remote islands and atolls within the Papahānaumokuākea Marine National Monument. Nihoa Island, Mokumanamana (Necker Island), French Frigate Shoals, Laysan Island, Lisianski Island, Pearl and Hermes Reef, Midway Atoll, and Kure Atoll each contain a semi-isolated subpopulation of seals that face unique ecological pressures and conditions. While fishing pressure and human activity have been minimized within this environment, the Hawaiian monk seal population as a whole continues to decline (NOAA, 2016a).

Over 50 animals died in a Hawaiian monk seal mortality event that occurred in Laysan Island in 1978 that may have resulted from exposure to ciguatoxins. High levels of ciguatoxin-like activity were observed in mouse bioassay of liver extracts from two animals, although ciguatoxins were not confirmed as the causative agent (Gilmartin and Antonelis, 1998). Ciguatoxins were detected in over half of fish tested during surveys of monk seal prey species in Midway lagoon conducted in 1986 (Wilson and Jokiel, 1986) and 1992 (Vanderlip and Sakumoto, 1993). The "stick test" immunoassay used for detection of ciguatoxins in this survey yielded a high rate of false-positives, so results were equivocal (Dickey *et al*., 1994; Wong *et al*., 2005). Relocation of a severely depleted monk seal stock from French Frigate Shoals to Midway Island in 1992 and 1993 resulted in only two of 18 translocated animals surviving beyond one year; one hypothesis is that animals were poisoned by high levels of ciguatoxins in the food web on Midway reefs (Gilmartin and Antonelis, 1998).

More recently, ciguatoxin activity was detected in blood from free-ranging Hawaiian monk seals from both northwest and main island populations who appeared to be healthy, and in brain, liver, and skeletal muscle from dead stranded animals on Midway Island (Bottein *et al*., 2011). The Neuro-2A cytotoxicity assay was used to quantify ciguatoxin activity, and analytical liquid chromatography–tandem mass spectroscopy (LC-MS/MS) confirmed the molecular structure in dead stranded Hawaiian monk seals. Ciguatoxin activity in blood samples from free-ranging animals ranged from 0.43 to 5.49 pg P-CTX-1 equivalents per milliliter blood; 11 of 55 blood samples (19%) had detectable levels of ciguatoxin activity. These toxin levels are comparable to levels necessary to elicit toxic symptoms in laboratory rats. This study confirmed for the first time that Hawaiian monk seals are exposed to significant levels of ciguatoxins and provided the first evidence of trophic transfer of ciguatoxins to marine mammals. The presence of ciguatoxins in food sources of Hawaiian monk seals could pose management challenges for this endangered marine mammal species (Bottein *et al*., 2011), and ciguatoxin exposure should be considered when investigating health or mortalities of marine mammals in all regions where *Gambierdiscus* spp. may be present.

5.2.3 Diarrhetic Shellfish Poisoning Toxins

Okadaic acid and its derivatives, the dinophysistoxins, are polyether compounds associated with diarrhetic shellfish poisoning (DSP) in humans, and are thus referred to as DSP toxins. These lipophilic toxins inhibit serine/threonine protein phosphatases, and can cause nausea, vomiting, and diarrhea lasting one to three days when contaminated shellfish are ingested by humans (Fire and Van Dolah, 2012). DSP toxins are produced by at least two disparate genera of dinoflagellates, *Dinophysis* and *Prorocentrum*. These organisms are widespread in temperate and tropical waters globally (Van Dolah *et al*., 2001). While DSP toxins have a lower toxic potency than the other lipophilic toxins, they have demonstrated tumor-promoting activity in mouse skin (Fujiki *et al*., 1988; Suganuma *et al*., 1988) and may promote fibropapilloma tumors in sea turtles (Landsberg *et al*., 1999). While effects of DSP toxins on marine mammals are not known, exposure to okadaic acid has been demonstrated in bottlenose dolphins (*Tursiops truncatus*) stranded during an unusual mortality event in Texas in 2008 and in live, apparently healthy Peruvian fur seals (*Arctocephalus australis*) and South American sea lions (*Otaria byronia*) sampled in Peru (Fire *et al*., 2011, 2017).

The unusual mortality event of Texas bottlenose dolphins in 2008 co-occurred with blooms of DSP toxin-producing *Dinophysis* spp. and *Prorocentrum* spp. and associated shellfish closures in the region (Deeds *et al*., 2010; Swanson *et al*., 2010). Over 100 dolphins stranded along the Texas coast from February to April 2008.

Okadaic acid concentrations detected in dolphin feces and gastric contents were very low relative to analytical detection limits (10 nanograms per gram sample, LC-MS/MS), and the role of DSP toxins as a factor in the mortality of these animals remains unclear. A bloom of the domoic acid–producing diatom *Pseudo-nitzschia pungens* was also detected toward the end of the stranding period, and concurrent exposure of dolphins to okadaic acid and domoic acid was observed in three animals (Fire *et al*., 2011).

Okadaic acid was evaluated in urine, stomach content, and feces sampled from Peruvian fur seals and South American sea lions during haul-out at a rookery habitat in Punta San Juan, Peru, during November 2012 (Fire *et al*., 2017). Punta San Juan is situated on the coast of Peru in the Humboldt Current region; this upwelling zone is the Southern Hemisphere counterpart to the California current region, where marine mammal populations experience repeated seasonal exposure to HAB toxins. Six of the 18 animals tested (33%) had okadaic acid in feces, with concentrations in toxin-positive animals ranging from 0.5 to 36 nanograms per gram (LC-MS/MS). Because okadaic acid is a lipophilic toxin that can have a longer biological residence time (Matias *et al*., 1999; Svensson, 2003), exposure may have occurred weeks or months prior to sampling these animals. Two of the Peruvian fur seals also tested positive for domoic acid, indicating exposure to multiple toxins in these pinnipeds. These toxin exposure data demonstrate the need for analysis of pinniped prey items for HAB toxins in the Humboldt Current region, to determine the vector species and timing of exposure relative to HAB events. While data for DSP toxins in marine mammals are extremely limited and effects are not known, it is possible these toxins have acute or chronic effects on marine mammal health, and they should be considered when evaluating potential causes of mortality or morbidity in regions where relevant HAB species occur.

5.2.4 Domoic Acid

Domoic acid is a water-soluble marine toxin produced by several diatom species in the genus *Pseudo-nitzschia*. This tricarboxylic amino acid mimics the neurotransmitter glutamate and is a potent activator of certain subtypes of glutamate receptor in the brain. Increased levels of calcium ions within brain cells result in neuronal cell death, and lesions occur in areas of the brain where glutamate receptors are heavily concentrated, particularly in regions of the hippocampus that are responsible for learning and memory processing (Van Dolah *et al*., 2003; Chandrasekaran *et al*., 2004). Symptoms of domoic acid poisoning, also known as amnesic shellfish poisoning (ASP), include nausea, vomiting, diarrhea, dizziness, disorientation, lethargy, seizures, and permanent loss of short-term memory in humans. Acute and chronic exposures to domoic acid pose a regular threat to marine mammals and other wildlife along the U.S. west coast, and toxin-producing diatom species are present on all U.S. coastlines and around the world (Fire and Van Dolah, 2012).

The first conclusive evidence of domoic acid poisoning in marine mammals followed an unusual mortality event during which over 400 California sea lions (*Zalophus californianus*) stranded along the central California coast during May and June 1998 concurrent with a bloom of the diatom *Pseudo-nitzschia australis* (Scholin *et al*., 2000). Animals were in good nutritional condition and displayed signs of neurological dysfunction, including scratching, head weaving, disorientation, ataxia, and seizures; histopathology revealed brain lesions characteristic of domoic acid poisoning in dead animals (Gulland, 2000). DNA probe-based tests were used to identify and quantify *Pseudo-nitzschia* species, and domoic acid was detected in anchovy samples (prey) and sea lion feces (2500–152,000 nanograms per gram) using a receptor binding assay. LC-MS/MS confirmed the presence of domoic acid in plankton, anchovy, and sea lion samples. This comprehensive investigation demonstrated trophic transfer of domoic acid from its algal source in a marine food web to marine mammals via a fish vector. Similar California sea lion strandings have recurred almost annually (Bejarano *et al*., 2008a, 2008b; Goldstein *et al*., 2009). In retrospect, it is likely that previous events involving mortalities of California sea lions and northern fur seals (*Callorhinus ursinus*) occurred on the California coast in 1978, 1986, 1988, and 1992 (Scholin *et al*., 2000), and in marine mammal mortalities associated with *Pseudo-nitzschia* spp. blooms in Mexico (Ochoa *et al*., 1998). The northern fur seal's diet is similar to that of the California sea lion, and domoic acid was detected in 83% of fecal samples (ranging from 2 to 18,600 nanograms per gram, ELISA) collected from northern fur seals (*Callorhinus ursinus*) stranded on the California coast between July 2005 and March 2009 (Lefebvre *et al*., 2010).

Classic signs of domoic acid intoxication in California sea lions include scratching behavior,

disorientation, ataxia, and seizures indicative of neurologic dysfunction (Gulland, 2000; Silvagni *et al*., 2005; Fire and Van Dolah, 2012). Histopathology typically reveals damage to both the brain (lesions and atrophy of the hippocampus and ischemic neuronal necrosis) and heart (pallor of the myocardium and fibrinous pericarditis). Domoic acid poisoning has been linked to abortion, premature births, or death due to pregnancy-related complications in California sea lions, as spring diatom blooms in the California current upwelling zone often occur when females are in the third trimester of pregnancy (Gulland, 2000; Gulland *et al*., 2002; Brodie *et al*., 2006; Goldstein *et al*., 2009). Sea lion pups exposed to domoic acid during gestation have an increased frequency of abnormalities and decreased survival rates (Goldstein *et al*., 2008; Ramsdell and Zabka, 2008; Goldstein *et al*., 2009). Animals that survive an acute domoic acid intoxication episode may have impaired survival and decreased reproductive potential, as these animals typically experience chronic neurological dysfunction and an increased likelihood of restranding (Goldstein *et al*., 2008). Two separate clinical syndromes are now recognized: acute domoic acid poisoning, which can result from a single feeding episode and may cause death, and a chronic domoic acid epileptic disease that results from one or more sublethal exposures (Goldstein *et al*., 2008; Ramsdell and Gulland, 2014). Domoic acid epileptic disease is characterized by spontaneous recurrent seizures and atypical behaviors (e.g., conspecific aggression) in animal subjects that occur weeks to months after exposure to domoic acid. This chronic syndrome was discovered while retrospectively examining California sea lion domoic acid exposure cases from 1998 to 2006 (Goldstein *et al*., 2008). Nearly one-quarter of the animals that stranded with neurological symptoms during this time did not fit the criteria for acute domoic acid poisoning events. These animals stranded individually rather than *en masse*, strandings occurred without concurrent domoic acid–producing algal blooms (often with individual strandings peaking approximately four months after an acute domoic acid poisoning event), and animals expressed intermittent seizures and unusual behaviors. Exposure studies in rats have confirmed that subsymptomatic doses of domoic acid produce delayed epileptic seizures similar to those seen in sea lions that survive an acute poisoning event (Ramsdell, 2010).

In California sea lions, acute domoic acid poisoning disproportionately affects adult females, whereas domoic acid epileptic disease is more often seen in young animals of both sexes. Additionally, disease presentation may differ with the developmental stage at which domoic acid exposure occurs (Ramsdell and Gulland, 2014). Factors influencing susceptibility include both environmental and physiological (e.g., age-dependent) components. Adult females reside year-round in rookeries near feeding zones impacted by toxic *Pseudo-nitzschia* blooms, while males spend a brief period at the rookeries to breed. Males typically do not feed during the breeding season and migrate out of the region heavily impacted by toxic blooms. As gestation lasts almost a year and animals often mate each summer, female sea lions spend much of their adult lifespan pregnant or nursing. Domoic acid has been measured in amniotic fluid from pregnant California sea lions, indicating that fetuses are at risk and that observed reproductive failures are associated with domoic acid–producing *Pseudo-nitzschia* blooms (Brodie *et al*., 2006). Spatial memory deficits observed in California sea lions treated at a marine mammal rescue facility result from disruptions in hippocampal connectivity that result from natural exposure to domoic acid (Cook *et al*., 2015). Progressive regional hippocampal damage that results from domoic acid–induced seizures alters behavior patterns, resulting in a lack of the behavioral flexibility essential for success in foraging. Because sea lions rely on spatial memory-related tasks to forage in a complex marine environment, sea lions with chronic domoic acid toxicosis may be less efficient predators (Cook *et al*., 2016). A degenerative cardiomyopathy observed in California sea lions has also been linked to domoic acid exposure (Silvagni *et al*., 2005); this represents a syndrome associated with toxin exposure beyond neurologic disease that may contribute to morbidity and mortality in all age classes of both sexes and in animals with both acute domoic acid poisoning and chronic epileptic disease (Goldstein *et al*., 2008; Zabka *et al*., 2009). The complex epidemiology of domoic acid intoxication in California sea lions raises concerns regarding the population-level consequences of repeated exposures that are known to occur on the U.S. west coast.

Other marine mammals on the California coast also experience domoic acid intoxications. California sea lions are readily observed and can serve as sentinels for other marine mammals affected by domoic acid–producing algal blooms, including sea otters and several species of dolphins and whales. A marked increase in southern sea otter (*Enhydra lutris nereis*) mortalities was observed following the peak of sea lion strandings

in 1998 (Kreuder *et al*., 2003). The lag time reflects a different dietary source of domoic acid, as otters primarily feed upon benthic invertebrates that become toxic following the downward transport of domoic acid in the water column (Kvitek *et al*., 2008; Sekula-Wood *et al*., 2009). An unusual mortality event of southern sea otters was declared by the U.S. Fish and Wildlife Service and NOAA Fisheries when otter deaths exceeded ten-year averages for three months in 2003; a toxic *Pseudo-nitzschia australis* bloom likely contributed to these otter mortalities (Jessup *et al*., 2007). Domoic acid poisoning in sea otters is associated with cardiac lesions similar to those observed in California sea lions, and domoic acid exposure was identified as a major risk factor for myocarditis and cardiomyopathy in sea otters (Kreuder *et al*., 2005). In the Southern Hemisphere, the Humboldt Current region on the Pacific coast of South America is, as mentioned in this chapter, a counterpart to the California current region. Domoic acid was detected in fecal samples from apparently healthy South American sea lions (*Otaria flavescens*) and Peruvian fur seals (*Arctocephalus australis*) sampled during haul-out at a rookery in Punta San Juan, Peru, in November 2011 and 2012. Prevalence of detectable domoic acid was 25% in Peruvian fur seals (11/44) and 31% in South American sea lions (4/13), although the maximum observed concentration (533 nanograms per gram feces; LC-MS/MS) was substantially lower than values reported for live-stranding pinnipeds with neurological symptoms of domoic acid toxicity. Clinical signs consistent with domoic acid–associated neurotoxicity were not observed in these animals (Fire *et al*., 2017).

Cetaceans have also suffered acute domoic acid poisoning resulting in mortalities. ln 2002, one of the largest documented marine mammal mass mortality events occurred in southern California, and strandings correlated temporally with *Pseudo-nitzschia* spp. blooms (Torres de la Riva *et al*., 2009). This unusual mortality event involved southern sea otters and multiple pinniped and cetacean species, including California sea lions, harbor seals (*Phoca vitulina*), common dolphins (*Delphinus capensis* and *Delphinus delphis*), minke whales (*Balaenoptera acutorostrata*), humpback whales (*Megaptera novaeangliae*), harbor porpoises (*Phocoena phocoena*), and Dall's porpoises (*Phocoenoides dalli*) (Heyning, 2003; Fire and Van Dolah, 2012). Toxin testing revealed domoic acid in urine and feces of stranded animals before there was evidence of toxin-producing *Pseudo-nitzschia* in nearshore waters. Domoic acid was detected in one individual each of Cuvier's beaked whale (*Ziphius cavirostris*), Risso's dolphin (*Grampus griseus*), and gray whale (*Eschrichtius robustus*); thus, exposure to domoic acid can occur in species that do not commonly forage in nearshore areas where blooms are easily detected. Cuvier's beaked whales and Risso's dolphins forage primarily on cephalopods in pelagic waters, and gray whales feed on benthic invertebrates, krill, and sardines (Torres de la Riva *et al*., 2009). Hundreds of seabird deaths and a rare mass mortality of Humboldt squid (*Dosidicus gigas*) occurred concurrent to the 2002 marine mammal unusual mortality event, and sampled individuals tested positive for domoic acid (Van Dolah, 2005). Another domoic acid–associated multispecies stranding occurred in the Gulf of California in January 2004 (Sierra-Beltran *et al*., 2005). Affected marine mammals included 112 common dolphins and 195 California sea lions; pelican (*Pelecanus occidentalis*) and sardine (*Sardinops* spp.) mortalities were also observed. Plankton samples were not collected concurrent with strandings, but domoic acid was detected in blood from stranded dolphins (three of four samples analyzed by LC-MS). The proximity of these potential domoic acid–induced marine mammal mortalities to the Vaquita refuge in the Gulf of California is of particular concern due to the gravely endangered status of the vaquita porpoise (*Phocoena sinus*), whose distribution is limited to the 4000 km^2 area of the refuge. As generalist feeders, vaquitas are susceptible to domoic acid poisoning if a toxic bloom should occur in their distribution range, and fewer than 30 vaquitas remain (NOAA Fisheries, 2017).

Humpback (*Megaptera novaeangliae*) and blue (*Balaenoptera musculus*) whale feces were sampled from August to November 2000 during blooms of toxic *Pseudo-nitzschia* around Monterey Bay, California (Lefebvre *et al*., 2002). Domoic acid was detected at levels ranging from 10,000 to 207,000 nanograms per gram, and further examination of the whale feces by scanning electron microscopy (SEM) revealed the presence of diatom frustules from *Pseudo-nitzschia australis*. During the study, humpback whales were seen feeding on sardines and anchovies, and domoic acid was detected at levels from 75,000 to 444,000 nanograms per gram fish viscera. Benthic and pelagic fish also tested positive for domoic acid, indicating the ubiquitous nature of this toxin in the marine food web during and after toxic *Pseudo-nitzschia* blooms. Acute domoic acid poisoning was demonstrated to be the cause of death when a minke whale (*Balaenoptera acutorostrata*)

stranded during an intense *Pseudo-nitzschia* bloom in southern California in 2007 (Fire *et al*., 2010). Unusually high levels of domoic acid (258,000 nanograms per gram, LC-MS/MS) were detected in feces, and frustules identified as cell fragments from *Pseudo-nitzschia australis* were observed in feces and gastric fluid. Trophic transfer was confirmed by the presence of otoliths from northern anchovy (*Engraulis mordax*), known to be a vector for domoic acid, in whale stomach contents. This case study provided the first direct evidence that baleen whales are susceptible to death from acute domoic acid poisoning.

A survey of 886 animals representing 13 marine mammal species sampled opportunistically from 2004 to 2013 in Alaskan waters revealed the presence of domoic acid in all species examined, with prevalence ranging from 5% (8/179) in northern fur seals to 68% (17/25) in bowhead whales (ELISA; Lefebvre *et al*., 2016). Overall, 21% (188/886) of the animals sampled contained detectable levels of domoic acid in at least one sample type. Sample matrices with detectable domoic acid included feces, stomach contents, intestinal contents, and urine. Marine mammal species sampled represented diverse life history and feeding behaviors, and included cetaceans, otariids, phocids, odobenids, and mustelids. The highest concentration of domoic acid (6457 nanograms per gram, ELISA) was detected in stomach contents from Pacific walrus (*Odobenus rosmarus*). This survey demonstrated domoic acid exposure in marine mammals from southeast Alaska to the Arctic Ocean for the first time, and reveals the potential vulnerability of northern marine mammal populations to toxic algal blooms that may occur more frequently and for a longer duration with increasing ocean temperatures. During the summer of 2015, a massive *Pseudo-nitzschia* bloom extended up the North American west coast from California to the Alaska peninsula, resulting in significant impacts to coastal resources and wildlife (NOAA, 2016b). Marine mammal and bird mortalities were reported in multiple states and Canada, but domoic acid was not confirmed as the cause. An unusual mortality event involving 30 large whales that stranded in the western Gulf of Alaska concurrent with the algal bloom is currently being investigated, but no conclusive evidence has linked the whale deaths to HAB toxins.

While toxin-producing *Pseudo-nitzschia* blooms are primarily associated with the large-scale marine mammal mortalities observed on the U.S. west coast, domoic acid has also been detected in samples from marine mammals stranding in other regions. A survey of cetaceans that stranded along the southeast U.S. coast revealed detectable levels of domoic acid in urine (0.4–1.8 nanograms per milliliter, LC-MS/MS) and feces (12–13,566 nanograms per gram, LC-MS/MS) recovered from pygmy sperm whales (*Kogia breviceps*) and dwarf sperm whales (*Kogia sima*) recovered from 1997 to 2008. The prevalence of domoic acid in *Kogia* spp. tested was 59% (24 of 41 samples) in animals recovered from Virginia to Florida. Forty stranded animals representing 11 other cetacean species recovered in the same geographical range from 2006 to 2008 were also tested; domoic acid was not detected in samples from these individuals (Fire *et al*., 2009). No observed *Pseudo-nitzschia* blooms in the region were spatially or temporally associated with any of these strandings. The *Kogia* spp. are elusive pelagic species with limited sighting data, as they reside offshore along the outer continental shelf and slope regions. Thus, the vector for *Kogia* spp. exposure to domoic acid is not known. It is possible that offshore blooms of toxin-producing *Pseudo-nitzschia* occur in remote pelagic locations not monitored for algal bloom activity, but other cetaceans sampled in the survey are also pelagic species and would likely be exposed similarly. Domoic acid exposure in *Kogia* spp. was distributed throughout the geographic range and across all seasons of the survey. The domoic acid concentrations in most of these stranded individuals likely indicate a repeated or chronic exposure, although it is not possible to ascertain possible health effects of the toxin exposure from these data. Given the myocardial damage observed in California sea lions exposed to domoic acid and the association of prior toxin exposure with myocarditis and cardiomyopathy in southern sea otters discussed above, it is possible that the cardiomyopathy or myocardial degeneration reported in over half of adult *Kogia* spp. strandings is a result of exposure to domoic acid (Bossart, 2011). Domoic acid–induced cardiomyopathy has been demonstrated in rat models, where cardiac dysfunction and morphological changes in cardiac cells are likely a result of severe seizure activity rather than a direct pathological effect of domoic acid on the myocardium (Vranyac-Tramoundanas *et al*., 2011).

Diatoms of the genus *Pseudo-nitzschia* spp. are present year-round in Gulf of Mexico waters (Dortch *et al*., 1997). Low levels of domoic acid were detected in blood, urine, and/or stomach contents from eight of nine bottlenose dolphins (*Tursiops truncatus*) stranded during the 2004 Florida panhandle bottlenose dolphin unusual

mortality event (89%, range, 2–9 nanograms per gram stomach contents; ELISA; Twiner *et al*., 2012). In dolphin health assessments conducted near St. Joseph Bay, Florida, during April 2005 and July 2006, domoic acid was detected in 43% and 29% of urine samples, respectively (Schwacke *et al*., 2010). The highest concentration measured was 201 nanograms domoic acid per milliliter urine. While this value is far lower than concentrations observed in west coast animals during acute poisoning episodes (10–3720 nanograms per milliliter urine), it exceeds values measured in urine from sea lions exhibiting chronic neurological effects (2–110 nanograms per milliliter; Goldstein *et al*., 2008). Elevated blood eosinophil counts, associated with decreased T-lymphocyte proliferation and increased neutrophil phagocytosis, were observed in 23% of the dolphins sampled in the St. Joseph Bay area captures. Elevated eosinophil counts have been documented in cases of acute domoic acid poisoning and chronic domoic acid epileptic disease in California sea lions (Gulland *et al*., 2002; Goldstein *et al*., 2008). During capture–release health assessments in the Sarasota Bay region of southwest Florida, the prevalence of animals testing positive for domoic acid in at least one tissue or fluid was 53% (44/83) over a ten-year study period from 2000 to 2009 (ELISA and LC-MS/MS; Twiner *et al*., 2011). These animals were exposed to domoic acid on an almost annual basis (2004, 2005, 2006, 2008, and 2009). A *Pseudo-nitzschia pseudodelicatissima* bloom was detected concurrent with the sampling period in 2008, and domoic acid was also detected in known bottlenose dolphin prey fish, but blooms were not detected in the other years. The highest domoic acid concentrations were observed in feces (up to 41.5 nanograms per gram), and values correlated with an increase in total white blood cell and eosinophil counts, although these hematological parameters did not exceed the reference threshold for these animals. These data support the hypothesis that domoic acid elicits an immunomodulatory response in dolphins, although implications of such a response on individual outcomes and population-level health effects are uncertain. Domoic acid was also detected in gastric/fecal samples (9–39 nanograms per gram; LC-MS/MS) from three of eight bottlenose dolphins stranded during an unusual mortality event in Texas from February to March 2008 (Fire *et al*., 2011). Of 27 bottlenose dolphins sampled during the ongoing northern Gulf of Mexico cetacean unusual mortality event that began in February 2010, three were positive at low concentrations (all 8 nanograms per gram feces; LC-MS/MS; Venn–Watson *et al*., 2015).

Domoic acid exposure has been assessed in several discrete marine mammal populations in the northern and southern Atlantic Ocean that are threatened or declining in numbers. The western North Atlantic population of right whales (*Eubalaena glacialis*) is of particular concern due to its critically endangered status under the Endangered Species Act. Potentially toxic *Pseudo-nitzschia* species have been identified in the Bay of Fundy, Canada, where North Atlantic right whales feed during summer months. Domoic acid was detected by surface plasmon resonance (SPR) in right whale feces and zooplankton prey species (copepods and krill) collected in April–September of 2005 and 2006 along the northeastern United States and eastern Canada (Leandro *et al*., 2010). Select domoic acid–positive samples were verified by receptor binding assay and confirmed using LC-MS/MS. Frustules of several potentially toxic *Pseudo-nitzschia* spp. were identified in whale feces and phytoplankton samples by light and electron microscopy; electron microscopy also revealed an abundance of *Calanus finmarchicus* mandibles in right whale feces. These right whales were exposed to domoic acid for a period spanning several months via ingestion of a domoic acid–contaminated copepod vector. A follow-up study examined 126 samples collected in the Bay of Fundy from 2001 to 2006 (Doucette *et al*., 2012). Prevalence of domoic acid–positive animals was 25% (annual average; range, 0–60% across years), with concentrations reaching 12,468 nanograms domoic acid per gram feces (RBA). Exposure occurred annually in multiple habitats for periods up to six months in duration (April–September), with similar exposure rates among the sexes (~25–30%). The effects of domoic acid exposure on the western North Atlantic right whale population is not known. Southern right whale (*Eubalaena australis*) calf mortalities observed around Península Valdés, Argentina, were investigated for a possible link to domoic acid exposure (Wilson *et al*., 2016); low levels of domoic acid were detected in two of 108 samples analyzed (3 and 7 nanograms per milliliter blood, LC-MS/MS), and correlations were observed between monthly *Pseudo-nitzschia* spp. densities and calf deaths in this region. The role of domoic acid in the right whale deaths at Península Valdés is not clearly understood.

Scottish harbor seal (*Phoca vitulina*) populations experiencing a marked decline in abundance that began in 2010 were evaluated to

determine whether decreasing population size was associated with biotoxin exposure from *Pseudo-nitzschia* blooms that occur in Scottish waters (Jensen *et al.*, 2015). Live-captured and stranded animals and feces from haul-out sites were sampled between 2008 and 2013; domoic acid was detected in feces at concentrations up to 100,460 nanograms per gram (by HPLC). Blooms of *Pseudo-nitzschia* spp. were observed in the sampling region during 2008 and 2010, and higher domoic acid concentrations were reported in samples collected during these years. Domoic acid levels observed in urine from live-captured harbor seals were higher than values reported for California sea lions suffering acute domoic acid toxicosis (3720 nanograms per milliliter; ELISA; Goldstein *et al.*, 2008), although live-captured Scottish harbor seals appeared healthy and seizures were not observed in stranded animals. Domoic acid was detected in prey species identified from fecal otolith identification; toxin concentration in prey fish sampled in the absence of any notable algae bloom reached 177,400 nanograms per gram viscera (by HPLC) in plaice (*Pleuronectes platessa*). Evidence of immunomodulatory effects in harbor seals including lymphocytopenia and monocytosis was observed in live-captured seals. It is possible that chronic or recurrent exposures of Scottish harbor seals to domoic acid have long-term effects to individual or population health that are not yet understood. Given the recurrent threats that acute and chronic exposures to domoic acid pose to marine mammals and other wildlife along the west coast of North America, and the distribution of *Pseudo-nitzschia* spp. in both Pacific and Atlantic oceans, domoic acid exposure should be considered when investigating health or mortalities of marine mammals.

5.2.5 Paralytic Shellfish Toxins

Saxitoxin (STX) and its derivatives form a suite of hydrophilic, tetrahydropurine neurotoxins. Over 20 toxin congeners are produced in varying amounts by marine dinoflagellates in three genera (*Alexandrium*, *Gymnodinium*, and *Pyrodinium*) and by several species of freshwater cyanobacteria (Landsberg, 2002). Individual toxin congeners have variable toxic potencies in mammals, which span more than two orders of magnitude across the various carbamate, decarbamoyl, and N-sulfocarbamoyl analogs; and toxin congener profiles differ among dinoflagellate species (Doucette *et al.*, 2006; Fire and Van Dolah, 2012). Shellfish toxicity levels fluctuate among species due to differences in retention of toxin congeners and variable rates of toxin depuration. When ingested by humans, these compounds cause the clinical illness known as paralytic shellfish poisoning (PSP), with symptoms that include tingling and numbness of the perioral area and extremities, and loss of motor control. The molecular target of these paralytic shellfish toxins (PSTs) is the voltage-gated sodium channel in nerve and muscle cells, where they bind to site 1 with high affinity, inhibiting channel opening and thereby blocking the passage of nerve impulses. Death may result from respiratory paralysis (Cusick and Sayler, 2013). Because humans can clear these water-soluble toxins from the blood in less than 24 hours, victims generally survive if they are put on life support (Fire and Van Dolah, 2012). PSP is a worldwide problem, and shellfish monitoring programs now exist on all continents except Antarctica. In the United States, paralytic shellfish toxins have historically threatened consumers of shellfish harvested along the northeastern and western coasts, including Alaska, but they have more recently been found in puffer fish from the Indian River Lagoon, Florida (Landsberg, 2002; Landsberg *et al.*, 2006). A comprehensive study of puffer fish sampled from eight regions spanning the Florida Gulf and Atlantic coastlines revealed the persistent presence of saxitoxins along both coasts (Abbott *et al.*, 2009). If paralytic shellfish toxins ingested by fish or other secondary producers are not lethal to an organism, toxins can bioaccumulate at higher trophic levels. Paralytic shellfish toxins have been implicated in wildlife mortalities involving sea birds and marine mammals (Cusick and Sayler, 2013).

The effects of paralytic shellfish toxins on marine mammals were first observed when 14 humpback whales (*Megaptera novaeangliae*) stranded dead along beaches of Cape Cod Bay and northern Nantucket Sound, Massachusetts, during a five-week period between November 1987 and January 1988 (Geraci *et al.*, 1989). Baleen whales had not previously been reported to mass-strand, and this event involved apparently healthy animals with stomachs full of undigested Atlantic mackerel (*Scomber scombrus*). Death appeared to have occurred quickly, so an acutely toxic substance was suspected as the cause. Because paralytic shellfish toxins were periodically present in New England waters (Shumway *et al.*, 1988), HAB

toxins were investigated as a potential causative agent. Saxitoxin-like activity was detected by mouse bioassay in stomach contents and tissues from the stranded whales and from planktivorous mackerel sampled in the same time and place where strandings occurred (but not in control samples), suggesting the whales were exposed to toxic levels of paralytic shellfish toxins via ingestion of mackerel. This was the first report of bioaccumulation of paralytic shellfish toxins in the marine food web, with transfer via a fish vector to marine mammals resulting in a mass mortality event.

Exposure to paralytic shellfish toxins also likely contributed to a mass mortality of Mediterranean monk seals (*Monachus monachus*) that occurred in northwest Africa during May and June 1997. More than 100 animals died along the coast of Mauritania, representing a loss of approximately 70% of the local population and about one-third of the world population of this endangered species (Harwood, 1998; Hernandez *et al*., 1998). Morbilliviruses, which had caused mass mortalities of other marine mammal species (Gulland and Hall, 2007), were detected in the monk seal carcasses and suspected to be the causative agent in this event (Osterhaus *et al*., 1997). But unlike previous morbillivirus-associated marine mammal mortality events, the stranded seals died quickly with few signs of disease, and carcass conditions were consistent with drowning from paralysis due to ingestion of paralytic shellfish toxins. Abundant concentrations of toxic dinoflagellate species were identified in waters near the seal colony, and both seal tissue samples and fish collected from seal feeding grounds tested positive for saxitoxin-like activity by mouse bioassay (Hernandez *et al*., 1998). The presence of decarbamoyl-saxitoxin (dcSTX) and 1-*N*-hydroxy-saxitoxin (neoSTX) was later confirmed in seal tissues and fish samples using tandem mass spectrometry (Reyero *et al*., 1999). A limited understanding of effects of paralytic shellfish toxins on marine mammals prevented conclusive determination that toxins were the cause of death, although both biotoxins and morbillivirus were likely contributors (Harwood, 1998). Mass mortalities from exposure to HAB toxins may have serious implications for long-lived marine mammals such as the Mediterranean monk seal; this event reduced the breeding population to fewer than 77 individuals and was believed to have seriously compromised the survival of the species (Forcada *et al*., 1999). A 2007 population study of the Cabo Blanco, Mauritania, monk seal population concluded that the population had recovered to numbers estimated before the 1997 mass mortality event, likely due to the high survival rates of females and high breeding potential observed in this population (Martínez-Jauregui *et al*., 2012).

A bottlenose dolphin (*Tursiops truncatus*) unusual mortality event in the Indian River Lagoon, Florida, during June and July 2001 was also suspected to involve dietary exposure to paralytic shellfish toxins (Fire and Van Dolah, 2012). Recovered dolphins were emaciated and displayed skin lesions, suggesting that multiple factors contributed to their overall poor health status (Bossart *et al*., 2003). A subsequent outbreak of human seafood poisoning cases in early 2002 prompted the detection of paralytic shellfish toxins in puffer fish (*Sphoeroides* spp.) from the Indian River Lagoon and the discovery of toxin production by the dinoflagellate *Pyrodinium bahamense* var. *bahamense* (Landsberg *et al*., 2002; Quilliam *et al*., 2002). These organisms were not previously associated with production or accumulation of toxin in the Indian River Lagoon, so dolphins from the 2001 unusual mortality event were re-examined for evidence of biotoxin exposure. Puffer fish were found in stomachs from at least two dolphins, and stomach contents from these and other animals recovered from this event tested positive for low concentrations of paralytic shellfish toxins (T. Leighfield, personal communication). Puffer fish are not known to be a regular component of the bottlenose dolphin diet (Barros and Odell, 1990). Because the levels of paralytic shellfish toxins that would result in dolphin mortalities are not known, it is not clear whether biotoxins played a role in the compromised health and mortality of these animals.

Exposure to paralytic shellfish toxins may also have more subtle effects on marine mammal health. Evidence of trophic transfer of toxins from blooms of *Alexandrium fundyense* to the North Atlantic right whale (*Eubalaena glacialis*) has raised questions regarding their potential role in fluctuating reproduction rates observed in this critically endangered whale species. In 2011, the North Atlantic right whale population was estimated to have fewer than 500 individuals (NOAA Fisheries, 2016), although recent data indicate a slight growth in population size among western Atlantic animals. The coastal waters of New England and the Bay of Fundy are major feeding grounds for these whales, and this region experiences toxic blooms of *A. fundyense* and associated

shellfish bed closures almost annually. The copepod *Calanus finmarchicus*, which dominates the right whale diet, grazes on toxic *A. fundyense* and has been found to contain high concentrations of paralytic shellfish toxins (Durbin *et al*., 2002). Paralytic shellfish toxins were detected in feces collected from at least 11 apparently healthy North Atlantic right whales in the Bay of Fundy between August and September 2001 at concentrations reaching 500 nanograms saxitoxin equivalents per gram (RBA, HPLC-FD; Doucette *et al*., 2006). A follow-up study assessed right whale exposure to paralytic shellfish toxins (and domoic acid) over a six-year period from 2001 to 2006 (Doucette *et al*., 2012). Prevalence of PST-positive animals was 73% (annual average; range, 45–100% across years, $n = 132$), with concentrations reaching 1198 nanograms saxitoxin equivalents per gram feces (RBA, LC-MS/MS). Exposure of right whales to paralytic shellfish toxins occurred annually in multiple habitats for periods up to six months in duration (April–September), with similar exposure rates among the sexes (~70–80%). Concurrent exposure to paralytic shellfish toxins and domoic acid was observed in over 20% of the fecal samples tested, thus the potential for interactive effects between biotoxins exists for animals feeding in this region. While the biological significance of biotoxin exposure in right whales is not clear with regard to circulating biotoxin levels in the blood or effects on behavior and physiology, it is possible that sublethal biotoxin exposure may affect whale behavior, leading to reduced feeding rate, fitness, and reproduction (Durbin *et al*., 2002). The susceptibility of the North Atlantic right whale population to biotoxin exposure remains a pertinent research question, and the extent of physiological, behavioral, and developmental effects related to these exposures is not known. Paralytic shellfish toxins were also detected (172 and 800 nanograms saxitoxin equivalents per gram feces; RBA) in two Southern right whales (*Eubalaena australis*) sampled during an investigation of right whale calf mortalities observed around Península Valdés, Argentina (Wilson *et al*., 2016). No correlation was observed between monthly *Alexandrium tamarense* densities and calf deaths in this region (as observed for *Pseudo-nitzschia* spp. densities over the same time period). As mentioned, what role biotoxins play in the right whale deaths at Península Valdés is unclear.

Paralytic shellfish toxins may also influence feeding behavior and population distribution of upper trophic-level predator species, as hypothesized for sea otters (*Enhydra lutris*) consuming bivalve shellfish on the Alaskan coast (Kvitek *et al*., 1991; Van Dolah *et al*., 2003). Alaskan sea otters feed on butter clams (*Saxidomus giganteus*), among other bivalves, and can consume 20–30% of their body weight per day. Butter clams have low rates of depuration for paralytic shellfish toxins, and sequester the toxins in the siphon where it may be retained for up to one year. In the Alaskan Kodiak region, butter clams and sea otters are co-distributed among inside passage waters and outer coastal areas. Butter clams are also abundant in southeast Alaska, but the clams in inner passage waters remain toxic year-round due to seasonal *Alexandrium* blooms, while clams along the outer coast are not toxic. Sea otter distribution in southeast Alaska is limited to the outer coast. Feeding studies on caged sea otters demonstrated the ability of the animals to detect and avoid toxic clams (Kvitek *et al*., 1991).

A comprehensive survey of Alaskan marine mammals representing cetaceans, otariids, phocids, odobenids, and mustelids sampled opportunistically between 2004 and 2013 demonstrated paralytic shellfish toxin exposure in ten of 13 marine mammal species (Lefebvre *et al*. 2016). Prevalence of exposure was highest in humpback whales (50%, 4/8) and bowhead whales (32%, 8/25), with the highest toxin concentration observed in Pacific walrus (240 nanograms per gram intestinal contents; ELISA). Overall, 13% (107/830) of the animals sampled contained detectable levels of paralytic shellfish toxins in at least one sample type. Sample matrices with the highest toxin concentrations were feces, stomach and intestinal contents, and urine. Domoic acid exposure was also evaluated in this survey, and 5% (46/830) of the animals tested had detectable concentrations of both domoic acid and paralytic shellfish toxins in at least one sample type, emphasizing the potential for interactive effects from multiple toxin exposure. Similar studies in recent years have focused on exposure of specific pinniped populations to biotoxins in coastal areas of Scotland and Peru. Live-captured and stranded Scottish harbor seals (*Phoca vitulina*) and feces from haul-out sites were evaluated to determine whether an observed decrease in population size was associated with biotoxin exposure (Jensen *et al*., 2015). Samples were collected between 2008 and 2013; paralytic shellfish toxins were detected in feces from live-captured seals (but not urine), and in feces collected from haul-out sites and dead-stranded seals. The highest concentration of paralytic shellfish

toxins (18 nanograms saxitoxin equivalents per gram; HPLC) was measured in feces from a dead-stranded animal. Prey species plaice (*Pleuronectes platessa*) and dab (*Limanda limanda*), identified from fecal otolith identification, were sampled and tested for paralytic shellfish toxins. Saxitoxin was the only congener detected, and concentrations were 1020 and 758 nanograms per gram viscera in plaice and dab, respectively. Thus, the Scottish harbor seals were exposed to biotoxins through consumption of contaminated prey species. Apparently healthy South American sea lions (*Otaria flavescens*) and Peruvian fur seals (*Arctocephalus australis*) sampled during haul-out at a rookery in Punta San Juan, Peru, in November 2011 and 2012 were evaluated for biotoxin exposure (Fire *et al*., 2017). Saxitoxin-like activity was detected by receptor binding assay in feces with concentrations ranging from 133 to 818 nanograms saxitoxin equivalents per gram, but was not detected in gastric or urine samples. Prevalence of paralytic shellfish toxin exposure was 8% (1/13) in South American sea lions and 6% (2/35) in Peruvian fur seals.

While knowledge of marine mammal exposure to paralytic shellfish toxins is increasing with recent studies, data regarding effect levels in specific populations are still lacking, and most investigations linking paralytic shellfish toxins to morbidity and mortality in marine mammals are circumstantial. While a preponderance of evidence exists to attribute the humpback whale strandings off Massachusetts in 1987–1988 to ingestion of paralytic shellfish toxins via mackerel, most other mortality event investigations are complicated by other factors such as morbillivirus disease (Mediterranean monk seals) and overall poor health status (bottlenose dolphins in Indian River Lagoon, Florida). Susceptibility of vulnerable populations such as the North Atlantic right whales in the western Atlantic to paralytic shellfish toxin exposure should be evaluated further. As for other HAB toxins, it is important to address effect levels in marine mammals and what concentrations are related to those known to cause clinical signs of toxicity (e.g., paralysis) in marine mammals exposed to paralytic shellfish toxins. Data on effect levels for paralytic shellfish toxins in marine mammals, including concentrations observed in specific sample matrices from marine mammals experiencing toxicosis, are lacking. A limitation in risk assessment is that toxin concentrations in marine mammal samples are not directly related to the magnitude of an animal's exposure (Lefebvre *et al*., 2016). Due to rapid clearance rates, not all animals experiencing toxicosis will have toxin in feces and urine. Efforts should also focus on secondary methods for confirmation of paralytic shellfish toxins in marine mammal sample matrices using LC-MS, as assay data should be confirmed using an analytical method in mortality investigations and exposure studies.

5.2.6 Other Algal and Cyanobacterial Toxins

Azaspiracids are nitrogen-containing polyethers that were first reported in 1995 during an outbreak of gastrointestinal illness in humans that followed consumption of shellfish from Ireland (James *et al*., 2003). Signs of azaspiracid poisoning are similar to diarrhetic shellfish poisoning and may include nausea, vomiting, diarrhea, and stomach cramps (Twiner *et al*., 2008). Azaspiracids are associated with the dinoflagellate *Azadinium spinosum* and have been reported in Europe, North America, and Africa. The mechanism of toxicity for azaspiracids is unclear, and impacts on marine mammals have not been reported.

Yessotoxin is a sulfated polyether produced by dinoflagellates from the genera *Gonyaulax* (aka *Protoceratium*) and *Lingulodinium*. Originally thought to be one of the diarrhetic shellfish poisoning toxins, yessotoxin has no diarrhetic activity and little toxic potency when administered orally to mice (Tubaro *et al*., 2008). When injected, yessotoxin is lethal to mice and induces neurological symptoms. Both *Lingulodinium polyedrum* and *Gonyaulax grindleyi* have been implicated in fish and shellfish mortality events, although the role of yessotoxin in those events is not known. Impacts of yessotoxin on marine mammals are not known.

Spirolides are macrocyclic amines produced by the dinoflagellate *Alexandrium ostenfeldii*; these compounds have been implicated in shellfish toxicity in northern Europe (Cembella *et al*., 2000). Injection of spirolides into mice causes rapid death following neurological symptoms, although the mode of action is not clear. The distribution of *A. ostenfeldii* in North America is limited to eastern Canadian waters and the Gulf of Maine. Impacts of spirolide exposure in marine mammals are unknown.

Cyanobacteria (aka blue-green algae) produce various types of biologically active compounds including hepatotoxins (microcystins, nodularins, and cylindrospermopsins), neurotoxins (anatoxins and saxitoxins), and irritants (lyngbyatoxin and

others). Cyanobacteria are prokaryotic photosynthetic organisms that grow in marine, brackish, and fresh waters worldwide; they form extensive blooms in both freshwater and estuarine habitats when conditions are favorable (Carmichael, 1992; Andrinolo *et al*., 2008). Microcystins are the most common cyanobacterial toxins; these cyclic heptapeptides are produced primarily by the freshwater cyanobacterium *Microcystis aeruginosa*, and also by other genera of cyanobacteria including *Oscillatoria*, *Anabaena*, *Cilindrospermopsis*, and *Planktothrix*. Human exposure to microcystins occurs with ingestion of contaminated drinking water and recreational contact with contaminated water. Acute illness and death due to microcystin exposure have been reported worldwide in humans and animals; chronic effects have been observed in experimental studies (Andrinolo *et al*., 2008). Deaths of 21 southern sea otters (*Enhydra lutris nereis*) along the shore of Monterey Bay in central California in 2007 were linked to microcystin intoxication via trophic transfer from marine invertebrates (Miller *et al*., 2010). A *Microcystis* bloom that originated in an inland freshwater lake discharged microcystins via three nutrient-impaired rivers flowing into Monterey Bay. Livers from affected otters tested positive for microcystins (range, 1–348 nanograms per gram; LC-MS/MS), and hepatic lesions consistent with microcystin intoxication were observed. Additional cases detected along the California coastline suggest multiple point-sources for microcystins along the U.S. west coast.

5.3 Impacts of Algal Toxins Specific to Marine Mammals

Marine mammals are sentinels of potentially dangerous changes in the ocean and coastal environment. Poisonings observed in marine mammals have alerted researchers to the presence of toxins in the marine environment to which humans are susceptible. Screening for algal toxins present in shellfish and other seafood protects humans from most illnesses associated with algal toxins, but animals living in oceans and coastal environments are routinely affected by exposure to biotoxins. Assessing impacts of marine biotoxins on marine mammals involves an intensive multidisciplinary approach using techniques ranging from satellite imagery to molecular DNA probes, toxin assays, and analytical techniques. To link a specific toxin to observed morbidity or mortality in marine mammals, it is ideal to identify a causative organism and toxins in water or prey sources in the proximity of animal strandings and to observe toxin-related clinical signs and histopathology in tissue samples collected from subject animals combined with chemical detection of toxin in tissues or excreta using biological assays with confirmed analytical chemistry (e.g., LC-MS). Linking exposure, tissue-level effects, and clinical signs is essential to proving the role of a toxin or ruling it out (O'Hara and O'Shea, 2001).

5.3.1 The Effects of Toxin Exposure Depend on Animal Physiology and Behavior

Cetaceans are completely aquatic animals that rely on the marine food web for sustenance. These specialized marine mammals have several physiological adaptations that may increase their susceptibility to toxic effects. A high proportion of blubber, in which water-soluble toxins (e.g., domoic acid and paralytic shellfish toxins) do not partition, relative to individual body mass, may concentrate toxins in metabolically sensitive tissues. Additionally, the *marine mammal diving response* in deep-diving cetaceans concentrates blood in the heart and brain; thus, circulating toxins may not reach organs that function in detoxification, and respiration is not under autonomic control in cetaceans (Van Dolah, 2005). Toxin exposure that would be sublethal in a terrestrial mammal may have more pronounced toxic effects in a diving mammal due to these adaptations to the marine environment (Fire *et al*., 2010).

Manatees live primarily in warm, tropical waters, and feed on plants. A theory on the manatee mass mortality events that occur frequently during toxic inshore blooms of *K. brevis* in southwest Florida links the increase in strandings to a unique combination of environmental, geographical, and biological factors that must co-occur to cause mass mortalities. In most years, red tide does not occur inshore during the winter/spring period when manatees are congregated and about to disperse. If present in large numbers, manatees are at high risk from February through April if salinities are higher than 28 and the *K. brevis* bloom enters nearshore waters. Concentration and geographic distribution of the *K. brevis* bloom, salinity, and bloom persistence in relation to the distribution of manatees mandate their length of exposure to brevetoxins. Individual manatees can be at risk anytime during exposure to red tide

(Landsberg and Steidinger, 1998). Similar environmental and behavioral differences may drive the observed differences in stranding patterns between bottlenose dolphin populations in the Florida panhandle versus southwest Florida, although other factors such as genetic adaptability could also play a role (Cammen *et al.*, 2015).

5.3.2 Emerging Issues: Non-acute and Multiple Toxin Exposure

Algal toxin impacts to marine mammals are primarily observed as acute intoxications, but the effects of chronic exposure to low levels of algal toxins on animal health are increasingly recognized (Van Dolah, 2000). HAB toxin exposure in marine mammals can be quite conspicuous, resulting in dramatic die-offs of large numbers of animals and associated marine fauna. Sudden death, respiratory paralysis, drowning, an otherwise healthy appearance, and abundant stomach contents containing phytoplankton-grazing fish or invertebrates indicate death from acute biotoxin exposure. Dense algal blooms and high concentrations of toxins present in seawater and prey species typically precipitate these events. In acute intoxications, biotoxins are usually detectable in marine mammal tissues and fluids if animals are sampled before decomposition sets in. Variability in the severity of HAB and the frequency in which they occur can also result in repeated, nonlethal exposures to one or more toxins that can also negatively impact marine mammal health. In California sea lions, repeated sublethal exposure to domoic acid has significant long-term effects, including chronic epileptic syndrome, degenerative heart disease, and in utero toxicity resulting in reproductive failure (Brodie *et al.*, 2006; Goldstein *et al.*, 2008; Ramsdell and Zabka, 2008; Zabka *et al.*, 2009; Ramsdell and Gulland, 2014). In southwest Florida, brevetoxins have been detected in bottlenose dolphins and their fish prey several months to over a year after cessation of a *K. brevis* bloom (Fire *et al.*, 2007; Naar *et al.*, 2007), suggesting potential year-round toxin exposure. Long-term impacts of repeated non-acute exposures on these populations are unknown. The effects of annual paralytic shellfish toxin–producing blooms or potential ciguatoxin exposure in marine mammals may negatively impact growth in depleted populations such as Northern Atlantic right whales or Hawaiian monk seals (Gilmartin and Antonelis, 1998; Durbin *et al.*, 2002), but few data exist to support this hypothesis, in part due to insufficiently sensitive detection methods, confounding factors such as infectious disease, or inadequate longitudinal data (Fire and Van Dolah, 2012). Evidence of HAB toxin exposure is seen in marine mammals even in the absence of observed toxic blooms. In a 1997–2009 survey of pygmy and dwarf sperm whales (*Kogia* spp.) stranded between North Carolina and Florida along the U.S. Atlantic coast, feces from nearly 90% of sampled animals were positive for domoic acid. No domoic acid–producing HAB were associated with any of the biotoxin-positive animals (Fire *et al.*, 2009). Undetected HAB activity occurring in remote regions may affect marine mammals; continued efforts to investigate such associations are necessary to understand impacts of HAB toxin exposures to these protected animals.

As HAB observation efforts become more widespread, toxic species are increasingly reported in new regions, resulting in increased awareness of marine mammal exposure to multiple HAB toxins. Northern Atlantic right whales experience frequent exposure to both domoic acid and paralytic shellfish toxins while at summer feeding grounds in New England and Bay of Fundy waters (Doucette *et al.*, 2006; Leandro *et al.*, 2010; Doucette *et al.*, 2012). Brevetoxins and domoic acid have been observed simultaneously in live-captured and dead-stranded bottlenose dolphins in Sarasota Bay, Florida, for several years (Twiner *et al.*, 2011) and in dolphins stranded during an unusual mortality event in the Florida panhandle during 2004 (Twiner *et al.*, 2012). Low levels of domoic acid, brevetoxins, and okadaic acid were all detected in bottlenose dolphins that stranded during an unusual mortality event occurring near Galveston, Texas, in 2008 (Fire *et al.*, 2011). Other recent studies have reported multiple toxin exposure in marine mammals in both northern and southern hemispheres in the Pacific Ocean (Fire *et al.*, 2017; Lefebvre *et al.*, 2016). It is not known whether exposure to multiple HAB toxins can result in additive or synergistic effects that increase the potency of one or more of the toxins present or whether multiple toxins suppress immunity to make animals more vulnerable to secondary stressors.

5.3.3 Prospects for Managing Impacts of HAB

Management of the impacts of algal toxins on marine mammals requires an understanding of the causes and consequences of HAB, their

dependence on large-scale oceanographic and climate changes, and the local and regional influences on their occurrence, severity, and duration (Fire and Van Dolah, 2012). Management options for HAB include strategies for prevention, control, and mitigation (see Chapter 12). As the HAB that have been associated with marine mammal mortality events are predominantly natural phenomena that occur in the absence of direct anthropogenic influences, prevention is not typically feasible or advisable (Van Dolah, 2005). Control strategies involve local regulation to avert HAB effects, and can include increasing freshwater outflow to decrease salinity in a local environment to a level that would not be tolerable to dinoflagellates and localized application of flocculants to remove algal cells from the water column. Both of these strategies may have unintended adverse effects that should be closely examined before taking action. Mitigation of HAB effects on human populations involves early detection by monitoring areas where HAB are known to occur. Coastal states have algal and shellfish monitoring programs that routinely test water and shellfish samples to detect HAB species and associated toxins; these programs have successfully prevented human illnesses in the USA. Programs are generally shore-based, so are not sufficient to protect marine mammals offshore who may be exposed before a toxic bloom was detected at coastal sampling sites. Satellite imaging and spectral analysis have successfully identified offshore blooms prior to HAB affecting coastal ecosystems, providing early warning for potentially toxic HAB.

Knowledge of the occurrence of toxic HAB is only useful when further mitigation strategies for marine mammals exist (Van Dolah, 2005). Physical removal of threatened or endangered animals may be a viable management option, although likely a costly one. Treatments for live-stranded animals involving supportive care and rehabilitation can mitigate the toxic effects of HAB on marine mammals. In some cases, animals can be sustained in treatment or rehabilitation facilities until they recover from toxicosis. For California sea lions, the large number of sea lions afflicted with domoic acid poisoning has permitted prognosis following rehabilitation after both acute toxicosis and chronic epileptic disease (Ramsdell and Gulland, 2014). Treatment of seizure due to acute domoic acid toxicosis generally requires symptomatic control with benzodiazepines. Short-term management with phenobarbital assists recovery of acute toxicosis cases, but treatment with benzodiazepines and phenobarbital was not effective in animals with chronic epileptic disease. Studies following rehabilitated animals returned to the wild have shown that chronic animals have a substantially poorer prognosis after rehabilitation in terms of both survival behaviors and progression of the epileptic state.

5.4 Considerations for the Evaluation of HAB Toxins in Marine Mammals

Current knowledge concerning the effects of HAB toxins on marine mammals comes from two sources: investigations of marine mammal strandings and mass mortality events, and studies of toxin exposures in living populations. While toxin data collected from stranded animals can be useful in determining relative tissue distributions and estimating toxin load necessary to cause death, live capture–release health assessment studies provide more accurate data (e.g., toxin levels, blood parameters, age, and sex) that can be used in conjunction with environmental parameters collected at the time of sampling. Live animal data provides insight into nonlethal exposures (acute and/or chronic) and the effects of these exposures on animal health (Twiner *et al.*, 2011). In the investigation of stranded animals, whether individual or *en masse*, it is important to determine whether toxic HAB species are present in the local environment and producing toxin, and whether toxins are present in marine mammal food sources and in select tissues sampled from stranded animals. In some cases, animals are stranded in remote locations or cannot be sampled without putting researchers at substantial risk. In these situations, environmental samples are the only source of information on whether biotoxins are involved, and it is imperative to obtain these samples as spatially and temporally close to the strandings as possible to determine whether biotoxins may be involved. The high potency of some classes of algal toxins results in symptomology or pathology at low concentrations, making confirmation of toxin presence difficult. Water-soluble toxins are cleared rapidly through urine and feces, and may not be detectable in tissues even when clinical signs are apparent (Rowles *et al.*, 2001). Thus, the failure to detect biotoxins in tissues from stranded animals does not necessarily rule out their role in morbidity or mortality. A drawback to carcass data is the potential lag time between exposure and sampling. Times between response and necropsy are usually

prompt, but the time between death and observation of the carcass is not known, and the effects of decomposition are not well understood. Health assessments of live animals routinely take blood, feces, and urine; these are not always successfully recovered during necropsy of stranded animals. An additional advantage of health assessments is the potential for citing history data for individual animals in well-studied marine mammal populations such as dolphins in Sarasota Bay, Florida, or North Atlantic right whales (Fire *et al*., 2008).

5.4.1 Sampling Marine Mammals for HAB Toxin Analysis

In marine mammal strandings, sampling of code 1 or 2 animals is preferred, although code 3 animals and later may still be useful for toxin analysis (see Geraci and Lounsbury, 2005, 177–178, for code definition). The most useful tissues and fluids for confirming biotoxin exposure are generally feces, urine liver, and stomach contents; however, samples from additional compartments (intestinal contents, kidney, lung, brain, whole blood, and serum) are also valuable depending on the toxins of interest, and are useful for metabolism and body burden studies. In animals where gastric contents or feces are available, this material can be processed and analyzed by electron microscopy for the presence of algal cell remnants or fragments as evidence for ingestion of potentially toxic HAB species as well as identifiable body parts of zooplankton (e.g., copepod mandibles) and fish (e.g., fish otoliths), all of which can be potentially identified to the species level. These qualitative data can provide insight into which prey species have been ingested and the potential for investigating biotoxin trophic transfer. All samples should be placed immediately in a cooler on ice and frozen (−20 °C to −80 °C) as soon as possible after collection. Samples should be transported or shipped on dry ice, with the transport or shipping container marked according to safety protocols and with the appropriate sample permits to allow transport of marine mammal samples under the Marine Mammal Protection Act. Sample types, and collection and storage information, are provided in Table 5.3. Urine and feces have proven to be the most informative samples for most toxin classes and should take priority when available (Rowles *et al*., 2001).

In addition to collecting samples from affected animals, prey species and water should be collected to identify whether harmful algal species are present. Plankton samples are collected with a plankton net in a vertical tow, and samples are preserved in Lugol's solution or in 2% glutaraldehyde. Water samples for biotoxin analysis should include multiple size fractions when possible (i.e., nets or filters sized appropriately for phytoplankton, zooplankton, and planktivorous fish), in order to provide a clear picture of biotoxin trophic transfer processes that may serve to expose marine mammals to biotoxins. Whole-water samples (two 1-L bottles)

Table 5.3 Sampling: Sample types for determination of biotoxins in marine mammals.

Sample type	Amount	Container	Comments
Urine	1–50 mL	Temperature-rated plastic tube	Store at −20 °C
Feces	5–50 g	Temperature-rated plastic tube or other	Store at −20 °C
Intestinal contents or bile	5–50 g	Temperature-rated plastic tube or other	Store at −20 °C
Stomach contents	5–50 g	Temperature-rated plastic tube or other	Collect undigested or partially digested prey or food items separately from gastric fluid; store at −20 °C
Liver, kidney, lung, spleen, brain	5–50 g	Temperature-rated plastic tubes, plastic bags, or other	Store at −20 °C
Serum	>0.5 mL	Temperature-rated plastic tube	Obtain serum (top layer) by centrifugation (1500–3000 × g; 5 minutes) of whole, heparinized blood; store at −20 °C
Whole blood		Blood collection cards	Store at room temperature with dessicant pouches

Note: if samples are to be analyzed for multiple algal toxins, a larger amount of sample is needed to perform multiple toxin extractions.

may be collected, preserved as above, and stored refrigerated when nets or filters are not available (Rowles *et al.*, 2001).

5.4.2 Priority Needs for Investigating HAB Toxin Involvement in Marine Mammal Morbidity and Mortality

Investigations into marine mammal mortality events over past decades have provided considerable insight into the environmental conditions, food web vectors, symptoms, pathology, and toxicokinetics associated with biotoxin exposure in marine mammals, but there is still much to learn. Confirmation of toxins as causative agents in marine mammal mortalities remains difficult due to the lack of knowledge of acute and chronic effect levels. Establishing background body burdens for specific toxin classes in the absence of detectable HAB has provided necessary baseline data for comparison and has been an integral step toward understanding what levels indicate adverse health effects. Coordinated, multidisciplinary responses to marine mammal mass mortality events remain necessary to obtain data needed to ascertain whether HAB species and toxin are present in the local environment and in food sources concurrent with observed marine mammal mortalities.

The development and validation of detection methods for biotoxins in marine mammal sample matrices remain a priority for ciguatoxins, paralytic shellfish toxins, and diarrhetic shellfish poisoning toxins. The high potency and complex structures of ciguatoxins and lack of toxin standards hinder detection capabilities; a detection infrastructure must be developed before implementing a management plan. Reference mass spectrometry methods for paralytic shellfish toxins in marine mammal sample matrices would allow confirmation of bioassay results and provide information on toxin congener profiles observed in marine mammal tissues and prey samples. Studies are necessary to gain additional insight into reproductive dysfunction in North Atlantic right whales, immunosuppression in animals exposed to brevetoxins, and differential sensitivity of individuals with repeated brevetoxin exposures compared with individuals from naive populations. Prey species in upwelling areas where animals may be exposed to diarrhetic shellfish poisoning toxins and on reefs where Hawaiian monk seals may be exposed to ciguatoxins should be evaluated as vectors for biotoxins.

Understanding the process and timeline for recovery in populations that experience losses from a major HAB event and the long-term consequences of repeated exposures will provide necessary knowledge as bloom events occur with greater frequency and in greater duration due to climate change. Data on exposure levels, body burdens, toxin effect levels, clearance rates, maternal transfer of toxin, reproductive failure, and chronic effects of toxin exposure are accumulating; we need a clear understanding of effects on individual and population fitness. A recent review of changes in marine mammal health from 1972 to 2012 identified difficulties in assimilating data from peer-reviewed communications to assess trends due to lack of detail and inconsistencies in reporting (Simeone *et al.*, 2015). A centralized reporting system for marine mammal health and disease data was suggested to allow detection of true trends in health and disease; the Marine Mammal Health Monitoring and Analysis Platform (MMHMAP) was proposed to address this goal (Marine Mammal Commission, 2016). Currently in the development stage, MMHMAP will standardize data reporting, integrate data sources, provide a summary of health and disease trends, and enable communication and analysis of health and environmental data. This platform will allow a formal risk assessment of the potential impacts of domoic acid on marine mammals on the U.S. west coast and brevetoxins in the Gulf of Mexico. These assessments can provide insight on effect levels, rates of metabolism and detoxification, and accumulated body burden resulting from repeated biotoxin exposures.

Abbreviations

ASP	Amnesic shellfish poisoning
AZP	Azaspiracid poisoning
BTX	Brevetoxin(s)
CFP	Ciguatera fish poisoning
CTX	Ciguatoxin
DA	Domoic acid
DSP	Diarrhetic shellfish poisoning
DTX	*Dinophysis* toxin(s)
ELISA	Enzyme-linked immunosorbent assay
FD	Fluorescence detection
HAB	Harmful algal bloom
HPLC	High-performance liquid chromatography
LC-MS	Liquid chromatography–mass spectroscopy

MMHMAP	Marine Mammal Health Monitoring and Assessment Platform
MMME	Marine mammal mortality event
NMFS	National Marine Fisheries Service (aka NOAA Fisheries)
NOAA	National Oceanic and Atmospheric Administration
NOS	National Ocean Service
NSP	Neurotoxic shellfish poisoning
OA	Okadaic acid
PbTx	Brevetoxin(s)
PSP	Paralytic shellfish poisoning
PST	Paralytic shellfish toxin
PTX	Pectenotoxin
RIA	Radioimmunoassay
RBA	Receptor binding assay
SEM	Scanning electron microscopy
UME	Unusual mortality event
YTX	Yessotoxin

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6

Interactions between Seabirds and Harmful Algal Blooms

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6.1 Introduction

Harmful algal blooms (HAB) are a regular occurrence worldwide in both freshwater and marine systems, with the frequency of toxic events increasing rapidly during the past few decades in magnitude, frequency, and intensity (Smayda, 1990; Hallegraeff, 1993; Burkholder, 1998; Shumway *et al*., 2003). This escalation has been broadly linked to warming temperatures, environmental degradation, and eutrophication, suggesting that HAB have the potential to become an even larger problem in the future (Guo, 2007; Paerl and Huisman, 2008; Kudela, 2011). Within marine ecosystems, harmful algal toxins can be transmitted trophically, from zooplankton grazers to apex predators including fishes, seabirds, marine mammals, and humans (DeMott and Moxter, 1991; Turner and Tester, 1997). The broad-scale effects of large harmful blooms may quickly impact local ecosystem function and stability, and have the potential to harm regional fisheries, aquaculture, and recreational industries.

Most ecological research on algal biotoxins has focused on their bioaccumulation and impact on fisheries species harvested for human consumption, particularly shellfish, crustaceans, and fishes (Novaczek *et al*., 1991; Robineau *et al*., 1991; Lewis and Holmes, 1993; Bricelj and Shumway, 1998; Tester *et al*., 2000; Bauder *et al*., 2001; Sipiä *et al*., 2001; Lefebvre *et al*., 2002; Jos *et al*., 2005). The adverse effects of biotoxins on marine mammals have also been well documented, reflecting the scientific and public interest associated with mass strandings and illness in these animals (Geraci *et al*., 1989; Flewelling *et al*., 2005). In contrast, HAB-associated impacts on seabirds have been less studied, particularly in the context of spatial and temporal links between seabird mortality events and HAB. This is somewhat surprising, considering that seabird mortality events (i.e., *wrecks*) are highly noticeable and frequently reported. Additionally, seabird carcasses can be tested for the presence of algal-derived biotoxins given adequate sampling and analyses. Recent advances and cost reductions in tagging technology also now provide a more comprehensive understanding of seabird distributions and foraging habits for many species, allowing researchers to both predict and monitor the spatiotemporal overlap between seabirds and algal blooms.

Several ecological factors render seabirds particularly vulnerable to the effects of HAB. As central place foragers, seabirds forage at sea and return to islands or offshore rocks for breeding, which are often located in coastal waters that may be more likely to promote HAB (Orians and Pearson, 1979; Heisler *et al*., 2008). Like marine mammals, seabirds are long-lived with a late age of first reproduction, making them susceptible to the bioaccumulation of toxins over time (Rowe, 2008). Unlike marine mammals, the comparatively small body masses of seabirds (less than 1000 g) may further increase the risk of intoxication and immediate negative effects of algal toxins. In addition to a delayed sexual maturity, most seabird species

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have a small clutch size, with many of the procellariforms and alcids producing only one chick per year (Warham, 1990). Consequently, major mortality events such as die-offs or wrecks can drastically impact the long-term viability of seabird populations (Croxall and Rothery, 1991). While most seabirds are believed to accumulate toxins through the ingestion of affected prey, seabirds have also been recently shown to be susceptible to nontoxic by-products of algal blooms such as surfactants that foul their feathers and strip them of their waterproofing capacity, resulting in documented mortality events (Jessup *et al.*, 2009; Phillips *et al.*, 2011; Jones *et al.*, 2017). Increased nutrient run-off and changing environmental conditions may amplify blooms of known harmful algal species, as well as introduce previously uncommon species, with a potential corresponding increase in toxic by-products. There is increasing concern that these conditions may represent an emerging threat to marine life.

Understanding the relationship between HAB and seabird mortality events is critical for the prudent monitoring and management of seabird populations in the future, and may potentially serve as a broader bellwether for the health of local marine environments. Given recent evidence that harmful blooms are increasing in severity, frequency, intensity, and composition, HAB likely pose an increasing and unappreciated threat to the health and wellness of marine bird populations. This chapter presents a summary of the types, locations, and severity of reported HAB–seabird interactions, and provides recommendations for improving management and conservation of seabird species.

6.2 Historical Interactions between HAB and Seabirds

Historically, HAB are known to have impacted seabirds since the Pliocene, with Emslie *et al.* (1996) implicating blooms of dinoflagellates in the deaths of cormorants and other seabirds through the examination of marine sediment beds. The earliest scientific observations include a few publications in the late 1800s (Glazier, 1882; Moore, 1882; Walker, 1883) that reported the existence of red tides in the Gulf of Mexico that led to major fish kills and corresponding seabird mortality. Seabird mortality events have been frequently documented since then, yet the cause of these events is often unclear and the oceanographic and environmental conditions are often poorly described. The most definitive description of HAB–seabird interactions was first compiled by Shumway *et al.* (2003), and is now updated here (Table 6.1). For the most part, however, it is important to emphasize that HAB–seabird interactions have largely been described via incident accounts of observed mortality events, and the field has lacked both a broader synthesis and experimental work on captive animals. The major harmful algal species and their known impacts on seabirds and waterbirds are reviewed here, and the known symptoms of the different toxins are briefly summarized in Table 6.2 (see also Chapter 9).

6.2.1 Paralytic Shellfish Poisoning (PSP)

Paralytic shellfish poisoning is a broad term for symptoms produced by neurotoxins produced by some dinoflagellates, particularly those in the genus *Alexandrium* (previously *Gonyaulax* and *Protogonyaulax*; Negri and Jones, 1995; Anderson *et al.*, 2002). The most common and dangerous of these toxins, saxitoxin, can quickly accumulate in the tissues of filter-feeding shellfish, which experience varying species-specific toxic effects (Widdows *et al.*, 1979; Shumway and Cucci, 1987; Gainey and Shumway, 1988; Shumway, 1990; Shumway *et al.*, 1990; Nielsen and Strømgren, 1991; Lesser and Shumway, 1993; Luckenbach *et al.*, 1993; May *et al.*, 2010). Early accounts from the 1940s and 1960s implicated *Gonyaulax* spp. as a dinoflagellate clade that produced PSP symptoms in birds that fed upon affected bivalves. These reports included the deaths of Common Murres (*Uria aalgae*), Pacific Loons (*Gavia arctica pacifica*), Northern Fulmars (*Fulmarus glacialis*), Herring Gulls (*Larus argentatus*), White-winged Scoters (*Melanitta fusca deglandi*), Tufted Puffins (*Lunda cirrhata*), Sooty Shearwaters (*Puffinus griseus*), and Black-footed Albatross (*Diomedea nigripes*) in Washington State; and Shags (*Phalacrocorax aristotelis*), terns (*Sterna* spp.), and Great Cormorants (*Phalacrocorax carbo*) on the Farne Islands in the United Kingdom (McKernan and Scheffer, 1942; Adams *et al.*, 1968; Coulson *et al.*, 1968a; Armstrong *et al.*, 1978). Reports of *Gonyalaux* blooms and PSP mortalities continued into the 1970s and 1980s, with one particular outbreak killing approximately 1600 Black Ducks (*Anas rubripes*) in New Hampshire and lesser numbers of terns (*Sterna* spp.) in Massachusetts (McKernan and Scheffer, 1942; Adams *et al.*, 1968; Coulson *et al.*, 1968a, 1968b). Effects of PSP are now associated with other dinoflagellate clades as well (for a review of

Table 6.1 A summary of seabird mortality events associated with harmful algal bloom outbreaks, summarized from incidence reports, published studies, and unpublished data.

Study	Year	Region	HAB species	Bird species
McKernan and Scheffer	1942	Washington State, USA	*Gonyalaux*	Pacific Loon
–	–	–	–	Northern Fulmar
–	–	–	–	Sooty Shearwater
–	–	–	–	Black-footed Albatross
–	–	–	–	White-winged Scoter
–	–	–	–	Common Murre
–	–	–	–	Tufted Puffin
–	–	–	–	Herring Gull
Bargu *et al.*	1961	California, USA	*Pseudo-nitzschia*	Sooty Shearwater
Coulson *et al.*	1968	Northeast England	*Alexandrium*	Shag
–	–	–	–	Cormorant spp.
–	–	–	–	Gannet
–	–	–	–	Herring Gull
–	–	–	–	Northern Fulmar
–	–	–	–	Atlantic Puffin
–	–	–	–	Razorbill
–	–	–	–	Common Murre
–	–	–	–	Duck spp.
Furphy	1969	Ireland	Unknown	Various alcid spp.
Bicknell and Collin	1972	New Hampshire, USA	*Gonyalaux*	Black Duck
Forrester	1974	Florida, USA	*Alexandrium*	Double-crested Cormorant
–	–	–	–	Red-breasted Merganser
–	–	–	–	Herring Gull
Nisbet *et al.*	1978	Massachusetts, USA	*Gonyalaux*	Common Tern
–	–	–	–	Arctic Tern
–	–	–	–	Roseate Tern
–	–	–	–	Laughing Gull
–	–	–	–	Herring Gull
Hockey *et al.*	1979	South Africa	*Gonyalaux*	Black Oystercatcher
–	–	–	–	Black-backed Gull
–	–	–	–	Hartlaub's Gull
Wranes	1981	Scandinavia	*Gyrodinium*	Eider spp.
Stroud and Lange	1983	Gulf Coast, USA	*Pseudo-nitzschia*	Common Loon
Gill-Darby	1989–1990	New Zealand	Unknown	Yellow-eyed Penguin
Work *et al.*	1991	California, USA	*Pseudo-nitzschia*	Brandt's Cormorant
–	–	–	–	Double-crested Cormorant
–	–	–	–	Pelagic Cormorant
–	–	–	–	Brown Pelican
–	–	–	–	Western Gull

(*continued*)

Table 6.1 (*Continued*)

Study	Year	Region	HAB species	Bird species
Jaspaerse	1993–1994	New Zealand	Unknown	Penguin spp.
Krokowski	1995–1997	Northeast coast, UK	*Dinophysis*	Common Murre
–	–	–	–	Black-legged Kittiwake
–	–	–	–	Herring Gull
Lavasseur	1996	St. Lawrence, Canada	*Alexandrium*	Herring Gull
Ochoa	1996	Baja, Mexico	*Pseudo-nitzschia*	Brown Pelican
Sierra-Beltran	1996	Baja, Mexico	*Pseudo-nitzschia*	Brown Pelican
Quintana	2000	Argentina	*Alexandrium*	Penguin spp.
Sepia *et al.*	2002–2004	Bering Sea, Alaska	*Nodularin*	Common Eider
Atwood	2001–2006	Florida, USA	*Karenia*	Cormorant spp.
–	–	–	–	Brown Pelican
–	–	–	–	Various shorebird spp.
–	–	–	–	Various gull spp.
–	–	–	–	Various loon spp.
Jessup *et al.*	2007	California, USA	*Akashiwo*	Common Loon
–	–	–	–	Pacific Loon
–	–	–	–	Red-throated Loon
–	–	–	–	Brandt's Cormorant
–	–	–	–	Double-crested Cormorant
–	–	–	–	Brown Pelican
–	–	–	–	Grebe spp
–	–	–	–	Northern Fulmar
–	–	–	–	Sooty Shearwater
–	–	–	–	Fork-tailed Storm-petrel
–	–	–	–	Common Murre
–	–	–	–	Rhino Auklet
–	–	–	–	Surf Scoter
–	–	–	–	Western Gull
–	–	–	–	California Gull
Shearn-Boschler	2008–2012	Alaska, USA	*Alexandrium*	Kittlitz Murrelet
Phillips *et al.*	2009	Oregon, USA	*Akashiwo*	Common Loon
–	–	–	–	Red-throated Loon
–	–	–	–	Common Murre
–	–	–	–	Surf Scoter
–	–	–	–	Grebe spp.
Gibble *et al.*	2016	California, USA	*Microcystis*	Surf Scoter
–	–	–	–	Common Murre
Jones *et al.*	2017	Washington, USA	*Akashiwo sanguinea*	Surf Scoters
–	–	–	–	White-winged Scoters,
–	–	–	–	Common Murre
Gibble *et al.*	2018	California, USA	*Pseudo-nitzschia*	Common Murre

Table 6.2 Physiological symptoms caused by harmful algal bloom toxins.

Syndrome	HAB species	Toxin produced	Symptoms
Paralytic shellfish poisoning (PSP)	*Alexandrium* spp., *Gymnodinium catenatum, Pyrodinium bahamense, and others*	Saxitoxin and derivatives	Neurologic symptoms including loss of motor coordination, impaired swimming and flying, vomiting, weight loss, gastrointestinal inflammation, impairment of respiration, paralysis, death
Neurotoxic shellfish poisoning (NSP)	*Karenia* (*Gymnodinium*) *brevis*	Brevetoxins	Gastrointestinal and neurological symptoms
Amnesic shellfish poisoning (ASP)	*Pseudo-nitzschia* spp.	Domoic acid and isomers	Neurologic symptoms, fine motor tremors, stargazing, impaired swimming and flying, vomitting, death
Diarrhetic shelfish poisoning (DSP)	*Dinophysis* spp., *Prorocentrum* spp.	Okadaic acid, dinophysis toxins	Gastrointestinal symptoms
Foam surfactants	*Akashiwo sanguinea*	Nontoxic	Loss of waterproofing, hyperthermia, death
Microcystis (cyanotoxin)	*Microcystis aeruginosa* also *Planktothrix, Anabaena Anthrospira, Oscillatoria, Nostoc, Microcystis* spp.	Microcystins	Liver function disruption
Nodularin (cyanotoxin)	*Nodularia spumigena*	Nodularin-R	Liver function disruption
Anatoxin-A (cyanotoxin)	*Anabaena, Aphanizomenon, Cylindrospermum, Oscillatoria Planktothrix, Raphidiopsis*	Anatoxin-a	Loss of muscle and neurological control, convulsions, paralysis, gasping, respiratory arrest, death

harmful marine dinoflagellates (see Faust and Gulledge, 2002), and some of the early *Gonyaulax* species associated with seabird mortality events have since been moved to the dinoflagellate genus *Alexandrium*. Correspondingly, broader ecological and spatial impacts of PSP have been reported in recent decades: in 1992, two gannets were found dead in North Carolina with PSP toxins confirmed in the tissues of these birds, illustrating the presence of PSP in a seabird species that predates fish rather than shellfish (Shumway *et al*., 2003). Similarly, in an unpublished report highlighted in Shumway *et al*. (2003), a large die-off (3000+ birds) of Magellanic Penguins (*Sphenicus magellanicus*), South American Terns (*Sterna hirundinacea*), Imperial Cormorants (*Phalacrocorax albivene*), and Great Grebes (*Podiceps major*) occurred in Chubut, Argentina, a region that frequently experiences large blooms of *Alexandrium tamarense* and *A. catenella*. Although testing for the presence of saxitoxins or *Alexandrium* was not conducted during this event, the symptoms found in normally healthy birds and the history of *Alexandrium* blooms in the area strongly imply that PSP was a likely factor in this mortality event (Shumway *et al*., 2003).

6.2.2 Neurotoxic Shellfish Poisoning (NSP)

Brevetoxins are neurotoxins primarily produced by the marine dinoflagellate *Karenia brevis* (formerly *Gymnodinium breve*), now known to also be produced by a few other related dinoflagellate species (Nakanishi, 1985; Baden, 1989). These toxins depolarize nerve cell membranes, disrupt normal neurological functions, and can accumulate quickly in invertebrates and shellfish (Hardison *et al*., 2013). The dinoflagellate *K. brevis* has a particular history of bloom production in Florida and the Gulf of Mexico, causing recreational and

economic upheaval (Walker, 1883; Quick and Henderson, 1974; Forrester *et al.*, 1977). NSP was suspected in an early incident report in 1974, when a large group (12,000–20,000) of Lesser Scaup (*Aythya affinis*) and smaller numbers of Double-crested Cormorants (*Phalacrocorax auritus*) and Red-breasted Mergansers (*Mergus merganser*) died during red tide events in Tampa, Florida; however, conflicting experimental analyses during the same decade failed to firmly establish associations between *K. brevis* and bird mortality (Quick and Henderson, 1974; Forrester *et al.*, 1977). For example, adult captive scaups fed toxic clams showed no adverse effects (Quick and Henderson, 1974), whereas Peking Duck chicks fed similarly weighted concentrations of toxic prey died and exhibited behavioral symptoms similar to those reported in the large scaup mortality event in Florida. A more recent study, however, examined ill Double-crested Cormorants on Sanibel Island in Florida that were sent to rehabilitation centers during a mass mortality event between 1995 and 1999 (Kreuder *et al.*, 2002). The authors identified brevetoxins in the organs of at least four individuals, and were able to demonstrate significant correlation between illness in birds and the presence of *K. brevis*. In 2005, Van Deventer (2007) examined gastrointestinal tissues and stomach contents from a large mortality event of marine birds in Florida and confirmed brevetoxins in both types of samples. The author asserted that trophic transfer through fish was likely the primary route of exposure for seabirds and shorebirds.

6.2.3 Amnesic Shellfish Poisoning

Domoic acid (DA), which causes amnesic shellfish poisoning (Jasperse, 1993), is produced by the diatom *Pseudo-nitzschia* and may be the most well-known harmful algal toxin associated with wildlife mortality in recent years. This is likely due to an increase in studies reporting the effects of this toxin on marine mammals (Lefebvre *et al.*, 1999; Scholin *et al.*, 2000; Gulland *et al.*, 2002; Silvagni *et al.*, 2005; Brodie *et al.*, 2006; Goldstein *et al.*, 2008). Perhaps the most famous account of the interaction between DA and seabirds was portrayed in Alfred Hitchcock's movie thriller *The Birds*. In 1961, there were many reports of seabirds (thought to be primarily Sooty Shearwaters) with neurologic symptoms in Monterey Bay, California. Although the cause of the symptoms was unknown at the time, the film dramatically portrays this event, and Bargu *et al.* (2012) later examined the gut contents of zooplankton and confirmed that *Pseudo-nitzschia* spp. were present at the time of the event. In the 1990s, there was a significant body of work that detailed several accounts of the interaction between seabirds and DA. The majority of this work was focused in California and Mexico (Fritz *et al.*, 1992; Work *et al.*, 1993; Ochoa *et al.*, 1996). The species implicated in these events primarily comprised Brown Pelicans (*Pelecanus occidentalis*) and cormorants (*Phalacrocorax* spp.). It was noted in Fritz *et al.* (1992) that during a bloom of *Pseudo-nitzschia* in northern Monterey Bay in 1991, many Brandt's Cormorants (*Phalacrocorax penicillatus*) and Brown Pelicans were reported to exhibit symptoms of central nervous system disorders. In this particular case, gut contents were analyzed that confirm that the birds had consumed large quantities of anchovies contaminated with DA. A similar event occurred in Cabo San Lucas, Mexico, in 1996, where 150 dead Brown Pelicans were found over a 5-day period (Beltrán *et al.*, 1997). Deaths were attributed to eating forage fish contaminated with *Pseudo-nitzschia* sp. after investigations using both microscopy and high-performance liquid chromatography (HPLC). Of the birds examined, many of the stomachs were empty, suggesting previous vomiting episodes, but stomach swabs and gastrointestinal tracts were analyzed for DA, as were potentially associated forage fish. Stomachs and fish showed similar HPLC signals for DA, and *Pseudo-nitzschia* sp. frustules were found via microscopy in both pelicans and forage fish. It was estimated that this event caused the demise of 50% of the breeding colony at this location (Beltrán *et al.*, 1997).

6.2.4 *Akashiwo sanguinea*

More recently, the dinoflagellate *A. sanguinea* has been implicated in the impairment and death of birds (Figure 6.1). While not toxic itself, the combination of senescent cells and wave action produces proteinaceous surfactant foam that can cause birds and other species of wildlife to lose waterproofing capabilities (Horner *et al.*, 1997; Jessup *et al.*, 2009; Trainer *et al.*, 2010). The slimy foam produced by this species coats the feathers of birds and causes them to become wet, cold, and often hypothermic (Jessup *et al.*, 2009; Phillips *et al.*, 2011; Jones *et al.* 2017). Generally, birds that encounter the foam come to shore for protection or wash ashore post-mortem. There were two recent notable events on the U.S. West Coast in 2007 and 2009 (Jessup *et al.*,

Figure 6.1 An example of seabird interactions with *Akashiwo sanguinea*. (a) A Pacific loon found dead in Monterey Bay, California, in November 2007 with yellow-green staining of ventral breast feathers typical of over 700 marine birds affected by this event. (b) Closer view of the stained feathers from (a), illustrating the yellow-green discoloration and the oily appearance of affected feathers. (c) Discolored, wet, and matted feathers on the ventral wing of a western grebe recovered during the same stranding event. *Source*: Jessup *et al*. (2009), http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0004550. Licensed under CC-BY 4.0.

2009; Du *et al*., 2011; Phillips *et al*., 2011; Jones *et al*. 2017). During both events, rehabilitation centers and beach survey programs were able to identify and track the increase in numbers of both live birds in rehabilitation and dead beachcast birds. The areas where these events occurred (Monterey Bay, and southern Washington/northern Oregon) are routinely monitored by beach survey programs (BeachCOMBERS, Beach Watch, and COASST), so the events did not go unnoticed, and many local and external rehabilitation centers were utilized to wash, care for, and often release affected birds. In both events, birds responded well to care. Blooms of *A. sanguinea* also occurred in 2004 and 2006, but birds were ostensibly not affected (Kudela *et al*., 2008). The species that were affected in the 2007 and 2009 events largely comprised nearshore feeders, which had a higher likelihood of encountering wave-produced foam. These events highlight the level of response that is possible when beach surveys coupled with wildlife rehabilitation efforts are engaged in tandem.

6.2.5 Diarrheic Shellfish Poisoning (DSP)

DSP is caused by okadaic acid (and the derivatives dinophysistoxins and pectenotoxins), a toxin produced by a number of dinoflagellates species, including *Dinophysis* sp. These toxins are often linked to human illness after shellfish consumption. It has as of yet not been directly implicated in the deaths of seabirds, but has been present during die-off events where mixed arrays of toxins were present. In the summer of 1992–1993 in New Zealand, there was a large suite of mixed HAB toxins present in the water. including those associated with NSP, PSP, ASP, and DSP, as well as others. At that time, there were several reports of human illness and contaminated shellfish in tandem with reports of deaths of Little Blue Penguins (*Eudyptula minor*), shags (*Phalacrocorax* spp.), gulls (*Larus* spp.), and Sooty Shearwaters among other species (Jasperse, 1993; Shumway *et al*., 2003); however, bird deaths were not directly linked to the HAB toxins. In 1996 and 1997, there

were noted large die-offs of Black-legged Kittiwakes (*Rissa tridactyla*) in the northeast United Kingdom (Coulson and Strowger, 1999). PSP toxins were believed to be the culprit, but mussels in the area did not test positive for these toxins. Algae that cause DSP are, however, known to be present in the area of the die-off and may have been affecting birds (Coulson and Strowger, 1999; Shumway *et al*., 2003).

6.2.6 CyanoHAB

Cyanobacteria (sometimes referred to as *blue-green algae*) can also produce harmful algal toxins, and such blooms (cyanoHAB) are increasing worldwide (Chorus and Bartram, 1999). *Cyanotoxin* is a term broadly used to describe toxins that may originate from various bacterial species; these toxins include neuro-, cyto-, and hepatotoxins, as well as irritants and gastrointestinal toxins (Codd *et al*., 1999, 2005). The most common cyanotoxins are microcystins, which are primarily produced by *Microcystis aeruginosa*; however, they are also produced by *Planktothrix*, *Anabaena*, *Anthrospira*, *Oscillatoria*, *Nostoc*, and other *Microcystis* spp. (Table 6.2). Nodularins are another type of cyanotoxin, produced by *Nodularia* spp. (Table 6.2). CyanoHAB are commonly associated with eutrophication spikes that promote algal scums and blooms in planktonic environments, and mats or films of benthic species (Codd *et al*., 2005). Most contemporary reported events involving bird mortality are caused by cyanobacteria in freshwater systems, although some toxin-producing cyanobacteria also occur in brackish or marine environments.

Eutrophic algal blooms are common ecological phenomena, and some studies have highlighted prehistoric indications of HAB-linked mortality events by linking preserved animal remains to calcified cyanobacteria layers in the sediment (Braun and Pfeiffer, 2002; Koenigswald *et al*., 2004). Humans have likely encountered the effects of cyanoHAB blooms since early antiquity, with Höger (2003) suggesting that Old Testament biblical accounts of mass fish kills are likely indicative of booms of the cyanobacteria *Planktothrix*. In the contemporary era, cyanobacterial poisoning in cattle and horses was first reported in a letter to the editor in the journal *Nature* by Francis (1878). The first report of birds experiencing negative interactions with freshwater algal toxins was in a review written by Fitch *et al*. (1934), which reviewed five incidences of toxin-producing freshwater blooms between the years 1918 and 1934, wherein poultry and other small experimental animals were affected by freshwater algal toxins. Later reports examined the physiological and behavioral effects of cyanobacterial intoxication on laboratory and domestic animals, including ducks and chickens (Steyn, 1945; Dillenberg and Dehnel, 1960).

In wild populations, most cyanoHAB-related mortality events are reported in freshwater waterbirds and shorebirds, rather than marine seabirds; however, the characteristics of these events are useful for establishing a broader understanding of the potential effects and symptoms of cyanoHAB, and are thus presented here. There have been several reports of mass mortality events in Lesser Flamingos (*Phoeniconaias minor*) in Tanzania and Kenya since 2003, all of which reported the cyanobacterium *Arthrospira fusiformis* in the guts of deceased flamingos (Krienitz *et al*., 2003; Lugomela *et al*., 2006). This cyanobacterium is known to produce the hepatotoxin microcystin and the neurotoxin anatoxin-a, both of which were implicated among the symptoms of flamingo deaths and were found to concentrate in flamingo feathers in a subsequent analysis (Metcalf *et al*., 2013). Another variety of microcystin was reported by Park *et al*. (2001) in a Canadian prairie lake, Pakowki Lake, with the study authors suggesting a previous mass waterbird mortality event in 1997 was plausibly caused by microcystin toxicity. Supporting this suggestion, dead birds were again reported near microcystin-containing blooms in Lake Ontario and Lake Erie, although bird carcasses were not examined for presence of toxins or toxicity effects (Murphy *et al*., 2003). In Belgium, the deaths of approximately 30 ducks and herons were associated with *M. aeruginosa* blooms in three adjacent lakes, and there was blood found in the intestinal tracts that the authors argued as indicative of microcystin ingestion (Wirsing *et al*., 1998). Other incidence reports suggesting microcystin or anatoxin poisoning are compiled in Table 6.1.

More recently, a cyanobacteria that grows on an introduced aquatic plant species (*Hydrilla verticillata*) has been implicated in avian vacuolar myelinopathy (AVM). AVM causes neurological symptoms in birds, and is caused by ingestion of the epiphytic cyanobacteria (Thomas *et al*., 1998; Rocke *et al*., 2002; Wilde *et al*., 2005; Backer and Miller, 2016). This has been noted in American Coots (*Fulca americana*) and Mallard Ducks (*Anas platyrhynchos*), as well as Bald Eagles (*Haliaeetus leucocephalus*) that fed on infected coots (Thomas *et al*., 1998; Rocke *et al*., 2002; Wilde *et al*.,

2005). Research by Wilde *et al.* (2005) reports that the previously unknown hazardous cyanobacteria is likely a species of *Stigonematales*. This is perhaps particularly interesting, because this phenomenon has been documented very few times, in very few places, and during times when bloom events are not otherwise apparent (Wilde *et al.*, 2005; Backer and Miller, 2016). The cryptic nature of these circumstances may mean that similar *Stigonematales* outbreaks are significantly underreported.

With increased eutrophication, warming temperatures, and increased nutrient loading, freshwater cyanoHAB have become an emerging issue in marine environments (Miller *et al.*, 2010; Gibble and Kudela, 2014; Fetscher *et al.*, 2015). *M. aeruginosa*, a common freshwater cyanobacterium discussed in this chapter, and associated toxins (microcystins) have recently been detected in the nearshore marine environment in central California (Miller *et al.*, 2010; Gibble and Kudela, 2014). This species can endure saline environments (Robson and Hamilton, 2003; *et al.*, Tonk 2007; Miller *et al.*, 2010; Ross *et al.*, 2010; Gibble and Kudela, 2014), giving it the potential to interact with wildlife in the marine environment (Ross *et al.*, 2010). Trophic webs may transport microcystin from coastal regions to marine systems, and the uptake and retention of microcystins in common marine prey items in the central California area have recently been confirmed through experimental trials (Miller *et al.*, 2010; Gibble *et al.*, 2016). Gibble *et al.* (2017) utilized blood collection cards to detect microcystin toxicity in marine and estuarine birds, and two species of seabirds (Common Murres and Surf Scoters) tested positive for microcystin in the blood. While this is the first documentation of microcystin toxin in the blood of marine seabirds, these results do not directly implicate this toxin as a cause of mortality. Further testing, as well as improvements in analytical tools and techniques, is required to understand the interactions between microcystin and marine birds, as well as to clarify the risk of potential detrimental effects.

Nodularins are another type of cyanoHAB-produced cyanotoxin that are generally associated with inland waters (McGregor *et al.*, 2012), but have recently been reported in the marine environment. These hepatotoxins attack the liver and have been shown to affect seabirds adversely at very low exposure levels. Sipiä *et al.* (2004) found that Common Eiders (*Somateria mollissima*) obtain this toxin by foraging on their most common prey item, Blue Mussels (*Mytilus edulis*), in the first demonstration of nodularins affecting seabirds. Sipiä *et al.* (2006) also found nodularins present in minor quantities in eider liver and muscle samples during die-off events in 2002 and 2004, with higher toxin concentrations in younger eiders, a pattern the authors attributed to migration patterns. Younger eiders tend to migrate in October, and are present during summer cyanobacteria blooms; older eiders leave between June and mid-August, and may avoid the blooms. A later study by Sipiä *et al.* (2008) examined breast feathers and liver samples from eiders in tandem with Blue Mussels, and found evidence of nodularins in both eider and mussel tissue. Interestingly, because the filaments of the cyanobacteria *Nodularia* stick to the feathers of eiders, the authors proposed that feather examination could provide a means for nonlethal examination of this toxin.

6.3 Improved Monitoring and Establishment of Causality

6.3.1 Coordinating Monitoring and Pathology to Confirm Relationships between HAB and Seabird Mortality

A common theme among many of these studies and incidence reports, unfortunately, is that biopsy sampling, necropsies, and testing for toxins are rarely conducted, and associations between bird mortalities and algal blooms often rely on anecdotal evidence, that is insufficient for clearly implicating algal toxins as the cause of death. In part, this reflects the nature of opportunistic incidence reports on seabird mortality events, which are typically descriptive in nature and often unable to mount any rigorous analytical inquiry into the cause of death. While these reports do aid in documenting the history and frequency of events, these reports are typically correlative, and rarely discuss how cyanotoxins may be causing death, or establish threshold toxin values in blood or organs that result in mortality. Similarly, toxin concentrations in blood, tissue, or feathers are rarely measured or sampled for later analysis, or are measured in just a few opportunistic samples that may not be representative of the overall effects of the toxins on a population in question. Furthermore, most incidence reports are likely to severely underestimate the number of affected individuals (Shumway *et al.*, 2003).

Incident reports that are largely qualitative in nature, rather than quantitative, make it difficult to compare the magnitude of mortality events. Some

reports may estimate mortality to be tens of thousands, while other reports may only mention the representative species affected; however, the prevalence of different seabird taxa affected by HAB can still be assessed (Figure 6.2). Figure 6.2 suggests that near-shore diving birds (grebes, loons, and duck) and gulls and terns are predominantly reported, with more pelagic seabirds not reported as frequently. This may reflect several possibilities: the nearshore foraging habits and ecology of some species bring them into increased exposure with coastal HAB blooms; the nearshore distribution of these species makes the beach deposition of carcasses more likely; and the species are easier to monitor and study relative to more pelagic species. Increased monitoring and testing of all recovered carcasses, whether beachcast or acquired via bycatch or at the colony, may help differentiate some of these possible explanations.

The historic difficulty of establishing causality between HAB events and seabird mortality may partly be due to poorly coordinated efforts linking stranding recoveries and systematic necropsy. Systematic beach surveys to assess trends in seabird carcass deposition are typically limited to populated and accessible coastlines. There are relatively

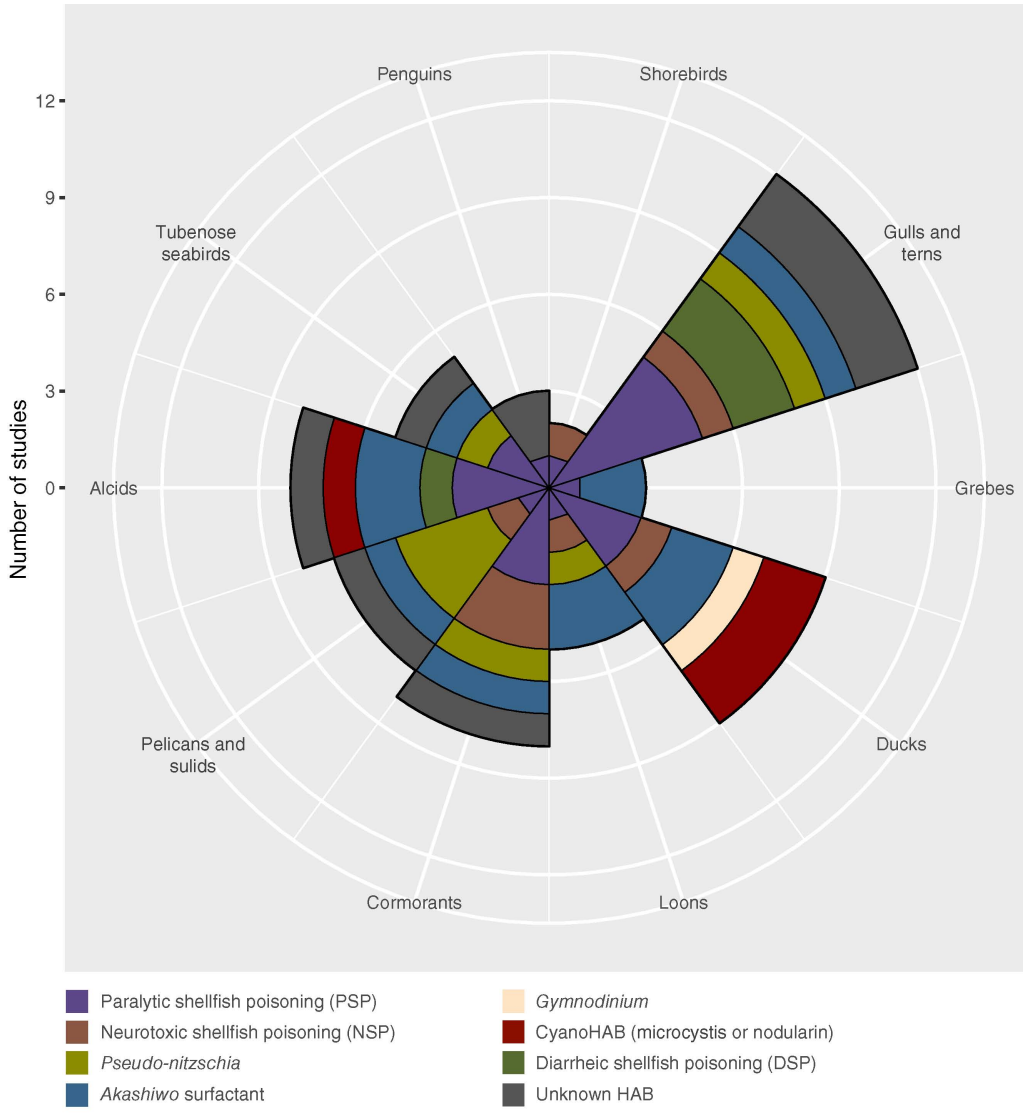

Figure 6.2 Pie chart depicting prevalence of different seabird taxa in cited seabird–HAB interactions. The polar axes show the number of times a seabird taxon was recorded in a study, incident report, or unpublished report. Colored stacks depict the HAB type associated with each study, organized by respective seabird taxon. Seabird taxa are used as a rough proxy for trends in life-history and ecological niches, and are ordered roughly here by nearshore to off-shore distribution.

few necropsy facilities that can process and examine dead birds, and it can be an arduous endeavor to legally obtain and transport beachcast birds to the nearest such facility. When surveys and facilities are available, precise coordination and adequate funding are required to integrate environmental monitoring and algal blooms. These types of collaborations are increasing, with beachcast bird survey programs and pathology labs now successfully running in parts of Europe, North America, New Zealand, and South Africa (Wiese and Elmslie, 2006). Monitoring efforts, however, are absent or infrequent in most other regions, leaving large gaps in coverage and likely resulting in a significant underestimate of global seabird mortality events (Benson *et al*., 1999; Camphuysen and Heubeck, 2001; Roletto *et al*., 2003; Parrish *et al*., 2007). Postmortem protocols for necropsy and histopathology procedures are not always standardized across regions, making it difficult to compare results.

There are some existing theories that marine birds have the ability to avoid consumption of contaminated food sources (Shumway *et al*., 2003). These reports are both anecdotal and experimental in nature. Perhaps the first documented anecdotal avoidance was noted by Coulson *et al*. (1968a), in which it was suggested that Common Eiders (*Somateria mollissima*) were able to diminish the toxic effects found in their prey. This conclusion was based on the fact that, when toxins were high in the mussels (*Mytilus edulis*) on which eiders normally feed, very few eiders were found dead. This was later followed up with reports in the 1990s that experimentally showed that eiders can actively select prey with the highest meat-to-shell ratio (Bustnes and Erikstad, 1990; Bustnes, 1998); and, later in unpublished laboratory studies, eiders refused toxic mussels when offered toxic and nontoxic mussels in the same trials. During these experiments, if the eiders were force-fed toxic mussels, they rapidly regurgitated the prey items (Shumway *et al*., 2003). Black Oystercatchers (*Haematopus bachmani*) were also shown to reject prey that was contaminated with algal toxins. Faxla (1992) documented both partial consumption of prey contaminated with PSP toxins, but also prey-switching behavior in oystercatchers. Shumway *et al*. (2003) suggested that since oystercatchers may have sensory nerves in their bills that may detect neurotoxins in their prey (Gill, 1995; Goss-Custard, 1996), this characteristic may aid in the active avoidance of contaminated food items. Glaucous-winged Gull (*Larus glaucescens*) chicks were also purported to have avoided PSP toxins as reported by Kvitek (1991), in which gulls that were fed toxic butter clams (*Saxidomus giganteus*) immediately regurgitated. Subsequent to being fed toxic clams, the chicks would refuse to eat any butter clam (toxic or nontoxic). During feeding trials in the same study, adult and juvenile gulls were also noted to forage on fewer butter clams in areas where these clams were known to be chronically toxic than areas where the clams were not known to be toxic (Kvitek, 1991; Shumway *et al*., 2003). If birds are able to actively avoid toxins, these studies may indicate that birds that do not have the same types of experiences with HAB toxins or are completely naïve to the experience, may not exhibit active avoidance, and therefore may be at greater risk for negative interactions (Shumway *et al*., 2003).

6.3.2 Seabirds as Biological Indicators

Indicator organisms are species characteristic of a particular environment, whose perturbations in population health, reproduction, and survival directly reflect changes in environmental variables and may consequently serve as early ecological warnings (Herricks and Schaeffer, 1985; Noss, 1990; Carignan and Villard, 2002). Small invertebrate organisms such as shellfish have typically been used to investigate and monitor the effects of HAB on water quality in marine and freshwater systems (Phillips, 1977; Powell, 2002; Shumway *et al*., 2003). For example, since 1985, bivalves in the United States have been routinely monitored for contaminants including HAB via the NOAA Mussel Watch Program (Wade *et al*., 1998). Using seabirds as indicators of water quality and biotoxins may provide a far larger and more wide-ranging perspective. Seabirds are already well-known biological indicators of ecosystem health and function (for discussions of biological indicators of habitat quality, see Herricks and Schaeffer, 1985; Noss, 1990; Carignan and Villard, 2002; for reviews of seabirds as indicator species, see Cairns, 1988; Piatt *et al*., 2007; Durant *et al*., 2009). Seabird distribution varies by species, but they are distributed throughout the world and encompass a wide variety of habitats and trophic niches. The behavior and population demographics of various species are well documented with many long-term data sets available, and fluctuations in these trends have been used to monitor for changing conditions in prey abundance, pollution, and oceanography (Boersma, 1978, 1986; Cairns, 1988; Becker, 1989; Furness and Camphuysen, 1997). Despite this utility, seabirds are currently underutilized resources for monitoring

environmental change pertaining to blooms of toxic and harmful algae. Any perturbations in colony health, foraging range, or population demographics are typically analyzed with respect to changing environmental conditions, and these conditions should include pertinent levels of toxin concentration or harmful algal species in the vicinity. For colony studies and routine bird captures, nonlethal sampling such as feather analyses and blood or fecal smears provides the means to analyze birds for cyanobacterial toxins in a nonlethal method (Metcalf *et al*., 2013; Gibble *et al*., 2017). With this in mind, some studies have recently advocated for a mandated variety of routine samples and analyses, including testing for the presence and effect of algal biotoxins, to be incorporated as a base monitoring standard during scientific studies or monitoring efforts on seabirds and waterbirds (Mallory *et al*., 2010).

6.4 Implications for Conservation

Approximately 25% of all seabird species are listed as threatened or species of special concern, representing one of the most threatened taxonomic groups (Croxall *et al*., 2012; IUCN, 2016). Furthermore, half of the world's seabird species are currently estimated to be experiencing a population decline (although some species have increased over the same time period, likely in response to increased fisheries offal and changing trophic niches; Croxall *et al*., 2012). These broad declines indicate that seabirds, as a taxon that relies on both marine and terrestrial habitats, have been significantly impacted by a broad spectrum of conservation threats. These challenges include the spread of invasive species, coastal development and colony habitat loss, bycatch mortality, pollution (including oil spills and plastic ingestion), prey depletion, hunting, and environmental factors associated with climate change (Anderson and Keith, 1980; Fowler, 1999; Votier *et al*., 2005; Jones *et al*., 2008; Grémillet and Boulinier, 2009; Anderson *et al*., 2011; Cury *et al*., 2011; Croxall *et al*., 2012). Climate change, in particular, may amplify the effects of many of the aforementioned factors, as well as facilitate local and global trophic changes that may indirectly benefit or harm specific seabird species (Sydeman *et al*., 2012). HAB clearly represent an emerging, yet infrequently quantified, threat to seabirds as well, particularly in response to changing climatic factors that may increase the magnitude and severity of bloom events (Anderson, 2009). Rising temperatures and increased eutrophication may increase the frequency of known recurring blooms, and stimulate blooms in new regions (Paerl and Huisman, 2008).

In general, seabirds are long-lived with relatively low fecundity and delayed sexual maturity, meaning adult survival is typically high and population changes occur slowly (Croxall and Rothery, 1991; Buckley and Downer, 1992; Weimerskirch *et al*., 2001). Consequently, adult mortality events can have long-lasting demographic effects and significantly delay population recovery (Nur and Sydeman, 1999). Seabird populations often retain a significant pool of adult nonbreeders, perhaps due to the constraints of appropriate breeding sites or available mates, and this pool provides a demographic buffer against some level of mortality loss (Weimerskirch *et al*., 2001). In the context of HAB, it is important to ascertain the effects on the adult breeding population in order to predict future demographic trends and recovery potential.

Seabirds also exhibit different life history strategies, in terms of clutch size, longevity, and habitat utilization, which may render them particularly vulnerable to the effects of HAB and subsequent population recovery. Some taxa, such as the shags and cormorants, lay a clutch of several eggs and exhibit a relatively k-selected life history tactic compared to long-lived and slow breeders such as the tubenose procellariforms or alcids (Weimerskirch *et al*., 2001). Cormorant populations can increase by 20% per year under ideal conditions (Frederiksen *et al*., 2001), and similar species with higher fecundity and population turnover may be able to recover more quickly from large mortality events. The foraging strategy and habitat associations of different seabird guilds may also affect the likelihood of exposure to HAB. For example, within a seabird community, specific species may preferentially utilize nearshore or offshore habitats, specialize in different prey types, and forage using plunge diving, pursuit diving, or surface foraging (Ainley, 1977; Ballance *et al*., 1997; Henkel, 2009). Foraging strategy and prey preference can increase time spent in a particular environment, and nearshore species may be more susceptible to HAB that are more prevalent in coastal regions. This is partially supported in Figure 6.2, showing a strong signal in coastal waterbirds such as grebes, ducks, and loons, suggesting that coastal species may be disproportionately affected. However, nearshore species are also likely to be disproportionately

Table 6.3 What to do if you find a bird with a potential HAB interaction.

Condition of bird	Resource	Instructions
Single bird or group of birds are alive and struggling	Local wildlife rehabilitation center	Contact your local wildlife rehabilitation center, and provide the exact location and status of the affected bird. Record as much information as possible.
Single bird is fresh dead*	Statewide or regional bird research facility	Contact your nearest statewide or regional bird research facility. You will likely be asked to leave the bird on the beach. You may be asked to collect and freeze or refrigerate the birds so that they can be sent to the facility.
Large group of birds are fresh dead*	Statewide or regional bird research facility	Contact your nearest statewide or regional bird research facility or National Wildlife Health Center. You may be asked to collect and freeze or refrigerate the birds so that they can be sent to the facility.
Single bird or group of birds are dead and scavenged	Statewide or regional bird research facility, or local beachcast bird survey program	Leave the carcass on the beach so that it may be recorded in the beach survey. Alert your nearest statewide or regional bird research facility of a potential HAB interaction event if there is a large group.

* Fresh dead: The eyes are still intact. The bird has died within a few hours. There is no scavenging.

observed, as pelagic species may be less likely to be deposited and quantified during beachcast mortality surveys.

HAB may also indirectly affect seabirds by poisoning prey resources, incurring starvation or relocation for resident seabirds. Such effects may be particularly pronounced on nearshore species that feed on benthic organisms that bioaccumulate toxins. While some shorebird and waterfowl species may be able to discriminate between prey that exhibits different concentrations of toxins (Kvitek, 1993; Bustnes, 1998; Kvitek and Bretz, 2005), large blooms may prohibit efficient relocation, and changes to foraging range and foraging efficiency are costs that affect stress levels and reproductive success (Suryan *et al*., 2000).

To better manage and ensure the future viability of seabird populations, it is imperative to investigate and incorporate the risks posed by HAB to regional seabird populations (Table 6.3). This could include the implementation of standardized necropsy protocols when seabird die-offs occur, and protocols for systematic testing of probably algal biotoxins. The future location and severity of HAB, and the effects on regional seabird populations, should also be investigated with the use of predictive models and spatial analyses of current trends. Spatial and temporal predictions of future "hotspots" of algal blooms can be overlaid on known spatiotemporal seabird distributions, to determine likely problem areas in the future. Using predictive models would ultimately help mobilize the implementation of policies to reduce nutrient run-off and other variables likely to increase the risk of algal blooms.

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7

Food Web and Ecosystem Impacts of Harmful Algae

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7.1 Introduction

This chapter considers food web and ecosystem-level impacts of major groups of harmful algae in U.S. and nearby waters, including high-biomass "blooms" (outbreaks) of nontoxic, allelopathic, or toxic algae that are visually noticeable and discolor the surrounding area (water or substrata); allelopathic algae in smaller populations whose bioactive substances have major impacts on other organisms; and low-biomass outbreaks of highly toxic microalgae can cause adverse effects on food webs at low cell concentrations without visible water discoloration. The cyanobacteria (blue-green algae) are included here, based on their major ecological role as primary producers (Burkholder, 2002, 2009 and references therein). Routes of exposure to harmful algae can be *direct*, via consumption of toxic algae, cell surface contact (sometimes causing physical damage), or exposure to algal toxins (e.g., in exudates or in the surrounding water), or *indirect* through related habitat degradation (e.g., oxygen deficits, shifts in community composition), transfer of toxins across trophic levels (bioaccumulation, biomagnification), or consumption of toxin-laced prey (e.g., Figures 7.1 and 7.2). Food web effects from exposure to harmful algae are also sustained by early life stages of grazers, with ramifications for recruitment and, therefore, future generations.

Harmful algal blooms (HAB) cause a "vast array" (Landsberg, 2002) of food web effects across multiple trophic levels. Surprisingly, such blooms have seldom been implicated in modifying trophic cascades, but there is increasing recognition that they do so through many mechanisms (Shumway *et al.*, 2003; Karjalainen *et al.*, 2007; Kvetik and Bretz, 2004, 2005; Branch, 2008; Casini *et al.*, 2008). They disrupt ecosystem function by decreasing biodiversity and altering energy flow within food webs, and they also cause low oxygen stress and/or destroy important habitats such as submersed vegetation meadows (below).

Research emphasis on impacts from harmful algae overwhelmingly has been anthropocentric. The published literature contains a wealth of information about effects on mammals (e.g., mice and rats as surrogates for humans) and modes of action of major classes of microalgal toxins, especially microcystins (MCs) from mostly freshwater cyanobacteria; saxitoxins (STXs), brevetoxins (BTXs), ciguatoxins, dinophysistoxins, and other toxins from marine dinoflagellates; and domoic acid (DA) from a small group of marine diatoms. Not until the studies by Shumway and colleagues in the early 1980s was there a focus on impacts of these toxins on invertebrates and other lower organisms (see Basti *et al.*, 2018 – Chapter 4). Moreover, nearly all of what is known about food web impacts from *toxigenic* (potentially toxic) microalgae is piecemeal, based on acute toxicity tests with one to a few strains (populations) of fauna or flora (other microalgae and macroalgae, aquatic vascular plants – Table 7.1).

These tests often have been conducted with purified toxins rather than with the toxic microalgae themselves. Use of purified toxins in such

Harmful Algal Blooms: A Compendium Desk Reference, First Edition. Edited by Sandra E. Shumway, JoAnn M. Burkholder, and Steve L. Morton.

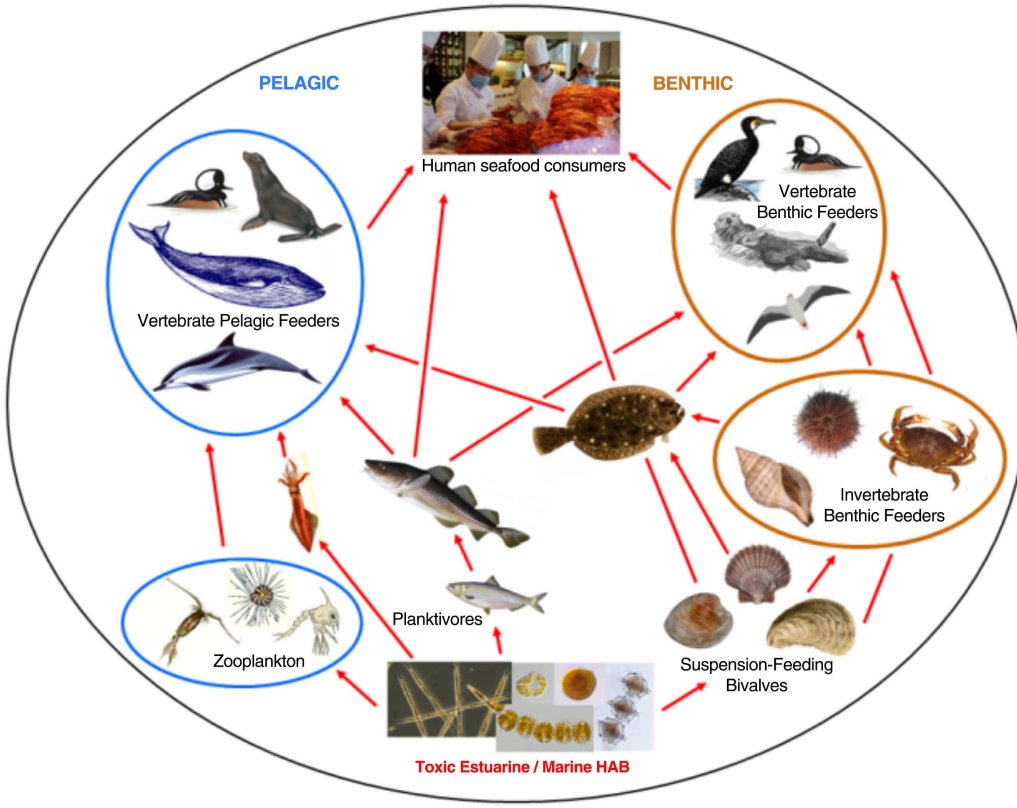

Figure 7.1 Simplified conceptual diagram showing how toxins from toxic algae can move through both pelagic and benthic communities of food webs, here depicting a north temperate marine coastal region. Note that arrows from the higher trophic levels in both pelagic and benthic routes can involve the same species, such as various ducks. Not shown are other complexities, such as when squid consume other squid, or squid eat fish, or invertebrate benthic feeders consume small flounder and small birds, etc.

research is important for verification purposes, but the data are difficult to extrapolate to the impacts of toxigenic algae in their natural setting (e.g., DeMott and Dhawale, 1995; Lürling and van der Grinten, 2003; Zurawell *et al.*, 2005; and see below). While there is ample evidence that *chronic/sublethal* effects of toxigenic microalgae are much more pervasive and ecologically important than acute impacts (Table 7.2), such effects generally are much more difficult to track or quantify (Burkholder, 1998). Mortality of aquatic fauna has been emphasized and (for cyanobacteria) of terrestrial animals of human interest such as livestock, based on acute exposures to selected purified toxins from harmful microalgae (Table 7.1).

Even less common than food web studies that include chronic/sublethal effects from harmful algae are *ecosystem-level analyses* of their impacts. High-biomass blooms can be more easily linked to ecosystem-level analysis than low-biomass outbreaks of some toxigenic algae, because high-biomass blooms often cause hypoxia or anoxia and some obvious, pervasive impacts across trophic levels (Figure 7.2). Thus, although it has been recognized that many HAB severely alter or degrade ecosystem function (Sunda *et al.*, 2006), the actual number of ecosystem-level analyses in the published literature remains very small (e.g., Landsberg *et al.*, 2009).

In this chapter, *food web impacts* are loosely defined as effects (mostly negative, but also sometimes positive) on organisms from one or more trophic levels. An overview is also provided of the present status of *ecosystem-level impacts*, considered as effects on the entire food web structure. Future directions are suggested to fill some major gaps in scientific understanding about influences of harmful algae on food webs and ecosystems from both ecological and economic perspectives.

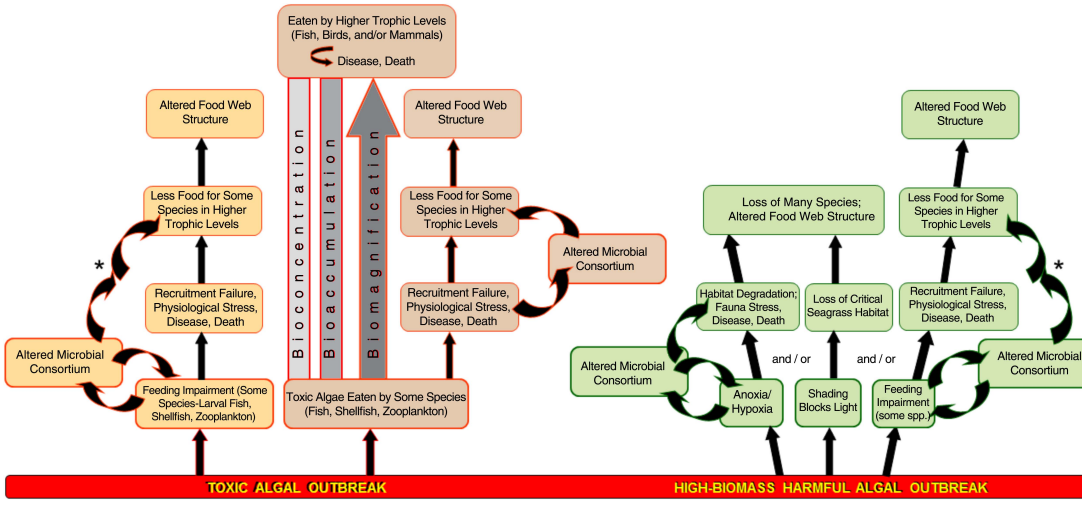

Figure 7.2 A conceptual model of food web effects and ecosystem-level changes from a toxic algal outbreak (bloom; left [gold boxes] and center [brown boxes], with toxin involvement or movement indicated by boxes and arrows, respectively, with red outlines) versus a high-biomass harmful algal outbreak (bloom; right, green boxes). Asterisk (*) indicates that multiple steps can be involved.

Left (gold boxes): Impacts that can occur, depending on the toxin(s), concentration(s), species, and life stage/physiological condition, when grazers avoid consuming the toxic algae or when feeding is inhibited.

Central (brown boxes): Impacts that can occur when toxic algae are eaten by grazers. Three processes (in shades of gray with red outline) involving the toxin(s) can occur depending on the organisms, their life stage/physiological condition, and the toxin congener(s)/concentration (from Gray, 2002; also see Doucette *et al.*, 2006b): *Bioconcentration* refers to uptake of the toxin directly from the water, resulting in a higher concentration of the toxin in the organism than in the water. *Bioaccumulation* is a process that causes an increased chemical concentration in an aquatic organism compared to the water, due to uptake by all exposure routes. *Biomagnification* is the process of transfer of the toxin from food to an organism, resulting in a higher concentration in the organism than its toxic food item. The overall result is a much higher concentration of the toxin as it moves up the food web to organisms at higher trophic levels. Note that one or more of these processes may be operable, depending on the toxin and the organisms.

Right (green boxes): Impacts that can occur from a high-biomass harmful algal bloom, here depicted as not involving toxin production.

In situations where toxic algae form high-biomass blooms, all of these pathways and impacts can occur. Not shown are all of the pathways involving toxic algae as mixotrophs consuming a wide array of other microbial prey (see Jeong *et al.*, 2010).

Table 7.1 Aquatic biota that have been killed by blooms of some selected harmful algae in U.S. and neighboring waters, emphasizing toxigenic microalgae but also including a few examples of otherwise-harmful microalgae and filamentous macroalgae as noted (Lapointe *et al.*, 2018 – Chapter 15). Note that the major algal group is indicated where the genus is first mentioned. This table is not meant to be complete but, rather, to provide examples of food web effects.

Taxon (taxa) and organisms killed
Cyanobacteria (blue-green algae, Cyanophyta)[a]
General: Cyanotoxins can bioaccumulate (Ettoumi *et al.*, 2011 and references therein).
Anabaena flos-aquae [F; T (strain-dependent), low DO]: MCs, anatoxins (also see impacts under *Microcystis aeruginosa*/other MC producers). From Schwimmer and Schwimmer (1968 and references therein), unless otherwise noted:
Mammals: Various wild animals – foxes, squirrels, mink, muskrat, skunks; domestic – cats, cows, dogs, horses, sheep, swine (also see Rose, 1953; Moore, 1977).
Birds: Wild birds – ducks, Franklin's gulls, coots, pheasants, hawks, herons, songbirds; domestic – chickens, ducks, turkeys (also see Rose, 1953; Moore, 1977).
Reptiles: Snakes.
Amphibians: Salamanders.
Fish: Buffalo fish, carp, black bullhead [low DO?]; perch (also see Rose, 1953; Hammer, 1968).
Aphanizomenon flos-aquae [F; T (strain-dependent), low DO] – Aphantoxins, CYL, STXs[b] (also see impacts under *Cylindrospermopsis raciborskii*, other CYL producers)
Mammals: Domestic cattle (Nelson, 1903, in Fitch *et al.*, 1934).
Amphibians: Tadpoles (Farrão-Filho and Kozlowski-Suzuki, 2011 and references therein).
Fish:
Massive fish kill following collapse of blooms in eutrophic lakes (Barica, 1978).
American eel, black bullhead, black crappie, bluegill sunfish, buffalo, carp, hog sucker, northern pike, perch, suckers, yellow pike (Mackenthum *et al.*, 1948).
Cylindrospermopsis raciborskii[c] (F; T [strain-dependent]: CYL, deoxy-CYL, STX, anatoxin; Zagatto *et al.*, 2012)
Mammals: Domestic cattle (Saker *et al.*, 1999).
Fish: Zebrafish larvae (Zagatto *et al.*, 2012).
Amphibians: Cane toad (White *et al.*, 2007).
Cnidarians: Larvae of the coral *Acropora surculosa* (Kuffner and Paul, 2004).
Arthropods: Brine shrimp (Metcalf *et al.*, 2002).
Zooplankton: *Daphnia magna* (Nogueira *et al.*, 2004).
Daphnia similis, Ceriodaphnia dubia (Zagatto *et al.*, 2012).
Lyngbya majuscula[c] [M, benthic macroalga; T (strain-dependent): aplysiatoxin, debromaplysiatoxin, kalkitoxin, LYNGTX; B = ~80 other biologically active chemicals (strain-dependent) – Wu *et al.*, 2000; Osborne *et al.*, 2001; Le Page *et al.*, 2005; Taylor *et al.*, 2014]
Fish: Goldfish (Wu *et al.*, 2000: toxin assays).
Arthropods:
Blue shrimp (raceway-reared); necrosis of the lining of the epithelium of the midgut, dorsal caecum, and hindgut, and hemolytic enteritis (Lightner, 1978; Osborne *et al.*, 2001).
Brine shrimp (Wu *et al.*, 2000: toxin assays).
Cnidarians: Larvae of staghorn coral, *Acropora surculosa*; more generally, scleractinian corals and gorgonians (Kuffner and Paul, 2004).
Seagrasses: Round-leaf seagrass, shoalgrass (Watkinson *et al.*, 2005; Tilling, 2007).
Red algae (Rhodophyta): Crustose coralline macroalgae (Kuffner and Paul, 2004 and references therein).
Microcystis aeruginosa (and various other ***Microcystis*** spp., and other MC producers.[b]) [Planktonic; can also be benthic; F, Br; T (strain-dependent) and low DO. MCs, cyanoginosins, cyanoviridin, LPSs, BMAA, anatoxin-a, unidentified volatile sulfur compounds (Fristachi and Sinclair 2008; Rastogi *et al.*, 2014 and references therein).] From Schwimmer and Schwimmer (1968), Codd (1995), and Codd *et al.* (1996) unless otherwise noted:

Table 7.1 (*Continued*)

Taxon (taxa) and organisms killed
Mammals: Southern sea otter (Miller *et al.*, 2010); domestic – cattle (Puschner *et al.*, 1998), dogs, horses, pigs, sheep.
Birds: Heron, snipe, ducks, geese, pheasants; domestic – chickens, ducks, geese, turkeys; waterfowl may be at higher risk because they feed on floating cyanobacterial scums (also see Ibelings and Havens, 2008).
Fish: Major fish kills; toxin-induced mortality of embryos of loach (Liu *et al.*, 2002), medaka (Jacquet *et al.*, 2004), and zebrafish (Oberemm *et al.*, 1999), and of juvenile carp (Osswald *et al.*, 2007).
Arthropoda: Crustacea other than zooplankton – Brine shrimp (Akin-Oriola and Lawton, 2006).
Zooplankton:
Microcrustaceans – *Daphnia ambigua* (Fulton and Paerl 1987), *D. galeata* (Rohrlack *et al.*, 1999), *D. magna* (Nizan, 1986), *D. parvula* (Fulton, 1988b), *Eucypris virens* (Stangenberg, 1968), *Monia macrocopa* (Yasuno and Sugaya, 1991), *M. micrura* (Liu *et al.*, 2006).
Rotifers – *Brachionus calyciflorus*: by consuming toxic cells, or by passive uptake of dissolved toxin from the surrounding medium (Starkweather and Kellar, 1987; Zhao *et al.*, 2014; but conflicting information depending on the *M. aeruginosa* strain and other factors). *Brachionus rubens* (Rothhaupt, 1991).
Benthic member(s) of the order Stigonematales (e.g., epiphyte of *Hydrilla*) [F; T: BMAA (Corbell *et al.*, 2014)][d]
Birds: Via avian vacuolar myelinopathy, mostly in bald eagles and American coots, from an uncharacterized cyanobacterial neurotoxin.[d]
Synechococcus (with ***Synechocystis* sp.** – Richardson, 2004) [T: BMAA (Cox *et al.*, 2005; also see Martins *et al.*, 2005); cultures produced substances with neurotoxic and hepatotoxic effects – Brand *et al.*, 2010; EDAB][e]
Crustaceans: Juvenile spiny lobsters (via loss of sponge habitat; Butler *et al.*, 1995).
Cnidarians: Reef-building corals on patch and bank reef ecosystems (e.g., *Montastrea annularis, Porites porites*; Tomascik and Sander, 1985, 1987).
Poriferans: Branch candle sponge, gray-purple sponge, loggerhead sponge, sheepswool sponge, stinker sponge, and vase sponge – implicated in mass mortalities (Tomascik and Sander, 1985, 1987; Lapointe and Clark, 1992; Butler *et al.*, 1995; Diersing, 2009 and references therein).
Trichodesmium spp. (e.g., *T. thiebautii*, *T. erythraeum*) [M, T (strain-dependent): ciguateratoxin-like activity and saxitoxins-like properties (neurotoxicity with paralytic symptoms) – Kerbrat *et al.*, 2010; trichamide, a cyclic peptide produced by senescing blooms, may have mild cytotoxicity and likely is used in anti-predation defense – Sudek *et al.*, 2006]
Zooplankton: *Macrosetella gracilis* (O'Neil and Roman, 1994), *Penaeus merguiensis* (Preston *et al.*, 1998).
Copepods: *Clausocalanus furcatus, Farranula gracilis* – Highly neurotoxic extracts from *T. thiebautii* blooms (but these species are not reported to consume *T. thiebautii* – Hawser *et al.*, 1992). *Note*: The natural grazers (*Macrosetella gracilis* and *Miracia efferata*) were not affected, suggesting that they may have developed resistance to the toxins.
Diatoms (Heterokontophyta – Bacillariophyceae)
Chaetoceros concavicornis (sometimes with ***C. convolutus***) [M: Str]
Fish:
Atlantic salmon (cultured in net pens). The primary mechanism appears to be reduction of gas exchange caused by mucus production when the gill epithelium is irritated by *Chaetoceros* setae wedged between the secondary lamellae.
Chinook salmon, Coho salmon, rainbow trout (Landsberg, 2002 and references therein).
Crustacea: Implicated in mortality of juvenile red king crabs, as *Chaetoceros* cells and setae were the dominant component of the debris in the mucus covering of damaged crab gills (Horner *et al.*, 1997 and references therein).
Toxic *Pseudo-nitzschia* complex [M; T (strain-dependent): DA, bioaccumulates]
Pseudo-nitzschia australis
Mammals: California sea lion, sea otter; multiple species of cetaceans and pinnipeds; minke whale; baleen whale, wherein DA was detected in the urine, gastric fluid, and feces. More than 560 otoliths from northern anchovies were identified from a whale's stomach, indicating recent feeding on that toxin vector prior to death (Scholin *et al.*, 2002; Fire *et al.*, 2010; Lewitus *et al.*, 2012 and references therein).
Birds: Brandt's cormorant, brown pelican, double-breasted cormorant, western gull (Fritz *et al.*, 1992; Work *et al.*, 1993).

(*continued*)

Table 7.1 (*Continued*)

Taxon (taxa) and organisms killed
Dinoflagellates (Dinophyta)
Akashiwo sanguinea[c] [M; B (strain-dependent); surfactant-like protein exudates, copious mucilage (Jessup *et al.*, 2009); T? (ROS hydrogen peroxide – Kim *et al.*, 1999); low DO – Mohamed and Al-Shehri, 2012; Badylak *et al.*, 2014a, 2014b and references therein]
General: Blooms have been the suspected cause of fish kills and marine mammal strandings (Badylak *et al.*, 2014a, 2014b).
Birds: Clark's grebe, common murre, northern fulmar, Pacific loon, red-throated loon, surf scoter, western grebe, white wing scoter; widespread seabird mortality (Landsberg, 2002 and references therein; Jessup *et al.*, 2009).
Fish:
Decomposition of a major bloom led to oxygen depletion and death of fish and shellfish (Horner *et al.*, 1997).
Anchovy, Atlantic bumper, Atlantic croaker, bluefish, Gulf menhaden, hardhead catfish, inland silverside, sand seatrout, silver perch, southern kingfish, southern stargazer, spadefish, spot, striped mullet, thread herring, threadfin (Harper and Guillen, 1989).
Molluscs:
Japanese littleneck clam, Olympia oyster, Pacific oyster – Larval stages (Shumway, 1990 and references therein).
Abalone – Larvae exposed to *A. sanguinea* blooms (Botes *et al.*, 2003).
Crustacea: Blue crab (Harper and Guillen, 1989).
Phytoplankton: *A. sanguinea* consumes microalgal prey such as *Alexandrium tamarense, Amphidinium carterae, Heterocapsa triquetra, Prorocentrum minimum*, and *Scrippsiella trochoidea* (dinoflagellates); *Isochrysis galbana* (haptophyte); *Rhodomonas salina* and a second unidentified taxon (cryptophytes); and *Heterosigma akashiwo* (raphidophycean; Jeong *et al.*, 2005a). Also consumes the cyanobacterium *Synechococcus* sp. (Jeong *et al.*, 2005b).
The ingestion rate of unicellular cyanobacteria by *A. akashiwo* was 62.9 ± 5.4 *Synechococcus* cells dinoflagellate^{-1} hour^{-1} (mean ± 1 standard error [SE]; Jeong *et al.*, 2005b).
Toxic *Alexandrium* complex [M; T (strain-dependent): STX and derivatives, which can bioaccumulate (e.g., Doucette *et al.*, 2006a)]
Alexandrium catenella [T: STX and derivatives, ROSs – Dorantes-Aranda *et al.*, 2015]
Fish: Post-smolt Atlantic salmon (Aguilera *et al.*, 2016).
Molluscs: Blue mussel, California mussel, eastern oyster, European flat oyster, gaper clam, littleneck clam, Nuttall's cockle, Olympia oyster, Pacific oyster, rock scallop, spiny scallop, weathervane scallop (Shumway, 1990).
Phytoplankton: The ingestion rate of unicellular cyanobacteria by *A. catenella* was 29.5 ± 6.7 *Synechococcus* cells dinoflagellate^{-1} hour^{-1} (mean ± 1 SE). The ingestion rate by *A. minutum* was much lower, 3.2 ± 2.2 *Synechococcus* cells dinoflagellate^{-1} hour^{-1} (Jeong *et al.*, 2005b).
Alexandrium fundyense [T – Bricelj *et al.*, 1990]
Fish:
Newly settled winter flounder, larval sheepshead minnow, and larval mummichug, after they consumed six or more toxin-contaminated copepod zooplankters (Samson *et al.*, 2008).
Shortnose sturgeon (mass mortality; high STX concentrations in stomach contents, liver, and gill tissues; Fire *et al.*, 2012).
Molluscs:
Eastern oyster, Pacific oyster: caused immunosuppression of hemocytes and increased hemocyte death (Hégaret *et al.*, 2007a).
Softshell clam: Depressed phagocytosis and adhesion (Hégaret *et al.*, 2007a).
Zooplankton: The copepod *Acartia hudsonica*, when *A. fundyense* was 25% or more of the diet by carbon content, and only 20–30% of males survived to adulthood. Males were more susceptible than females. Differential mortality and skewed sex ratios acted as feedback mechanisms that potentially can affect the population dynamics of grazers and toxic algal bloom development (Avery *et al.*, 2008).
Alexandrium monilatum[c] [T (strain-dependent): STXs, gonyautoxins (Schmidt and Loeblich, 1979), goniodomin A (Hsia *et al.*, 2005); bioaccumulate]
Fish: General (fish kills), crested blenny, jack species, needlefish, pinfish, sheepshead minnow, stippled clingfish, whip eel, whiting (Wardle *et al.*, 1975; Gunter, 1942; Connell and Cross, 1950; Howell, 1953; Gates and Wilson, 1960; Williams and Ingle, 1972).
Invertebrates (general): Ichthyoplankton, zooplankton (International Council for the Exploration of the Sea, 1999).

Table 7.1 (*Continued*)

Taxon (taxa) and organisms killed
Molluscs:
Atlantic surfclam, auger snail, Brazil arc, calico crab, double moonshell, dwarf crab, eastern oyster, Florida coquina, Florida dogwinkle, gray augur, green mussel, green porcelain crab, hermit crab, hooked mussel, lettered olive, northern quahog (juveniles), shark eye, spotted porcelain crab, striped false limpet, stone crab, surfclam (Wardle *et al.*, 1975).
Larval eastern oyster, northern quahog (May *et al.*, 2010).
Veined rapa whelk (in association with a bloom of *A. monilatum*; eastern oysters and northern quahogs in the same flow-through system apparently were unaffected). External signs of stress included reduced ventilation, inability to attach to hard substrata, periodic pumping of the opercular plate, and increased mucus production over a 24- to 48-hour period prior to death. High concentrations of toxin goniodimum A were measured in bivalves attached to rapa whelk shells (Harding *et al.*, 2009).
Annelids: Polychaetes *Americonuphis magna* and *Nereis* sp. (Wardle *et al.*, 1975).
Crustacea:
Blue crab, calico crab, dwarf crab, Florida stone crab, green porcelain crab, saltwater porcelain crab, sand flea, speckled swimming crab, surf hermit, thinstripe hermit crab (Wardle *et al.*, 1975).
Daggerblade grass shrimp (*note*: not affected – depressed mud crab, ivory barnacle) (Sievers, 1969).
Cnidarians: Sea anemone *Bunodosoma cavernata* (Wardle *et al.*, 1975).
Echinoderms: Brittle star, six keyhole sand dollar, holothuroids (sea cucumbers) (Wardle *et al.*, 1975).
Alexandrium tamarense [T (strain-dependent) – major STXs: C2, gonyautoxin 4, neosaxitoxin and gonyautoxin 3 bioaccumulate (Shimada *et al.*, 2011 and references therein); also ROS hydrogen peroxide – Kim *et al.*, 1999]
Mammals: Humpback whale (Geraci *et al.*, 1989).
Birds: Black duck, black-legged kittiwake, common tern, other terns, gulls, shag, shorebirds, waterfowl (Shumway *et al.*, 2003 and references therein).
Fish: Larval and adult Atlantic herring; bluefish, larval capelin monkfish, sand lances (sand eels), skates, spiny dogfish (White, 1980; Smayda, 1991 and references therein).
Molluscs: Atlantic deep-sea scallop (juveniles – Lesser and Shumway, 1993), blue mussel (Desbiens *et al.*, 1989), softshell clam (juveniles – Lesser and Shumway, 1993).
Zooplankton:
Ciliates – *Favella ehrenbergii* (with toxic strain; Hansen, 1989); *Favella taraikaensis* and *Eutintinnus* sp. (with toxic strain; Fulco, 2007).
Heterotrophic dinoflagellates – Lysis of *Polykrikos kofoidii* occurred when fed (presumed toxic) *A. tamarense* (Cho and Matsuoka, 2000).
Phytoplankton:
A. tamarense consumes *Isochrysis galbana*, *Rhodomonas salina* and an unidentified cryptophyte taxon, *Heterosigma akashiwo*, *Amphidinium carterae*, and *Prorocentrum minimum* (Jeong *et al.*, 2005a).
The ingestion rate of unicellular cyanobacteria by *A. tamarense* was 13.7 ± 0.9 *Synechococcus* cells dinoflagellate^{-1} hour^{-1} (mean ± 1 SE; Jeong *et al.*, 2005b).
Ceratium tripos (high-biomass bloom) [M; low DO]
Fish: Fish kills (anoxic conditions, hydrogen sulfide at lethal levels over large areas, e.g., 14,000 km^2); massive mortalities of finfish (Mahoney, 1978 and references therein; Mahoney and Steimle, 1979).
Molluscs: Ocean quahog, sea scallop, surfclam (Mahoney and Steimle, 1979; anoxic conditions were also associated with the bloom).
Crustacea: American lobster (Mahoney and Steimle, 1979).
Cochlodinium polykrikoides (or *Margalefidinium polykrikoides* – Gómez *et al.*, 2017) [Br, M; T (strain-dependent), but toxin(s) uncharacterized (Dorantes-Aranda *et al.*, 2010); toxicity to fish could be caused by non-hydrogen peroxide, highly reactive labile toxins such as other ROS-like chemicals: Tang and Gobler, 2009a; Kim *et al.*, 2002, but see Onoue and Nozawa, 1989 (zinc-bound paralytic shellfish poisoning toxins)].

(*continued*)

Table 7.1 (*Continued*)

Taxon (taxa) and organisms killed

Fish:

In bioassays, sheepshead minnows (1 week old), adult striped killifish and silverside. Moribund fish had epithelial proliferation of gills with focal areas of fusion of gill lamellae, suggesting impaired gill function (Gobler *et al.*, 2008; also see Tang and Gobler, 2009a).

Cyprinodon variegatus (age 1 week); adult *Fundulus majalis, Menidia menidia,* and *Fundulus heteroclitus* (Gobler *et al.*, 2008).

Molluscs:

Bay scallop, eastern oyster, and northern quahog larvae (Tang and Gobler, 2009b); eastern oyster larvae (Ho and Zubkoff, 1979: as *Cochlodinium heterolobatum*).

Juvenile bay scallop, eastern oyster (Gobler *et al.*, 2008).

Bay scallop larvae, eastern oyster: Moribund animals exhibited hyperplasia, hemorrhaging, and apoptosis in gill and digestive tissues, with gill inflammation specifically associated with areas containing *C. polykrikoides* cells (Gobler *et al.*, 2008).

Zooplankton: Female copepods (*Acartia tonsa*) at low *C. polykrikoides* densities; both male and female *A. tonsa* at bloom densities. Earlier nauplii stages were most susceptible (Jiang *et al.*, 2009).

Phytoplankton:

C. polykrikoides consumed an unidentified cryptophyte: The maximum ingestion rate was 9.4 cells grazer^{-1} day^{-1}, and the calculated grazing coefficients ranged from 0.001 to 0.745 hour^{-1}; up to 53% of cryptophyte populations were removed by *C. polykrikoides* in 1 hour. The data suggest that *C. polykrikoides* can have considerable grazing impact on cryptophytes (Jeong *et al.*, 2004a).

C. polykrikoides consumed small phytoplankton prey including *Isochrysis galbana*, *Rhodomonas salina*, *Heterosigma akashiwo*, and *Amphidinium carterae* (Jeong *et al.*, 2004a).

Various phytoplankton species: Allelopathic; cell lysis (Fistarol *et al.*, 2004; Ma *et al.*, 2009, 2011); distortion of cell shape, loss of motility, death (Tang and Gobler, 2010).

The ingestion rate of unicellular cyanobacteria by *C. polykrikoides* was 38.7 ± 1.1 *Synechococcus* cells dinoflagellate^{-1} hour^{-1} (mean ± 1 SE; Jeong *et al.*, 2005b).

Bacteria: Maximum ingestion and clearance rates by *C. polykrikoides* were 17.4 bacteria and 1.0 nL algal cell^{-1} hour^{-1}, respectively (Seong *et al.*, 2006).

Gambierdiscus toxicus [M; T (strain-dependent): CTXs, bioaccumulate in fish – Lewis, 1992; Naar *et al.*, 2007]

Mammals: Hawaiian monk seal (CTXs in the liver, muscle, and brain of five dead stranded animals – Dechraoui *et al.*, 2011).

Fish: Medaka (experimental data); the dose response for lethal adverse effects was within the range of the CTX load found in adult reef fish (Edmunds *et al.*, 1999).

***Hematodinium perezi*, *Hematodinium* spp.** [F, Br, M; Par]

Arthropods: Crustacea – blue crab, Tanner crab (bitter crab disease or bitter crab syndrome: Horner *et al.*, 1997 and references therein). *Note*: Crabs are often the dominant prey in the diets of various invertebrates, fish, marine mammals.

Karenia brevis[c] [M; T (strain-dependent): BTXs, bioaccumulate (e.g., Naar *et al.*, 2007)]. From Landsberg (2002 and references therein), National Oceanic and Atmospheric Administration (2006), and Gannon *et al.* (2009) unless otherwise noted:

General: The most affected organisms are fish; it is unclear whether invertebrate mortalities are due to BTXs or to hypoxia resulting from the blooms, or both (Landsberg *et al.*, 2009).

Cascading effects, ecosystem level: During intense, protracted blooms (1971, 2005), entire benthic communities over thousands of km^2 (eastern Gulf of Mexico) were killed (Smith, 1975; Dupont *et al.*, 2010). Contributing factors – Unusually high surface water temperatures/strong thermoclines, isolated *K. brevis* populations at depth, fish and invertebrate kills from bloom toxicity and/or hypoxia, increased biochemical oxygen demand from decomposition of dead organisms, increased hydrogen sulfide levels, and decreased light availability at depth (Landsberg *et al.*, 2009).

Mammals:

Bottlenose dolphin, manatee (O'Shea *et al.*, 1991; Bossart *et al.*, 1998; Twiner *et al.*, 2012).

Coyotes, dogs near a bloom (Castle *et al.*, 2013).

Birds: Double-crested cormorant, ducks, frigate birds, gulls, lesser scaup, red-breasted merganser, terns, vultures.

Reptiles: Sea turtles (loggerhead, Kemp's ridley, and green. During two intense *K. brevis* red tides (Feb.–Dec. 2005, Aug.–Dec. 2006), sea turtle strandings were much higher in the affected areas during Jan. 2005–Dec. 2006 (174 in 2005, 144 in 2006) than the 12-year average (43 ± 23, mean ± 1 SE; Fauquier *et al.*, 2013).

Table 7.1 (*Continued*)

Taxon (taxa) and organisms killed

Fish (114 taxa): Atlantic bumper, Atlantic menhaden, Atlantic midshipman, Atlantic moonfish, Atlantic needlefish, Atlantic spadefish, balloonfish, bank cusk-eel, barracuda, bay anchovy, belted sandfish, black drum, black grouper, blackcheek tonguefish, black tip shark, bluerunner, bluestriped grunt, catfish, checkered puffer, cobia, cowfish, crevalle jack, damselfish, eel, gafftopsail catfish, gag, gar, goldspotted killifish, grass carp, gray angelfish, gray snapper, gray triggerfish, graysby, groupers, grunts, Gulf flounder, Gulf kingfish, Gulf menhaden, halfbeak, hardhead catfish, harvestfish, hogfish, inshore lizardfish, jacks, jack crevalle, jewfish, ladyfish, lancelet, leatherjacket, leopard searobin, lined sole, longnose batfish, longnose killifish, northern puffer, orange filefish, oyster toadfish, palespotted eel, planehead filefish, perch, pikeperch, pinfish, pompano, porgy, porcupine fish, puffer, purplemouth moray, queen triggerfish, rays, red drum, red grouper, red snapper, redfin needlefish, redfish, round scad, sailfish, sailors choice, sand perch, sand seatrout, scaled sardine, scamp, sharksucker, sheepshead, shiners, short bigeye, shortnose batfish, silver jenny, silver perch, silver trout, snook, sole, sooty eel, southern kingfish, southern seabass, southern stargazer, spadefish, Spanish mackerel, speckled worm eel, spinner shark, spiny boxfish (butterfish), spot, spotted moray (?), spotted seatrout (or speckled trout), striped burrfish, striped mullet, tarpon, thread herring, tidewater silverside, toadfish, tomtate, tripletail, trunkfish, Warsaw grouper, white grunt, yellowtail amberjack.

Chordata: Ascidians (sea squirts).

Invertebrates (general): Mortality of benthic invertebrates, from a combination of BTX neurotoxicity and anoxia due to oxygen depletion from decomposition of dead organisms and respiration of *K. brevis* blooms at night (Simon and Dauer, 1972).

Molluscs:

Bay scallop [mortalities and failed recruitment], bruised nassa, conch, dwarf surfclam (or coot clam), Florida coquina, minor jacknife, oysters, quahogs. In laboratory experiments, larval bay scallop, eastern oyster, northern quahog.

Eastern oyster and northern quahog – When larvae (age 3 days) were exposed to toxic *K. brevis* (10^3 cells mL^{-1}), survival was significantly less in lysed culture than whole culture (Leverone *et al.*, 2006).

Bay scallop, eastern oyster, and northern quahog – Mortality of older larvae (age 7 days) from exposure to 5000 cells mL^{-1} of toxic *K. brevis* (Leverone *et al.*, 2006).

Brachiopod: *Glottidia pyramidatum.*

Echinoderms: Sea urchins.

Poriferans: Sponges (Smith, 1975).

Annelids: *Onuphis eremita oculata*, common clam worm, and polychaetes (*Clymenella mucosa, Diopatra cuprea, Glycera americana, Glycera capitata, Laeonereis culveri, Scoloplos fragilis, Scoloplos rubra, Scolelepis squamata*).

Crustaceans (other than zooplankton) – Barnacles, blue crab, horseshoe crab, lady crab, pea crab, shrimps; amphipods (*Acanthohaustorius* sp.).

Cnidaria: Corals (Smith, 1975).

Phoronids: *Phoronis architecta.*

Zooplankton:

Microcrustaceans

Acartia tonsa – Lethargy, paralysis (Turner *et al.*, 1996).

Calanus pacificus – Reduced development, lethargy (Huntley *et al.*, 1986, 1987); rapid heart rate, paralysis, lethargy, regurgitation (Sykes and Huntley, 1987).

Phytoplankton:

Competitors *Asterionellopsis glacialis (diatom), Prorocentrum minimum*, and *Skeletonema costatum* (diatom), from exposure to extracts from *K. brevis* natural blooms; and these taxa as well as *Akashiwo* cf. *sanguinea* were killed from exposure to extracts from toxic culture (Prince *et al.*, 2008).

The ingestion rate of unicellular cyanobacteria by *K. brevis* was 5.0 ± 0.1 *Synechococcus* cells dinoflagellate^{-1} hour^{-1} (mean ± 1 SE; Jeong *et al.*, 2005b), and up to 84 *Synechococcus* cells dinoflagellate^{-1} hour^{-1} (Glibert *et al.*, 2009).

Karlodinium veneficum[c] [Br; T (strain-dependent): KTXs, linear polyketides that cause membrane permeabilization in other protists, and interact strongly with certain membrane sterols which form stable complexes with KTSs, increasing ionic permeability of affected membranes (Deeds and Place, 2006); also can act as ichthyotoxins; hemolysins, ROSs (Yamasaki *et al.*, 2004, Van Wagoner *et al.*, 2008 and references therein). Low DO can develop in association with large blooms.]

(*continued*)

Table 7.1 (*Continued*)

Taxon (taxa) and organisms killed

Fish:

Black drum, bluegill sunfish, common snook, grass carp, hardhead catfish, red drum, sheepshead, hybrid striped bass, striped mullet (Deeds *et al.*, 2002, Kempton *et al.*, 2008 and references therein).

Cod – Before death the juvenile fish became lethargic. Affected fish showed increased plasma osmolality; gill tissue showed severe pathological changes, with extensive separation of the respiratory epithelium from the underlying pillar cells (Nielsen, 1993).

Sheepshead minnow, zebrafish – In laboratory experiments, larvae and juveniles exposed to KTXs (Deeds *et al.*, 2006).

Molluscs:

Eastern oyster larvae (Glibert *et al.*, 2007; Stoecker *et al.*, 2008).

Common whelk, lamellibranch bivalve *Lasaea rubra*, and great scallop (Abbott and Balantine, 1957); juvenile Atlantic surfclam, bay scallop, blue mussel, European flat oyster, northern quahog (Lesser and Shumway, 1993).

Crustacea: Blue crab, lady crab, shrimps (Landsberg 2002 and references therein), barnacles (Günter *et al.*, 1948).

Zooplankton: Ciliates – *Favella ehrenbergii* (Hansen, 1995).

Phytoplankton: Consumes cryptophytes; abundant cryptophyte prey can trigger toxic blooms (Adolf *et al.*, 2008). KTXs react strongly with high concentrations of desmethyl sterols in some phytoplankton (e.g., the heterotrophic, herbivorous dinoflagellate *Oxyrrhis marina*), resulting in their death (Adolf *et al.*, 2007).

Lingulodinium polyedrum[c] [M; T (strain-dependent): STXs, yessotoxins; Paz *et al.*, 2004, 2008]. From Landsberg (2002 and references therein) unless otherwise noted:

Fish: Blindfish, California anchovy, dogfish, guitarfish, Haller's round ray, horn shark, red perch, smelt, stingray, thornback guitarfish. Other descriptors: dead bottom fauna including fish and shellfish; fish and shore fauna.

Holothurians: *Trachostoma arenata.*

Molluscs: Limpets, banded pheasant, barrel-bubble, beatic dwarf olive, blister glassy-bubble, California cone, California semele, California tagelus, California venus, Californian beanclam, frilled venus, Gould beanclam, kelp scallop, Mexican pyramidella, moonsnail, octopus, onyx slippersnail, Pacific eggcockle, purple dwarf olive, straight horsemussel, western mud nassa.

Crustacea: Brown rock crab, flat porcelain crab, morbid sand crab.

Phytoplankton:

Consumes microalgal prey such as *Isochrysis galbana*; *Heterosigma akashiwo*; *Rhodomonas salina* and an unidentified cryptophyte taxon; and dinoflagellates *Alexandrium tamarense, Amphidinium carterae, Heterocapsa triquetra, Prorocentrum minimum,* and *Scrippsiella trochoidea*. Its maximum specific growth rates on *P. minimum* or *S. trochoidea* were much higher than in the same light regime without prey. The data suggest that *L. polyedrum* can have a potentially significant grazing impact on some algal prey populations (Jeong *et al.*, 2005a).

The ingestion rate of unicellular cyanobacteria by *L. polyedrum* was 64.2 ± 2.2 *Synechococcus* cells dinoflagellate^{-1} hour^{-1} (mean ± 1 SE; Jeong *et al.*, 2005b).

***Noctiluca* spp. (e.g., *N. miliaris, N. scintillans*)** [M; allelopathic/ammonia toxicity, NH_3/NH_4^+; Okaichi and Nishio, 1976]

General: Blooms have been linked to massive fish and invertebrate kills (Tirkoglu, 2013 and references therein).

Fish: These heterotrophic dinoflagellates can consume fish eggs and larvae (e.g., anchovy eggs: Hattori, 1962), and produce NH_3/NH_4^+ following deamination of ingested prey; may become progressively more toxic (10-fold range) with more food intake (Okaichi and Nishio, 1976).

Zooplankton: *N. miliaris* consumes copepod eggs of *Acartia clausi, A. tonsa, Calanus euxinus, Centropages hamatus,* and *Temora longicornis* (Kimor, 1979; Daan, 1987; Quevedo *et al.*, 1999; Nikishina *et al.*, 2011) and copepod nauplii (Dela-Cruz *et al.*, 2002), with resulting NH_3/NH_4^+ production as above.

Phytoplankton:

Species with an equivalent spherical diameter (ESD) > 10 µm were consumed as prey; species with an ESD < 5 µm did not support growth. Growth rates of *N. scintillans* fed toxigenic raphidophyceans *Chattonella antiqua* or *Heterosigma akashiwo* increased linearly with prey concentration, without an obvious threshold. This dinoflagellate is well adapted to eutrophic environments (Nakamura, 1998).

Noctiluca spp. may act as vectors of toxigenic microalgae (e.g., *Dinophysis, Pseudo-nitzschia*; Escalera *et al.*, 2007).

Table 7.1 (*Continued*)

Taxon (taxa) and organisms killed

In laboratory trials, *N. scintillans* consumed *Dunaliella marina* (small chlorophyte flagellate), *Rhodomonas baltica*, *Ditylum brightwelli* and *Thalassiosira weisflogii* (diatoms), and *Prorocentrum minimum*, *Heterocapsa triquetra* and *Prorocentrum micans* (dinoflagellates; Kiørboe and Titelman, 1998).

Lowest growth rates occurred when *N. scintillans* was given very small algal prey (haptophyte *Isochrysis galbana* and pelagiophycean *Aureoumbra lagunensis*). Moderate growth rates were supported by consumption of *Dunaliella tertiolecta* (small chlorophyte flagellate), and *Gyrodinium dorsum* and *Prorocentrum minimum*. Highest growth rates occurred when *N. scintillans* was given diatom (e.g., *Thalassiosira* sp.) and prasinophyte prey (Buskey, 1995).

N. miliaris also consumed toxic *Alexandrium minutum*; the maximum ingestion rate was 466 prey cells *N. scintillans* cell^{-1} day^{-1} (Frangópulos *et al.*, 2011).

Bacteria: *N. miliaris* consumed live cells of *Vibrio* sp. and *Serratia plymuthica* at a rate of 10^4 to 10^6 bacteria *N. scintillans* cell^{-1} hour^{-1} (Kirchner *et al.*, 1996).

Ostreopsis ovata [M, benthic; T (strain-dependent): palytoxin and analogs (Ciminiello *et al.*, 2010, 2011, 2012)]

Fish: European seabass juveniles (Fiamali *et al.*, 2011).

Crustacea: Brine shrimp (instars, stages 2–3), striped barnacle larvae (Faimali *et al.*, 2011).

Zooplankton: Crustaceans – *Tigriopus fulvus* nauplii (Faimali *et al.*, 2011).

Pfiesteria piscicida*, *Pfiesteria shumwayae [planktonic, some stages benthic; Br; T: PfTXs (strain-dependent); Burkholder *et al.*, 2005; Marshall *et al.*, 2006; Moeller *et al.*, 2007, Burkholder and Marshall 2012; can also kill via physical attack alone: Burkholder *et al.*, 2001]

Fish (death via toxin alone, or toxin along with physical attack): American eel, Atlantic croaker, Atlantic menhaden, channel catfish, hogchoker, hybrid tilapia, striped bass, sheepshead, southern flounder, spot, spotted sea trout, striped mullet, white perch (Burkholder and Glasgow 1997); Atlantic croaker and spot (Gordon *et al.*, 2002); cultured hybrid striped bass, tilapia (both species: Burkholder and Glasgow, 1997), hybrid tilapia (Gordon *et al.*, 2002), pikeperch (Moestrup *et al.*, 2014 – *Pfiesteria shumwayae*).

Molluscs (same mechanisms as for fish): Bay scallop (cultured adults, larvae), eastern oyster (cultured adults, larvae), green mussel (cultured adults), northern quahog (wild adults, cultured larvae – Springer *et al.*, 2002; Shumway *et al.*, 2006).

Crustacea: Blue crab (Burkholder and Glasgow, 1997).

Zooplankton: Ciliates – *Euplotes vannus, Euplotes woodruffi* (Lewitus *et al.*, 2006).

Phytoplankton: Toxigenic strains in the absence of live fish (TOX-B functional type), and NON-IND strains consume cryptophytes and other small microalgae (Burkholder and Marshall, 2012, and references therein).

Benthic *Prorocentrum* complex[f] [T (strain-dependent): DTXs, OA, diol esters of OA, 7-deoxy-OA, D8- and/or D-9 congeners of OA]. From Glibert *et al.* (2012 and references therein), unless otherwise noted:

Fish: European sea bass – Feeding *P. lima* complex cells to juveniles, either directly or by feeding them brine shrimp that had been consuming *P. lima*, led to fish death (Ajuzie, 2008).

Crustacea: Brine shrimp grazed continuously on *P. lima* complex cells until they died (Ajuzie, 2007).

Prorocentrum micans [M; T? (reported to produce hydrogen peroxide ROS – Kim *et al.*, 1999); low DO]

Fish: High-biomass blooms can deplete oxygen and cause major fish kills (Red-Tide, 2011; Smithsonian Institution, 2011).

Phytoplankton:

Allelochemicals from *P. micans* may kill other phytoplankton (Smithsonian Institution, 2011).

P. micans consumes *Isochrysis galbana*; *Rhodomonas salina* and an unidentified cryptophyte taxon; *Heterosigma akashiwo*; and *Amphidinium carterae, Prorocentrum minimum,* and *Heterocapsa triquetra* (Jeong *et al.*, 2005a).

The ingestion rate of unicellular cyanobacteria by *P. micans* was 35.4 ± 2.1 *Synechococcus* cells dinoflagellate^{-1} hour^{-1} (mean ± 1 SE; Jeong *et al.*, 2005b).

Prorocentrum minimum [T (strain-dependent): venerupin shellfish poisoning; Grzebyk *et al.*, 1997); low DO]

Molluscs: Bay scallop, eastern oyster (adults and larvae; Wikfors 2005 and references therein).

Eastern oyster – When fed a diet consisting of only *P. minimum* (1.6×10^3 cells mL^{-1}; Luchenbach *et al.*, 1993).

(*continued*)

Table 7.1 (*Continued*)

Taxon (taxa) and organisms killed

Phytoplankton:

Consumes *Isochrysis galbana*, *Rhodomonas salina* and an unidentified cryptophyte taxon, *Heterosigma akashiwo*, and *Amphidinium carterae* (Jeong *et al.*, 2005a).

P. minimum is consumed by various mixotrophic and heterotrophic dinoflagellates (e.g., *Akashiwo sanguinea, Alexandrium tamarense, Gonyaulax polygramma, Gymnodinium catenatum, Gyrodinium resplendens, Heterocapsa triquetra, Lingulodinium polyedra, Polykrikoides kofoidii, Prorocentrum micans*) (Skovgaard, 2000; Burkholder *et al.*, 2008 and references therein).

The ingestion rate of unicellular cyanobacteria by *P. minimum* was 5.9 ± 1.2 *Synechococcus* cells dinoflagellate^{-1} hour^{-1} (mean ± 1 SE; Jeong *et al.*, 2005b).

Bacteria: In stationary phase under nutrient-replete conditions, *P. minimum* engaged in bacterivory (Wikfors and Fernandez, 2013).

Scrippsiella trochoidea [T – but the toxin(s) have not been isolated or chemically characterized (see Tang and Gobler, 2012); low DO]

Molluscs: Eastern oyster and northern quahog larvae (at environmentally relevant densities, 10^4 cells mL^{-1}). Cultured *S. trochoidea* in later growth stages was more toxic than exponential growth stages. Mortality may have been caused in part via a physical/chemical mechanism such as clogging of larval feeding apparati by materials (e.g., lipids, extracellular polysaccharides, and/or cell debris) produced by *S. trochoidea* (Tang and Gobler, 2012).

Phytoplankton:

S. trochoidea consumes *Isochrysis galbana*; *Rhodomonas salina* and an unidentified cryptophyte taxon; *Heterosigma akashiwo*; and *Amphidinium carterae, Prorocentrum minimum,* and *Heterocapsa triquetra* (Jeong *et al.*, 2005a).

The ingestion rate of unicellular cyanobacteria by *S. trochoidea* was 7.1 ± 1.1 *Synechococcus* cells dinoflagellate^{-1} hour^{-1} (mean ± 1 SE; Jeong *et al.*, 2005b).

Bacteria: Maximum ingestion and clearance rates were 21.9 bacteria and 2.3 nL, respectively, algal cell^{-1} hour^{-1} (Seong *et al.*, 2006).

Haptophytes (Haptophyta – golden algae)

Prymnesium parvum[c] [Br; T (strain-dependent): many different toxins, e.g., hemolysins (lipopolysaccharides, a galactoglycerolipid, polene polyethers, polyketides such as prymnesins 1 and 2, cycloamines ["fast-acting ichthyotoxins"]), ROSs, dimethylsulfonio-propionate, polyunsaturated fatty acids, and fatty acid amides). Some of these toxins form micelles and require activation by various cofactors (Henrikson *et al.*, 2010; Manning and Claire, 2010; Bertin *et al.*, 2012a, 2012b and references therein). Also, various allelochemicals such as glycolipids, galactolipids, proteolipids, and lipid-carbohydrate complexes (Manning and Claire, 2010). Low DO can develop in association with large blooms. EDAB (Sunda *et al.*, 2006).]

General: Blooms of *P. parvum* have caused death of gill-breathing organisms such as fish (all species present), mussels, and larval amphibians (Burkholder, 2009 and references therein).

Fish:

Massive fish kills; primary uptake of toxins by gill filaments (Van Landeghem *et al.*, 2013 and references therein).

Massive fish kill affecting all fish species present in the area (blue catfish, bluegill sunfish, channel catfish, common carp, carpsucker, flathead catfish, freshwater drum, gar, largemouth bass, longnose gar, mosquitofish, Rio Grande darter, white bass, white crappie (James and de la Cruz, 1989).

Molluscs: *Corbicula fluminea* – Although this exotic/invasive species had previously been common in an affected river with densities as high as 1076 individuals m^{-2} (100 individuals ft^{-2}), no live animals were observed for several years following a massive fish kill (James and de la Cruz, 1989).

Zooplankton:

Copepods – *Eurytemora affinis*: Cell-free filtrates from toxic *P. parvum* cultures negatively affected survivorship (Sopanen *et al.*, 2008).

Ciliates – *Euplotes affinis* (Granéli and Johansson, 2003a).

Favella ehrenbergii, Eutintinnus pectinis, Metacyclis angulata, Strombidium conicum, and *Strombididinopsis* sp. (Rosetta and McManus, 2003).

Table 7.1 (*Continued*)

Taxon (taxa) and organisms killed
Phytoplankton:
Oxyrrhis marina – Consumed by *P. parvum* as prey, especially rounded and partly lysed cells (toxin effect; Tillmann, 2004).
Rhodomonas sp.: Consumed as prey (Fistarol *et al.*, 2003).
Rhodomonas cf. *baltica*, *Rhodomonas salina*, and *Thalassiosira weissflogii* (Granéli and Johansson, 2003; Barreiro *et al.*, 2005; Uronen *et al.*, 2007).
Heterocapsa rotundata (Skovgaard and Hansen, 2003).
Minidiscus trioculatus, *Thalassiosira* sp. (small diatoms, maximum cell dimension 5 µm): High interim grazing rates during the first eight hours (0.30 *M. trioculatus* and 0.74 *Thalassiosira* sp. *P. parvum* cell^{-1} hour^{-1}). When bacteria were added as potential prey, prey-switching from diatom cells to bacteria occurred in cultures with *M. trioculatus* (Martin-Cereceda *et al.*, 2003).
Bacteria: Bacterial grazing was similar with or without diatom prey (0.17 bacteria *P. parvum* cell^{-1} hour^{-1}) (Martin-Cereceda *et al.*, 2003).
Euglenoids (Euglenophyta) [F, Br, M; T: EUGL (Zimba *et al.*, 2010, 2017)]
Euglena sanguinea[g]
General: The toxin structure is similar to that of alkaloids produced in fire ant venom. This class of alkaloids has strong biological activity ranging from necrotoxicity and hemolysis to phytotoxicity, as well as insecticidal and antibiotic activities. Cultured euglenoids apparently produce EUGL regardless of growth phase, suggesting use of the toxin in defense against herbivores (Zimba *et al.*, 2010 and references therein). Toxic euglenoid blooms have only recently begun to be examined.
Fish: Blue tilapia, channel catfish, sheepshead (scientific naming information not included), and striped bass. Tilapia appeared to have euglenoid cells associated with gills, resulting in distressed breathing (indicated by surface porpoising; Zimba *et al.*, 2010).
Pelagophyceans (Heterokontophyta: brown tide algae)
Aureococcus anaophagefferens [T-Br (strain-dependent) – see Robbins *et al.*, 2010; note that the toxin(s) are not chemically characterized. Low DO can develop in association with large blooms. EDAB (Sunda *et al.*, 2006).]
Fish: Failure of bay anchovies to spawn (Smayda, 1991).
Molluscs: Bay scallop, blue mussel, northern quahog (Tracey 1988; Smayda and Villareal, 1989); bay scallop larvae: Gallager *et al.*, 1989; Gainey and Shumway, 1991).
Zooplankton: Failure of the cladoceran community to develop during a brown tide bloom (Smayda, 1991).
Seagrasses: *Zostera marina*, via light reduction – large-scale die-offs of eelgrass, a critical habitat species for shellfish, larval fish, and many other fauna (Dennison *et al.*, 1989).
Beneficial macroalgae: Macroalgal die-off (kelps, others) occurred during a brown tide (Dennison *et al.*, 1989; Smayda, 1991).
Aureoumbra lagunensis [B? T? (strain-dependent): e.g., Liu and Buskey 2000; or, from Buskey *et al.*, 1996, p. 43: "there is evidence that brown tide [*A. lagunensis*] may be directly toxic to some species of zooplankton at cell concentrations similar to those found in nature." Low DO can develop in association with large blooms. EDAB (Sunda *et al.*, 2006).]
Fish: Massive fish kills (Gobler *et al.*, 2013).
Molluscs: Bay scallop, blue mussel, northern quahog (Smayda *et al.*, 1991; Gobler *et al.*, 2013 and references therein).
Seagrasses: Reduced biomass and areal extent of seagrass meadows (Onuf, 1996); steady decline in the biomass of roots and rhizomes after several years of the brown tide bloom, and major reduction in the ratio of root+rhizome biomass to shoot biomass, indicating a loss in critically important food reserves (data of K. Dunton in Buskey *et al.*, 1996).
Raphidophyceans (Heterokontophyta)
Heterosigma akashiwo [Br, M; T (strain-dependent): ROSs (e.g., superoxide, hydrogen peroxide), BTX like compound(s), hemagglutinating and hemolysing compounds, and an uncharacterized polysaccharide–protein complex (Yamasaki *et al.*, 2009, Mohamed and Al-Shehri, 2012 and references therein unless otherwise noted). Low DO can also develop during/following large fish kills linked to *H. akashiwo*.]
Fish: Atlantic salmon (wild and cultured), Chinook salmon (including the endangered Wild White River spring chinook salmon), Coho salmon, chum, sockeye salmon, and rainbow trout (Rensel *et al.*, 2010 and references therein). Massive fish kills (Liu *et al.*, 2008 and references therein).

(*continued*)

Table 7.1 (*Continued*)

Taxon (taxa) and organisms killed
Note: Blooms occurred in coastal waters of British Columbia, Canada, every year beginning in the 1960s, and fish kills linked to *H. akashiwo* were reported in most years from 1986 through the following decade. In addition, fish kills linked to *H. akashiwo* were reported in Washington state waters during some years, with substantial economic losses (Horner *et al.*, 1997 and references therein). *Zooplankton*: Ciliates *Tintinnopsis tubulosoides, Favella* sp., and *Synchaeta cecilia* (Verity and Stoecker, 1982; Egloff, 1986). *Flavella* sp. and *Metacylis* sp., when *H. akashiwo* was the only prey offered; with mixed algal prey, however, toxic effects of *H. akashiwo* were not apparent, probably because of selective feeding on the nontoxic prey (Graham and Strom, 2010). *Bacteria*: The maximum ingestion rate and maximum clearance rate were 11.7 bacteria and 2.6 nL, respectively, algal cell^{-1} hour^{-1} (Seong *et al.*, 2006).

Note: Plankters are listed unless otherwise indicated. T-toxigenic, B-impacts known from other bioactive allelopathic compound(s); low DO-high-biomass blooms cause anoxia/hypoxia; Str-structural feature of the algal cell causes impacts, e.g., needle-like extensions; EDAB – ecosystem-disruptive algal bloom; Par, parasitic. F-freshwater, Br-brackish, M-marine. Toxins: BMAA, β-methylamino-L-alanine; BTXs, brevetoxins; CTXs, ciguatoxins (ciguateratoxins); CYL, cylindrospermopsin; DA, domoic acid; DTX, dinophysis toxin; EUGL, euglenophycin toxin; KTXs, karlotoxins; LPS, lipopolysaccharides; LYNGTX, lyngbyatoxin; MC, microcystin; TMCs, total microcystins; OA, okadaic acid; PfTXs, *Pfiesteria* spp. toxins; PTX, palytoxin and analogs; ROSs, reactive oxygen species; STXs, saxitoxins and derivatives. *Bacteria* refers to eubacteria. See Appendix 1 for scientific names if not included here. Taxonomy is based on AlgaeBase (http://algaebase.org), the World Register of Marine Species (WORM: http://marinespecies.org/), and recent science publications.

[a] Throughout this table for some taxa, the species listed is not the only harmful species in the genus. For example, toxic strains of *Anabaena circinalis* produce STXs (Llewellyn *et al.*, 2001); *Cochlodinium* cf. *fulvescens* has been linked to mortality of California mussels (Curtiss *et al.*, 2008), etc.

[b] Microcystins are known to be produced by some strains within the cyanobacteria *Microcystis* spp., *Anabaena* spp., *Planktothrix/Oscillatoria* (*P. agardhii, P. rubescens*), *Anabaenopsis* spp., *Nostoc rivulare,* and *Hapalosiphon* spp. (de Figueiredo *et al.*, 2004a). The species *Aphanizomenon flos-aquae* is also sometimes listed as a MC producer (e.g., de Figueiredo *et al.*, 2004a), but that information apparently resulted from a long-standing error in the literature wherein tested *Aphanizomenon flos-aquae* material had been contaminated with MC-producing, cryptic *Microcystis* spp. (W. Carmichael, pers. comm., 1999). For toxins of *Aphanizomenon flos-aquae*, see http://www.env.gov.bc.ca/wat/wq/reference/cyanophytes.html#m1.

[c] Engene *et al.* (2012) suggested renaming the cyanobacterium *Lyngbya majuscula* as *Moorea producens*, but the issue has not been resolved. Since *Lyngbya majuscula* is so widely used by resource managers and scientists, we have retained that name here. Among various former names used for the following species, the most common previous names are as follows:

Cyanobacteria – *Cylindrospermopsis raciborskii:* previous name *Anabaenopsis raciborski*i.

Dinoflagellates –
Akashiwo sanguinea: previous names *Gymnodinium sanguineum, G. splendens, G. nelsonii*;
Alexandrium monilatum: previous name *Gonyaulax monilata*;
Alexandrium tamarense: previous names *Alexandrium excavata, Alexandrium excavatum, Gonyaulax tamarensis*;
Cochlodinium polykrikoides: previous name *Cochlodinium heterolobatum*;
Karenia brevis: *Gymnodinium breve, Gymnodinium brevis, Ptychodiscus brevis*;
Karlodinium veneficum: previous names *Gymnodinium veneficum, Gymnodinium galatheanum, Gyrodinium galatheanum, Karlodinium micrum*;
Lingulodinium polyedrum: previous name *Gonyaulax polyedra*.

Haptophytes –
Prymnesium parvum: previous names *Prymnesium patelliferum, P. parvum* f. *patelliferum*.

Raphidophyceans –
Heterosigma akashiwo: previous names *Olisthodiscus luteus, Entomosigma akashiwo*.

[d] "Avian vacuolar myelinopathy (AVM) is a neurological disease that produces uncoordinated behavior in affected birds in wetland ecosystems of the southeastern U.S. Feeding and sentinel trials, field surveys, and genetic studies have implicated the introduced flowering plant species hydrilla (*Hydrilla verticillata*) and an associated epiphytic cyanobacterial species (Order Stigonematales) as a causal link to AVM. All morphotypes of cyanobacteria have been shown to produce the neurotoxic amino acid BMAA, including cyanobacteria of the Stigonematales that are epiphytic on hydrilla. If biomagnification of BMAA occurs in other ecosystems, as has been observed in the Guam ecosystem, then the consumption of fish (e.g., shad and herring) and waterfowl (e.g., Canada geese and mallards) from AVM-confirmed reservoirs in Arkansas, Texas, Georgia, North Carolina and South Carolina, U.S., could represent a significant human health risk" (Bidigare *et al.*, 2009, p. 71; also see Cox *et al.*, 2005; Lürling *et al.*, 2011; Holtcamp, 2012; Al-Sammak *et al.*, 2014). Importantly, BMAA has been found in nearly all cyanobacteria that have been assessed from terrestrial, freshwater, brackish, and marine environments (e.g., Cox *et al.*, 2005; Wilde *et al.*, 2005; Brand *et al.*, 2010; Lürling *et al.*, 2011; Al-Sammak *et al.*, 2014). Its structural isomer, 2,4-diaminobutyric acid (DABA), frequently co-occurs with BMAA (Banack *et al.*, 2010; Al-Sammak *et al.*, 2014).

[e] After reviewing the available information about *Synechococcus* blooms, Beardall (2008, p. 3) wrote, "While reports of toxicity in *Synechococcus* blooms are rare, this is largely because investigators have not been attuned to the possibility of this genus producing toxins." The toxicity of *Synechococcus* blooms likely has been underestimated.

[f] Examples of toxigenic benthic species: *Prorocentrum belizeanum*, *P. concavum*, *P. faustiae*, *P. hoffmannianum*, *P. lima* complex (likely several cryptic species), *P. maculosum*, and *P. rhathymum* (see Glibert and Burkholder 2018b, and references therein).

[g] The toxin EUGL is also produced by other euglenophytes including, thus far, *Euglena clavata*, *E. socialis*, *E. stellata*, *Euglenaria anabaena*, *Lepocinclus acus*, *Strombomonas borysteniensis*, and *Trachelomonas ellipsodalis* (Zimba *et al.*, 2017).

Table 7.2 Reported sublethal or chronic lethal effects of selected harmful algae on other organisms.[a]

Organism(s) impacted and reported effect(s)
Cyanobacteria
Anabaena flos-aquae (also see impacts under *Microcystis aeruginosa* and other MC producers)
Zooplankton:
Microcrustaceans
- *Daphnia pulex*: Lower fitness based on reduced filtering rate, smaller brood size, depressed survivorship, reduced rate of increase, and depressed net reproductive rate (Gilbert, 1994 and references therein).
- *Daphnia hyalina, D. pulicaria*: Feeding was inhibited (DeMott *et al.*, 1991).
- *Daphnia parvula, D. pulex*: Reduced feeding (Fulton, 1988a).
- *Diaptomus reighardi, Eurytemora affinis*: Avoided feeding (Fulton, 1988a).
- Rotifers: *Asplanchna girodi*, *Brachionus calyciflorus*, *Keratella cochlearis*, *Synchaeta pectinata* reduced fecundity, and reproduction was inhibited (Gilbert, 1994 and references therein).
Phytoplankton:
- *Chlamydomonas reinhardtii* (chlorophyte flagellate): Allelopathic; crude extracts decreased the number of motile cells, inhibited growth, and induced settling (Kearns and Hunter, 2000, 2001); and crude extracts or MC-LR increased the settling rate. Such effects would help to reduce inter-algal competition for resources such as water-column nutrients (Zurawell *et al.*, 2005 and references therein).
- *Thalassiosira weissflogii*, *Rhodomonas* sp., *Prymnesium parvum*: Allelopathic; inhibited growth (Suikkanen *et al.*, 2004).
Aphanizomenon flos-aquae
Fish:
- Common carp (three months old): Exhibited rapid opercular movement and abnormal swimming, suggested to have negative consequences on fish populations due to changes in reproductive and predator–prey interactions (from exposure to freeze-dried cells; Osswald *et al.*, 2007).
- Pacific herring: Severe reductions in spontaneous swimming behavior and touch response (Lefebvre *et al.*, 2005).
Zooplankton:
Microcrustaceans
- *Acartia bifilosa*: Reduced feeding and fecundity (Sellner *et al.*, 1996).

(*continued*)

Table 7.2 (*Continued*)

Organism(s) impacted and reported effect(s)

- *Daphnia longispina, D. magna*: Impaired reproduction (de Figueiredo *et al.*, 2004b).
- *Daphnia carinata*: Reduced appendage beat rate (Haney *et al.*, 1995).
- *Diaptomus reighardi, Eurytemora affinis*: Avoided feeding (Fulton, 1988a).
- *Eurytemora affinis*: Reduced fecundity and feeding (Fulton, 1988a).

Phytoplankton:

- *Rhodomonas* sp.: Inhibited growth (Zanchett and Oliveira-Filho, 2013 and references therein; Suikkanen *et al.*, 2004).

Cylindrospermopsis raciborskii

General: Other bioactive compounds present in *C. raciborskii* extracts and cells along with CYL likely contribute to its toxicity (Seifert, 2007). Toxins CYL and deoxy-CYL concentrate and bioaccumulate in a range of aquatic flora and fauna (Seifert, 2007; Kinnear, 2010).[b]

Mammals: *C. raciborskii* was implicated in cattle mortality (Hawkins *et al.*, 1997).

Fish:

- General: "In every investigated ontogenic stage, reports of the biological effects of CYL on fish species are scarce" (Sotton *et al.*, 2015, p. 5). Nearly all available information is for exposure to CYL rather than exposure to toxic *C. raciborskii* (Sotton *et al.*, 2015). CYL has been shown to accumulate in various fish species (e.g., brown trout; Sotton *et al.*, 2015).
- Zebrafish: Exposure to STX-producing *C. raciborskii* increased the mean swimming distance covered and the mean swimming velocity (Ferrão-Filho *et al.*, 2007).
- Zebrafish: Embryos were malformed (e.g., lateral and ventral body curvature and edema; Zanchett and Oliveira-Filho, 2013).
- Tilapia: Adults exposed by immersion to environmentally relevant concentrations of CYL sustained damage especially to the liver and kidneys, but also to heart, intestine, and gill (Gutiérrez-Praena *et al.*, 2012; Puerto *et al.*, 2012).

Amphibians: Cane toads – Tadpoles exposed to whole toxic cell extracts survived, but decreased relative growth rates and time spent swimming. Exposure to live toxic cultures led to bioaccumulation of CYL (~19-fold) (White *et al.*, 2007).

Molluscs:

- CYL bioaccumulated in tested mussels (*Anodonta cygnea*; Metcalf *et al.*, 2004).[c]
- Malaysian trumpet snail: Number of hatchlings decreased when exposed to live toxic culture. In contrast, exposure to whole-cell extracts with extracellular CYL increased hatchling numbers, and adults were not affected. Since CYL is a protein synthesis inhibitor, it may be especially toxic to rapidly developing tissues such as in snail embryos (Kinnear *et al.*, 2007).
- Swan mussel: Exposure of adults to CYL-producing cultures of *C. raciborskii* for 16 days led to CYL accumulation (up to 2.52 mg g tissue dry wt^{-1}). Most toxin was in the hemolymph (~68%), viscera (~23%), and foot and gonad (~8%). CYL was not detected in gill or adductor muscle tissue. After 2 weeks of depuration, ~50% of the toxin still remained in the tissues. The animals appeared to be unaffected (Saker *et al.*, 2004).

Arthropods other than zooplankton: CYL bioaccumulated in crayfish, especially in the hepatopancreas. The animals appeared unaffected, without histological abnormalities (Saker and Eaglesham, 1999; Nogueira *et al.*, 2004; Saker *et al.*, 2004).

Zooplankton:

- The community was more diverse, with larger species, when *C. raciborskii* abundance was low or undetectable. As abundance of *C. raciborskii* increased, micrograzers such as rotifers increased and became dominant (Leonard and Paerl, 2005).
- Small trichomes of *C. raciborskii* tended to clog cladoceran filters, reducing food intake and decreasing daphnid body size (Hawkins and Lampert, 1989; Nogueira *et al.*, 2004).
- Zooplankton diversity increased during and after a *C. raciborskii* bloom; copepods and rotifers apparently were able to sever the long filaments and shorten them to edible size for small-bodied herbivorous cladocerans (Bouvy *et al.*, 2001).
- *Ceriodaphnia dubia:* Animals were immobilized (Zagatto *et al.*, 2012).
- Daphnids sustained high mortality and low fecundity. Toxic blooms may reduce grazing pressure, giving the toxic strains a survival advantage over nontoxic strains (Nogueira *et al.*, 2004).
- *Daphnia magna:* Both fitness (fecundity) and growth potential decreased during *C. raciborskii* blooms (Nogueira *et al.*, 2004).
- *Daphnia pulex:* Decreased swimming velocity, mean swimming distance covered, and mean swimming velocity (Ferrão-Filho *et al.*, 2007, 2008).
- *Daphnia pulex, Moina micrura:* Reproduction rates decreased (Zanchett and Oliveira-Filho, 2013, and references therein).
- *Daphnia similis*: Fitness decreased (Zagatto *et al.*, 2012).
- *Eudiaptomus gracile:* Feeding was depressed on a toxic strain relative to feeding on a nontoxic strain (Rangel *et al.*, 2016).

Table 7.2 (*Continued*)

Organism(s) impacted and reported effect(s)

Phytoplankton:

- Decreased assemblage diversity (Dobberfuhl, 2003).
- *Microcystis aeruginosa* (cyanobacterium): Allelopathic; inhibited growth and MC-LR production, and promoted upregulation of alkaline phosphatase activity (Rzymski *et al.*, 2014).
- *Microcystis wesenbergii* and *Coelastrum sphaericum*, *Monoraphidium contortum* (chlorophytes): Allelopathic; inhibited photosynthesis (Figueredo *et al.*, 2007).

Bacteria: Total biomass and abundance of larger size classes increased post-bloom (Bouvy *et al.*, 2001).

Lyngbya majuscula

General:

- Significantly alters marine ecosystem dynamics. The many bioactive compounds known to be produced by toxic strains include tumor promoters, substances that enhance oncogene-induced cell transformations, and immunosuppressants. Three toxins are tumor promotors (Osborne *et al.*, 2001).
- Specific metabolites produced by *L. majuscula* act both as feeding attractants (e.g., to the long-tailed sea hare, a specialist herbivore) and as effective feeding deterrents to generalist fishes (Charpy *et al.*, 2012).

Birds: Altered foraging behavior (Estrella *et al.*, 2011).

Reptiles: Tumors developed in sea turtles that consumed *L. majuscula* (Arthur *et al.*, 2006). Toxins of *L. majuscula* are a suspected cause of the debilitating neoplastic disease of marine turtles known as fibropapillomatosis (Osborne *et al.*, 2001).

Fish:

- Altered feeding behavior and reduced biomass in the fish community; also, depressed species richness and reduced growth of juveniles, and decreased carrying capacity for fish (Gilby *et al.*, 2011; Hudon *et al.*, 2014).
- Parrotfish: Three bioactive substances produced by *L. majuscula* deterred feeding by juveniles (Thacker *et al.*, 1997; Kuffner and Paul, 2004; Kuffner *et al.*, 2006).

Invertebrates:

- Reduced recruitment (and survival – Table 7.1) of scleractinian corals and gorgonians, depressed species diversity, and decreased biomass (Kuffner and Paul, 2004, and references therein):
- Coral *Pocillopora damicornis*: The presence of *L. majuscula* significantly adversely affected recruitment. Larvae also exhibited avoidance behavior. *Note*: Certain species of crustose coralline red algae contain compounds that attract coral larvae and, thus, provide positive chemical cues for scleractinian settlement. *L. majuscula* mats have killed the crustose coralline algae beneath.

Seagrasses:

- Blooms decreased the habitat nursery capacity, and blooms have overgrown and smothered seagrass beds (Watkinson *et al.*, 2005; Tilling, 2007; Ng *et al.*, 2012).
- Adverse effects on shoalgrass occurred after declines in bloom biomass, indicating that *L. majuscula* can cause prolonged effects on shoalgrass production (Tilling, 2007).

Lyngbya wollei [T – aplysiatoxins, CYL, deoxy-CL, LYNGTX, STX analogs (e.g., decarbamoylsaxitoxin, decarbamoylgonyautoxin) (Camacho and Thacker, 2006; Seifert, 2007; Foss *et al.*, 2012)]

General: A diverse assemblage of invertebrate mesograzers lives on and within *L. wollei* mats. Trichomes of *L. wollei* are surrounded by a prominent extracellular polysaccharide sheath (~55% of the dry weight – apparently functions as a structural defense against herbivory; Camacho and Thacker, 2006).

Fish: Wetlands dominated by *L. wollei* have supported lower biomass of large fish, lower fish species richness, and more slowly growing juvenile perch (*Perca flavescens*) than macrophyte [vascular plant]-dominated wetlands (Hudon *et al.*, 2012).

Invertebrates:

- Apparently toxic to some amphipod species; eaten (but not preferred) by the amphipod *Hyalella azteca* (Camacho and Thacker, 2006).
- Acetylcholinesterase and glutathione-S-transferase (GST) activities were higher for amphipods (*Gammarus fasciatus*) within *L. wollei* mats, suggesting toxicity to the amphipods (Gélinas *et al.*, 2013).
- Wetlands dominated by *L. wollei* had lower invertebrate biomass than macrophyte-dominated wetlands (Hudon *et al.*, 2012).
- Grazer biomass was significantly less in areas with dominant *L. wollei*, apparently related to reduced food and habitat availability via declines in macrophytes and epiphytes (Lévesque *et al.*, 2012).

(*continued*)

Table 7.2 (*Continued*)

Organism(s) impacted and reported effect(s)

Habitat:

- There was an inverse relationship between abundance of *L. wollei* and abundance of macrophytes (e.g., freshwater eelgrass); blooms are considered symptomatic of ecosystem degradation (Hudon *et al.*, 2014). Dominance of *L. wollei* coincided with low macrophyte biomass, yielding a simplified, less productive ecosystem (Hudon *et al.*, 2014).
- *L. wollei* appears to proliferate when native primary producers are limited by unfavorable light or nutrient conditions, or physically removed (Evans *et al.*, 2007).
- Macrophytes suppressed benthic *L. wollei* mats (Doyle and Smart, 1998).

***Microcystis aeruginosa* (and various *Microcystis spp.* and *other MC producers*)**

General: Example, San Francisco Bay – Total microcystins [TMCs] were in all levels of the food web; higher TMC concentrations in striped bass than their prey suggested that MCs accumulated in biota of higher trophic levels. "This study suggests that even at low abundance, *Microcystis* may impact estuarine fish production through toxic and food web impacts at multiple trophic levels" (Lehman *et al.*, 2010, p. 229). MCs can bioaccumulate in many biota (White *et al.*, 2006 and references therein).

Mammals:

- The liver-to-body mass ratio increased due to liver hemorrhaging (Zurawell *et al.*, 2005).
- MCs bind specifically to hepatic cells, irreversibly inhibiting serine/threonine protein phosphatases PP1 and PP2 (important enzymes involved in tumor suppression), and causing disintegration of hepatocyte structure, apoptosis, liver necrosis, and internal hemorrhage. MC-LR may also bind to ATP synthetase, potentially leading to cell apoptosis. Some symptoms characteristic of MC poisoning include weakness, anorexia, gastroenteritis, vomiting, and diarrhea. Chronic exposure to MCs promotes liver cancer by inducing DNA damage. Other chronic effects such as increased liver weight, liver tissue damage, and kidney damage have been detected in mammals after treatment with low concentrations of MCs in drinking water (de Figueiredo *et al.*, 2004a – mostly based on mouse models).

Birds:

- Black-crowned night heron, mallard duck: High levels of MCs in gonad, egg yolk, and egg white, suggesting potential effects of MCs on waterbird embryos; also high MC content in spleens of both species (Ferrão-Filho and Kozlowsky-Suzuki, 2011).
- Piscivorous birds were considered to be at risk due to high MC levels in planktivorous smelt prey (but see Ibelings *et al.*, 2005).

Reptiles: European pond turtle, Mediterranean turtle – High MC content was found in liver; MCs also were detected in viscera and muscle tissues from fresh carcasses; and "liver crumbling" occurred during necropsy (Nasri *et al.*, 2008).

Fish:

- In various species, MCs have modified immunological and blood indices, causing increased activities of ALT (alanine amino-transferase), AST (aspartate aminotransferase), and LDH (lactate dehydrogenase). There has been damage to the liver, kidneys, heart, digestive tract, gills, spleen, and skin (Malbrouck and Kestemont, 2006 and references therein). Respiration and behavior also have been adversely impacted (Wiegand and Pflugmacher, 2005 and references therein). Younger stages have been more adversely affected than adults, and the toxins have been rapidly cleared from fish tissues (Malbrouck and Kestemont, 2006; Ibelings and Havens, 2008 and references therein). Oral and immersion exposure is slow to induce adverse effects, so acute toxic episodes are rare (Zurawell *et al.*, 2005).
- Increased MC production has occurred when *M. aeruginosa* is exposed to certain fish; apparently, blooms can respond to a chemical signal related to feeding, even when fish are not vigorously consuming the cyanobacteria (Jang *et al.*, 2004).
- Histopathology of liver tissue (striped bass, Mississippi silverside) suggests that fish health was adversely affected by tumor-promoting substances, especially in areas where TMC concentrations were elevated (Zurawell *et al.*, 2005).
- Lipopolysaccharides present on the cell surface of cyanobacteria such as *Microcystis* can stimulate drinking in fish, which would increase the potential for toxin exposure and promote osmoregulatory imbalance (Ferrão-Filho and Kozlowsky-Suzuki, 2011 and references therein).
- Atlantic salmon, Chinook salmon, steelhead trout: MCs have been linked to "net-pen liver disease;" histopathological changes have included diffuse necrosis and hepatic megalocytosis (Zurawell *et al.*, 2005 and references therein).
- Brown trout: Exposure to lysed *Microcystis* cells indicated an energy allocation from growth processes toward stress responses (Bury *et al.*, 1996b).
- Carp: MC-LR blocks gill activity: Ion pumps (e.g., Na^+-K^+, Na^+, HCO_3^-, and Ca^{+2}-ATPases) in gill tissue were directly inhibited by MC-LR. This toxin also blocks the hydrolysis of phosphorylated protein and inhibits the aspartic dephosphorylation step of the sodium pump enzymes (Gaete *et al.*, 1994; Zambrano and Canelo, 1996).
- Carp: Embryotoxic effects have included delayed hatching, low number of hatched embryos, suppressed embryonic development, disturbance of air bladder filling, significant inhibition of GSTs; missing eye pigmentation at 48 hours post-fertilization; an incomplete filling of air bladder after 120 hours of exposure to MCs; and atrophy of hepatocytes. In addition, gills exhibited pinpoint necrosis, epithelial ballooning, folded lamellar tips, exfoliation of the lamellar

Table 7.2 (*Continued*)

Organism(s) impacted and reported effect(s)

epithelium, elevated aspartate aminotransferase activity, and elevated serum bilirubin concentrations (Chorus and Bartram, 1999).

- Carp: In field studies during toxic *M. aeruginosa* blooms, fish consumed the toxic cells and appeared healthy but showed signs of hepatocyte atrophy; and 37% of the collected fish had gills displaying folded lamellar tips with epithelial ballooning and localized necrosis (Zurawell *et al.*, 2005 and references therein).
- Loach: Exhibited abnormalities such as pericardial edema and tubular heart, bradycardia, homeostatis, poor yolk resumption, small head, curved body and tail, and abnormal hatching. Overall, exposure to crude MC extracts led to gross malformations in embryo development, such that progression to the larval stage ceased (Zurawell *et al.*, 2005 and references therein).
- Medaka: Sustained hepatobiliary damage (hepatobiliary hypertrophy, hepatic hemorrhage, and necrosis at late development stages in embryos; Jacquet *et al.*, 2004).
- Roach: When fed sublethal cell densities of *Microcystis* cells, fish were unable to digest and lyse enough cells to release a harmful amount of toxin. Culture experiments with roach feces revealed that most *Microcystis* cells were not digested, and *Microcystis* grew exponentially after passing through the gut (Kamjunke *et al.*, 2002).
- Silver carp, tilapia: In laboratory studies, fish selectively consumed nontoxic strains *of M. aeruginosa*. Ingestion decreased as the proportion of toxic cells increased; grazing response decreased linearly as the proportion of toxic cells increased above 25% (Keshavanath *et al.*, 1994). Fish exposed to nontoxic strains had higher opercular beat rates (which effectively maintain the flow of water and suspended food particles over the gills; Beveridge *et al.*, 1993; Keshavanath *et al.*, 1994).
- Silver carp (80-day sub-chronic experiment): Fish fed *Microcystis viridis* that was collected from a eutrophic pond had measurable MC-RR in their blood (highest levels), liver, and muscle. In contrast, most MC-LR was in the intestines, and MC-LR was not detected in blood or muscle. The date suggest that silver carp have a mechanism to degrade MC-LR actively and to inhibit MC-LR transport across the intestines (Xie *et al.*, 2004).
- Silver carp, goldfish: MCs accumulated in the liver (up to 150 ng g dry wt^{-1}; Chen *et al.*, 2009).
- Tilapia (*Oreochromis mossambicus*): *Microcystis* cell lysate inhibited ion pumps (e.g., Ca^{+2}) in the gills more effectively than exposure to pure MC-LR, due to fatty acids present in the cells which interacted with the membranes of gill epithelial cells. These fatty acids inhibited the p-nitrophenol phosphatase activity of the gill basolateral membrane (Bury *et al.*, 1996a, 1998).
- Tilapia (*Oreochromis mossambicus*): Chronic exposure to low MC-LR levels (0.5 μg L^{-1}) reduced growth in larval fish, perhaps by increasing energy demands as required by detoxication processes (Zurawell *et al.*, 2005 and references therein).
- Zebrafish: Reduced growth and reduced weight by 25%. Environmentally relevant concentrations of MC-LR (0.5 to 50 μg L^{-1}) influenced the diurnal rhythm, leading to impaired food uptake and depressed spawning success (Baganz *et al.*, 1998).
- Zebrafish: Vitellogenin genes were highly upregulated in fish exposed to *Microcystis* but not to MC, suggestive of potential endocrine disrupting effects of *Microcystis* blooms. There was a significant decrease in the percentage of adults that spawned when exposed to *Microcystis*, but fecundity and larval survival were not affected (Rogers, 2010).

Molluscs:

- *Anodonta simpsonina* (freshwater clam): This species, an important food source of muskrats, bioaccumulated high amounts of MCs. The clams were suggested as a route of toxicity for muskrats and their predators (Prepas *et al.*, 1997).
- Asian clam (exotic/invasive): Cytotoxic effects occurred from exposure to toxic strains of *M. aeruginosa* (Martins *et al.*, 2009).
- Blue mussel: After being fed toxic *M. aeruginosa* for 3 days, MCs bioaccumulated (Williams *et al.*, 1997).
- Freshwater clam: Bioaccumulated MCs from phytoplankton containing MC-LR (~0 to 8.3 μg L^{-1} as cellular toxin); after ~12 days, the animals contained from ~24 to 527 ng g^{-1} MC-LR equiv. MC-LR concentrations were usually higher in the visceral mass than in gill and muscle tissues. After clams were placed in toxin-free water, MC-LR equiv. levels in tissues rapidly decreased for 6 days (by ~70%), but remaining levels were relatively stable for the following 15 days (Prepas *et al.*, 1997).
- Great pond snail: Sustained a marked decrease in egg production. Snails accumulated MC-LR; the amount accumulated depended on the toxin content and *Microcystis* abundance in the phytoplankton assemblage (Zurawell *et al.*, 1999).
- Mediterranean mussel: Accumulated MCs when fed *M. aeruginosa*, especially in the digestive tract (muscle, gill, and foot had very little toxin). When transferred to nontoxic phytoplankton food, depuration occurred with a 50% decrease within 2 days, but MCs were still detectable after 2 weeks and increased via consumption of feces (Vasconcelos, 1995; Amorim and Vasconcelos, 1999).

(*continued*)

Table 7.2 (*Continued*)

Organism(s) impacted and reported effect(s)
- Swan mussel: Accumulated high amounts of *Oscillatoria agardhii*, mainly into the hepatopancreas. The mussels apparently were not harmed, but MCs extracted from them were toxic to mice, suggesting that the MCs were not metabolized (Eriksson *et al.*, 1989).
- Zebra mussel: Decreased food intake, filtration, absorption, and fecal loss, and showed significantly lower net energy balance in growth (Juhel *et al.*, 2006). Pseudofeces production was higher in the presence of toxic than nontoxic *Microcystis* strains, but pseudofeces contained more living cells of co-consumed nontoxic green algae than of the cyanobacterium (Pires and Van Donk, 2002). After exposure to *Microcystis* crude extracts, zebra mussels increased GST and glutathione peroxidase activities, accompanied by short-term depletion and oxidation of the glutathione pool (Peuthert and Wiegand, 2004). In a food web study, MCs were found in ~90% of all zebra mussel samples, indicating transfer of MCs within the food web (Zurawell *et al.*, 2005 and references therein).
- Zebra mussel (exotic/invasive, Hudson River): Preferentially ingested *Microcystis* (Baker *et al.*, 1998).
- Zebra mussel (exotic/invasive, Lake Erie): Given credit for promoting toxic blooms, and also reject live *Microcystis* cells in their pseudofeces (Vanderploeg *et al.*, 2001).
- Zebra mussel: Biotransformed MC-LR by conjugation to a glutathione/MC-LR conjugate, apparently the first step in detoxication (also found in the macrophyte coontail, the crustacean zooplankter *Daphnia magna*, and zebrafish; Pflugmacher *et al.*, 1998).
Note: Freshwater and marine mussels and clams mostly have been found to be relatively resistant to MCs and other cyanotoxins (Wiegand and Pflugmacher, 2005).
Crustacea other than zooplankton:
- Brine shrimp: Exposure to MC-LR increased GST activity of the detoxification system and conjugation of the toxin to GST (de Figueiredo *et al.*, 2004a).
- Red swamp crayfish: Accumulated MCs in the intestine and hepatopancreas (de Figueiredo *et al.*, 2004a).
- Signal crayfish: Accumulated MCs both during a benthic *Oscillatoria* bloom and in laboratory experiments. Harmful effects were not detected (Liras *et al.*, 1998).
- Southwestern Atlantic burrowing crab: Sustained physiological impacts from exposure to extracts of *M. aeruginosa*, including inhibition of Na^+ and K^+ ATPase, increased GST activities, and enhanced oxygen radical scavenging capacity (Vinagre *et al.*, 2002).
Note: In general, crayfish grow well on toxic *Microcystis*; MCs accumulate in intestine and hepatopancreas, but not in muscle (Vasconcelos *et al.*, 2001).
Zooplankton
Microcrustaceans
- Responses are highly species- and strain-specific. MCs have accumulated in natural zooplankton communities (Ferrão-Filho *et al.*, 2002 and references therein).
- Declines in biomass and altered species composition of zooplankton communities have occurred during blooms, attributed to difficulty in filtering large colonies, indigestibility, poor nutritional quality, and/or toxicity. Large-bodied cladocerans (e.g., larger *Daphnia* spp.) seem more susceptible than smaller species (e.g., *Ceriodaphnia reticulata, Bosmina longirostris*), perhaps because their feeding behavior may limit their ability to avoid the toxic cyanobacteria. Unlike cladocerans, copepods appear to feed size-selectively on colonial cyanobacteria, and to avoid toxic strains (Zurawell *et al.*, 2005 and references therein).
- MCs significantly decreased beat rates of thoracic legs, mandibles, foregut, and second antennae. Stimulation of gut muscles led to permanent contraction of the midgut (interfered with digestion, nutrient assimilation, and uptake of ions; caused exhaustion, loss of cell-to-cell contact within the digestive epithelium, and inhibition of protein phosphatases. In a food web study, MCs were found in 80% of all zooplankton samples. Thus, transfer of MCs within the food web occurred, as well as temporary feeding inhibition in *Daphnia* (Zurawell *et al.*, 2005 and references therein).
- Highest *M. aeruginosa* abundance coincided with a low ratio of cladocerans-to-calanoid copepods (Lehman *et al.*, 2010).
- *Acartia tonsa*: Reduced fecundity (Schmidt and Jónasdóttir, 1997).
- *Bosmina longirostris*: Reduced feeding (Jiang *et al.*, 2013; but there was conflicting information depending on the cyanobacterial strain and the zooplankton strain).
- *Bosmina longirostris, Ceriodaphnia quadrangula, Daphnia ambigua, Diaptomus reighardi, Simocephalus serratulus*: Reduced feeding (Fulton and Paerl, 1987).
- *Ceriodaphnia quadrangula*: Reduced feeding (Watanabe *et al.*, 1997).
- *Cyclops vicinus*: Avoided contact with *Microcystis* colonies (Watanabe *et al.*, 1997).
- *Daphnia ambigua*: Reduced feeding (Zhang *et al.*, 2009). Abundance decreased during blooms of toxic *M. aeruginosa* in laboratory experiments (Fulton and Paerl, 1987).
- *Daphnia galeata*: Feeding was inhibited (Rohrlack *et al.*, 2001). Toxic *Microcystis* cells in the midgut caused the epithelium to lose cohesion. Loss of cell-to-cell contact may facilitate MC uptake into the blood (Rohrlack *et al.*, 2005).

Table 7.2 (*Continued*)

Organism(s) impacted and reported effect(s)
- *Daphnia hyalina, D. pulcaria*: Feeding was inhibited (De Mott *et al.*, 1991; Rohrlack *et al.*, 2001).
- *Daphnia longispina*: Reduced growth and clutch size (Reinikainen *et al.*, 1999; Hietala *et al.*, 1995).
- *Daphnia magna, Moina macrocopa*: Avoided feeding (Yasuno and Sugaya, 1991).
- *Daphnia magna*: Reduced grazing activity (Łotocka, 2001).
- *Daphnia pulex*: Reduced feeding (DeMott, 1999); also exhibited reduced growth, depressed reproduction rate and clutch size (DeMott *et al.*, 1991; Reinikainen *et al.*, 1999); formed ephippia (molted carapaces enclosing one or more fertilized eggs, resistant to harsh conditions; Zurawell *et al.*, 2005 and references therein). Effects differed depending on the zooplankton clone (Hietala *et al.*, 1997). Outcompeted *Bosmina longirostris* in the absence of *M. aeruginosa*, but the competitive outcome was reversed when *M. aeruginosa* was added, and competitive reversal was more pronounced both when more *M. aeruginosa* was added and when the temperature was increased from 20 to 28 °C (Jiang *et al.*, 2014).
- *Daphnia pulicaria*: Feeding was inhibited (Rohrlack *et al.*, 2001).
- *Diaphanosoma*: Did not consume *Microcystis* (Watanabe *et al.*, 1997).
- *Diaptomus reighardi* (and other small copepods): Increased abundance during blooms of toxic *M. aeruginosa* (laboratory experiments; Fulton and Paerl, 1988).
Rotifers
- *Brachionus rubens*: Reduced ingestion rates (Rothhaupt, 1991).
Macrophytes:
- Duckweed (*Spirodela oligorrhiza*): MC-LR levels as low as 10 μg L^{-1} inhibited growth and reduced plant chlorophyll *a* and chlorophyll *b* content. MC-LR also caused a reduction in the number and mass of fronds, and plants concentrated the toxin (Romanowska-Duda and Tarczynska, 2002; de Figueiredo *et al.*, 2004a).
- Coontail: MC-LR at environmentally relevant concentrations (0.1 to 5 μg L^{-1}) decreased the chlorophyll *a*-to-chlorophyll *b* ratio (considered to be a stress reaction that reduces photosynthetic efficiency; de Figueiredo *et al.*, 2004a; Wiegand and Pflugmacher, 2005 and references therein).
- Coontail: Exposure to MC-LR led to enhanced formation of hydrogen peroxide, in turn causing elevation of anti-oxidative enzymes. Elevation of superoxide dismutase (SOD), glutathione peroxidase, and ascorbate peroxidase indirectly indicated formation of ROS and ongoing detoxification in the plants (Ou *et al.*, 2005; Wiegand and Pflugmacher, 2005 and references therein).
- Duckweeds (*Lemna minor, Wolffia arrhiza, Spirodela oligorrhiza*): Reduced growth in the presence of small quantities of MC-LR, usually 5 μg L^{-1} or less (Mitrovic *et al.*, 2005; Saqrane *et al.*, 2007; de Figueiredo *et al.*, 2004a; Wiegand and Pflugmacher, 2005 and references therein).
- Duckweed (*Lemna japonica*): Reciprocal allelopathic responses occurred between axenically cultured *L. japonica* and two toxic strains of *M. aeruginosa*. Exposure to toxic *M. aeruginosa* inhibited *L. japonica* growth, whereas exposure to axenic duckweed increased MC production and also inhibited growth of the cyanobacteria (Jang *et al.*, 2007).
- Elodea, coontail, Eurasian watermilfoil: After 24 hours of exposure to 0.5 μg MC-LR L^{-1}, photosynthesis decreased by 50–90% relative to pre-treatment values, and there was an overall reduction in growth (de Figueiredo *et al.*, 2004a; Wiegand and Pflugmacher, 2005; Zurawell *et al.*, 2005 and references therein).
- Phragmites: After 24 hours of exposure to 0.5 μg MC-LR L^{-1}, photosynthesis decreased by 10%. MC-LR was absorbed especially by the stems and rhizomes, which had elevated soluble GSTs (de Figueiredo *et al.*, 2004a and references therein).
- Java moss: Bioaccumulated high levels of MC-LR (de Figueiredo *et al.*, 2004a, and references therein).
Filamentous Macroalgae:
- *Cladophora fracta*: After 24 hours of exposure to 0.5 μg MC-LR L^{-1}, photosynthesis decreased by 10% (de Figueiredo *et al.*, 2004a; Wiegand and Pflugmacher, 2005 and references therein).
Phytoplankton:
- *Chlorella vulgaris*, *Oocystis marssonii* (chlorophytes), and *Microcystis wesenbergii*: Allelopathic; *M. aeruginosa* inhibited growth (Zak and Kosakowska, 2014; Dunker *et al.*, 2013; and Yang *et al.*, 2014, respectively).
- *Chlamydomonas neglecta* (chlorophyte flagellate): The allelochemical kasumigamide, produced by *M. aeruginosa*, inhibited growth and flagellar movement (Ishida and Murakami, 2000).
- *Chlamydomonas reinhardtii*: Was paralyzed by MC-LR, and settlement to the bottom of the culture vessels was enhanced (Engelke *et al.*, 2003).
- *Nephroselmis olivacea* (chlorophyte flagellate): Reproduction was inhibited by MC-LR (Christoffersen, 1996).
- *Nostoc muscorum, Anabaena* sp. (cyanobacteria): Growth and photosynthesis were inhibited, and cell lysis increased, after exposure to MC-LR for 6 days (Singh *et al.*, 2001).

(*continued*)

Table 7.2 (*Continued*)

Organism(s) impacted and reported effect(s)

- Addition of nontoxic *Planktothrix agardhii* enhanced MC production by *M. aeruginosa* (Sukenik *et al.*, 2002).
- *Peridinium gatunense* (dinoflagellate): Allelopathic; growth and photosynthesis were depressed by *Microcystis* sp. (Sukenik *et al.*, 2002).

Synechococcus elongatus (with *Synechocystis* sp. – Richardson, 2004)

Zooplankton: Macrozooplankton in *Synechococcus* blooms switched to micrograzers as their food resource. This change, along with increased egg production and high hatching success rates, apparently enabled them to maintain their populations (described as a food web effect; Vargo *et al.*, 1996).

Invertebrates: Longstanding blooms in south Florida (Keys, Florida Bay – Lapointe *et al.*, 1994; Butler *et al.*, 1995) have caused widespread loss of spiny lobsters and multiple sponge species (food web effect; see Table 7.1).

Seagrasses: Longstanding blooms in south Florida have exacerbated loss of seagrasses (turtlegrass, shoalgrass, and manateegrass) via light reduction (Phlips *et al.*, 1995, 1999; Hall *et al.*, 1999). "Algal blooms [largely *Synechococcus*] in Florida Bay have had a number of negative impacts on the ecosystem, such as . . . increased light attenuation which has reduced the distribution of seagrass beds" (Berry *et al.*, 2015, p. 362).

***Trichodesmium* spp.**

Zooplankton:

- *Acartia tonsa*: Reduced fecundity; also exhibited lethargy and paralysis (Guo and Tester, 1994).
- *Clausocalanus furcatus*: Reduced feeding (Hawser *et al.*, 1992).
- *Farranula gracilis*: Did not feed (Hawser *et al.*, 1992).
- *Labidocera* sp.: Did not feed (O'Neil and Roman, 1994).
- *Macrosetella gracilis*: Were lethargic (O'Neil and Roman, 1994).
- *Penaeus merguiensis*: Starved (Preston *et al.*, 1998).
- *Temora turbinata*: Reduced feeding (O'Neil and Roman, 1994).
- *Tigriopus californicus*: Did not feed (O'Neil and Roman, 1994).

Diatoms

Toxic *Pseudo-nitzschia* complex, e.g., *P. australis, P. delicatissima* (Lefebvre and Robertson, 2010; Prince *et al.*, 2013)

General: DA is a polar, water-soluble amino acid (rapidly depurated from the digestive tract, within 2–3 days) that interacts with glutamate receptors in the central nervous system, leading to overstimulation of excitable tissues, neurotoxicity, and neuronal cell death (Iverson *et al.*, 1990; Tryphonas *et al.*, 1990; Lefebvre *et al.*, 2007). A short food chain is required to transfer DA efficiently through the food web, such as large immediate consumers with a direct link between toxic *Pseudo-nitzschia* and large predators. A wide array of pelagic fauna as well as some benthic fauna have been found to contain DA (Bargu *et al.*, 2011), including zooplankton, shellfish, crustaceans, worms, marine mammals, and birds. DA has also been measured in sediments. These data demonstrate the stable transfer of DA through the marine pelagic food web and to the benthos (Trainer *et al.*, 2012). Low-level exposure (that is, less than doses that do not cause obvious symptoms) may result in different whole-body responses than high-level exposure. Low-level exposure to DA over several weeks has made zebrafish more sensitive to subsequent exposures. Chronic, low-level (apparently asymptomatic) exposure to DA has caused an immune response and production of a DA-specific antibody in serum (Landsberg *et al.*, 2014 and references therein).

Mammals:

- California sea lion: A chronic neurological syndrome was characterized by epilepsy and abnormal behavior long after the initial exposure, due to lasting damage in the central nervous system and progressive, cumulative effects from seizure propagation (Goldstein *et al.*, 2008). Reproductive failure occurred with increased abortion rates and premature live births (Goldstein *et al.*, 2009), as well as degenerative cardiomyopathy from chronic exposure to DA (Zabka *et al.*, 2009).
- California sea lion, sea otter: Degenerative cardiomyopathy occurred from chronic exposure to DA (Kreuder *et al.*, 2005; Zabka *et al.*, 2009).

Fish:

- In general, toxicity to fish from DA has not been documented, but fish can be important vectors of DA in the food web (Lefebvre *et al.*, 2012). Plankton grazers (e.g., West Coast-Pacific sardine, northern anchovy, jack smelt; Gulf (Gulf Coast) menhaden) can contain high levels of DA. Example: the viscera of northern anchovies associated with mass mortality of California sea lions contained 220 μg DA L^{-1}; Lewitus *et al.*, 2012 and references therein). Non-planktivorous fish such as chub mackerel and jack mackerel have contained detectable levels of DA during *Pseudo-nitzschia* blooms (Busse *et al.*, 2006; Del Rio *et al.*, 2010).
- Diverse benthic fish taxa can also accumulate high levels of DA in association with blooms, partly because as the blooms wane, toxic "marine snow" settles out with dying *Pseudo-nitzschia* cells (Parsons and Dortch, 2002; Bates and Trainer, 2006; Sekula-Wood *et al.*, 2009). Examples: Pacific sanddabs accumulated DA to levels 25-fold greater than the federal regulatory limit for seafood (Kvitek *et al.*, 2008); and eight species of commercially valuable benthic fish species (soles, turbots, halibut) contained detectable levels of DA even during non-bloom periods (Vigilant and Silver, 2007).

Table 7.2 (*Continued*)

Organism(s) impacted and reported effect(s)

Molluscs: Pacific oyster: Reduced the number of hemocytes (*P. multiseries* – Jones *et al.*, 1995a); shell valves closed (Jones *et al.*, 1995b).

Other Invertebrates aside from Zooplankton: Diverse filter-feeding bivalve species accumulated elevated concentrations of DA when in the vicinity of blooms (*P. australis*; predatory Pacific sanddabs contained up to 514 μg DA L^{-1}) (Goldberg, 2003).

Zooplankton: Reduced feeding and reduced fecundity were sustained by the rotifer *Brachionus plicatilis* (*P. multiseries*; Whyte *et al.*, 1996).

Phytoplankton:

- *Akashiwo sanguinea*: Allelopathic; inhibited growth (*P. multiseries*; Xu *et al.*, 2015).
- *Chattonella marina* (toxigenic raphidophycean), *Rhodomonas salina*: Allelopathic; inhibited growth (*P. pungens*; Xu *et al.*, 2015).

Dinoflagellates

Akashiwo sanguinea

Birds: Clark's grebe, Pacific loon, red-throated loon, surf scoter, western grebe – Developed subtle, gross, nonspecific lesions. Affected birds also had slimy yellow-green material on their feathers, which were saturated with water, and they were severely hypothermic (Jessup *et al.*, 2009). Live-stranded birds responded well to rinsing, rehydration, warming, and nutritional supplements using standard treatment protocols for rehabilitating birds oiled with petroleum products (Jessup *et al.*, 2009; Gaydos, 2012).

Zooplankton: Natural assemblage – Total abundance of herbivorous zooplankton was lower within a layer of *A. sanguinea* than in any other depth interval between 0 and 40 m; 96% of all herbivores sampled belonged to zooplankton groups that exhibited patterns of avoidance behavior as indicated by their vertical distributions (Fíedler, 1982).

Microcrustaceans:

- *Acartia tonsa*: Reduced feeding and egg hatching (Fíedler, 1982; Turner *et al.*, 1998).
- *Calanus pacificus*: Reduced feeding (Fíedler, 1982).
- *Paracalanus parvus*: Reduced feeding (Fíedler, 1982).
- *Centropages hamatus*: Survival of nauplii was low when fed *A. sanguinea* in comparison to survival when feeding on benign algal controls (Murray and Marcus, 2002).

Phytoplankton: *A. sanguinea* consumes various microalgal prey (see Table 7.1).

Toxic *Alexandrium* complex: *A. catenella, A. fundyense, A. minutum* (and various other species)

Fish:

- Newly settled winter flounder, larval sheepshead minnow, larval mummichog: Sublethal exposure via consumption of toxin-contaminated copepod prey caused reduced swimming performance. Larval sheepshead minnows also exhibited reduced prey capture and reduced predator avoidance (*A. fundyense*). "Adverse effects on prey capture or predator avoidance may reduce larval survival and facilitate the transmission of STXs through the food web" (Samson *et al.*, 2008, p. 168).
- Cultured post-smolt Atlantic salmon exposed to *A. catenella*: Exhibited convulsions; significant increase in blood levels of sodium, potassium, and chloride; and, in gill tissue, congestion, telangiectasia, blanching epithelia, cell hypertrophy, and lamellar hyperplasia, suggesting disruption of osmoregulatory capacity (Aguilera *et al.*, 2016).

Molluscs:

- Eastern oyster: The adductor muscle was paralyzed from exposure to bloom densities of *A. fundyense*, but there was no significant effect on hemocyte numbers, morphology, or functions. These findings are consistent with known interference of STXs with sodium channel function in neural tissues (Hégaret *et al.*, 2007a).
- Pacific oyster: Reduced pumping and increased pseudofaeces production (*A. catenella*; Dupuy and Sparks, 1967); reduced clearance rate (*A. fundyense*; Lassus *et al.*, 1996); reduced clearance rate with inhibited shell valve activity, and reduced rate of biodeposition (*A. minutum*; Lassus *et al.*, 1999). STXs bioaccumulated, with no significant effect on hemocytes (Hégaret *et al.*, 2007a).

Zooplankton:

- *Acartia clausi*: Decreased fecundity (*A. minutum*; Guisande *et al.*, 2002); delayed development (*A. minutum*; Frangópulos *et al.*, 2000).
- *Acartia tonsa*: Inhibited grazing (*A. fundyense*; Marcoval *et al.*, 2013).
- *Acartia tonsa*: Waterborne chemicals produced by *A. tonsa* caused a 2.5-fold increase in STX production by *A. minutum* in nitrate-rich medium (but not in low nitrate treatments) relative to control cultures without the grazers, and further grazing was inhibited (Selander *et al.*, 2006). The magnitude of grazer-induced STX production was directly proportional to the degree of N availability (Selander *et al.*, 2006; Bergkvist *et al.*, 2008).
- *Acartia tonsa, Eurytemora herdmanii*: Reduced feeding, and STXs bioaccumulated (*A. fundyense*; Teegarden and Cembella, 1996).

(*continued*)

Table 7.2 (*Continued*)

Organism(s) impacted and reported effect(s)

- *Centropages typicus*: Waterborne chemicals from this grazer caused a 20-fold increase in STXs production (similar to the dinoflagellate response to *A. tonsa*, but much more so), versus negligible change in toxin production when exposed to the grazer *Pseudocalanus* sp. (*A. minutum*; Bergkvist *et al.*, 2008).
- *Euterpina acutifrons*: Greatly reduced nauplii activity (*A. minutum*; Bagøien *et al.*, 1996).

Phytoplankton:

- *Thalassiosira* cf. *gravida*: Allelopathic; culture filtrate inhibited growth and nutrient utilization (*A. fundyense;* Lyczkowski and Karp-Boss, 2014).
- *Chaetoceros neogracile* (diatom): Allelopathic; inhibited photosynthesis and decreased cell size (*A. minutum*; Lelong *et al.*, 2011).

Alexandrium monilatum

Molluscs:

- Hooked mussel: Inhibited byssus production (Sievers, 1969).
- Eastern oyster: Shell valves closed (no filtration), shell valve gape decreased, and clearance rate was depressed (May *et al.*, 2010).
- Green mussel: Shell valve gape decreased, and clearance rate was depressed (May *et al.*, 2010).
- Northern quahog: Shell valve gape decreased, and clearance rate was depressed (May *et al.*, 2010).

Alexandrium tamarense

Mammals:

- North Atlantic right whale: Trophic transfer of STXs, accumulated from *A. tamarensis* via zooplankton prey, was suggested to be a factor contributing to failure of a population of this endangered mammal to recover (Doucette *et al.*, 2006a).
- Sea otter: Foraging behavior (food preference, foraging efficiency, and distribution) of this keystone marine predator was altered by toxic *A. tamarense*. Butter clams are preferred prey of sea otters in the southeastern Alaska area. Otters foraged on butter clams in areas of intermediate prey toxicity (200–500 µg STX eq. 100 g^{-1}), but discarded the most toxic body parts. At highly toxic sites (prey toxicity > 500 µg STX eq. 100 g^{-1}), sea otters avoided butter clams and other large, abundant but toxic bivalve mollusc prey, and consumed smaller and/or less abundant, nontoxic prey (Kvitek and Bretz, 2004). Earlier laboratory experiments with analogous findings guided the field research (Kvitek *et al.*, 1991).

Birds:

- Common eider: Avoided toxic blue mussels as prey under field conditions. In laboratory experiments, eider were offered toxic versus nontoxic mussel meats and refused the toxic meats. If force-fed toxic mussel meat, the food was regurgitated almost immediately. "This selective behavior could have long-term implications for the nutrition of the ducks. While ducks would normally choose large mussels low on the shore . . . the presence of red tide in Maine appears to drive the ducks higher up the shore, where they must settle for smaller, less toxic mussels or cease feeding altogether. . . . The ratio of shell to meat is higher, forcing ducks to be less effective predators. In some areas the eiders switch their prey to sea urchins" (Shumway *et al.*, 2003, p. 12, and references therein).
- Shag: Lost equilibrium and staggered, and many vomited sand eel (sand lances) prey that had accumulated STX (Wood and Mason 1968).
- Black oystercatcher: Dropped or rejected mussel meat (levels > 1500 µg 100 g^{-1}) during a bloom of toxic STX dinoflagellates, but did not engage in that behavior when exposed to nontoxic prey. Birds also switched to nontoxic prey and only partly consumed mussel prey with high levels of STXs (Shumway *et al.*, 2003 and references therein).
- Glaucous-winged gull: Initially regurgitated toxic butter clams within 5 minutes of ingestion, whereas nontoxic butter clams were not regurgitated. In experiments, gulls previously conditioned with toxic butter clams refused to eat either toxic or nontoxic butter clams, but ate other bivalve mollusc species. In the field, gulls at a highly toxic site consumed significantly fewer butter clams than at a nontoxic site. Gulls foraging at a toxic site discarded the siphons (major site of toxin storage) of both toxic and nontoxic butter clams, but did not discard the siphons of other bivalve molluscs that were eaten. In contrast, gulls feeding at a nontoxic site did not discard the siphons from butter clams (Shumway *et al.*, 2003 and references therein).
- Cormorants: Recovered birds that were banded and released during *K. brevis* outbreaks were readmitted to the clinic with the same cerebellar ataxa noted during their first admittance within 5 or more days after release, suggesting either no learned response to the presence of toxins in their food source, or a role of aerosol-borne toxins (which they could not avoid; Shumway *et al.*, 2003 and references therein).

Fish:

- Atlantic herring, American pollock, winter flounder, Atlantic salmon, cod: When experimentally dosed, fish exhibited loss of equilibrium (sideways or upside-down swimming), immobilization, and shallow arrhythmic breathing (White, 1981b).
- Larvae of many fish species directly depend on dinoflagellates as food items for the first week of feeding or slightly longer. Experiments based on use of toxic cells as a food source indicated that "the quality, density, and patchiness of prey, and larval behavioral feeding mechanisms, are significant to successful larval feeding process and growth" (Smayda, 1991, p. 284). Larval fish can serve as vectors for biomagnification and food web transfer of the toxins (Smayda, 1991 and references therein).

Table 7.2 (*Continued*)

Organism(s) impacted and reported effect(s)

- Neurotoxic effects and behavior modification occurred following ingestion of toxic cells (Robineau *et al.*, 1991a, 1991b).
- Atlantic cod, Atlantic herring, Atlantic mackerel, capelin, red sea bream, winter flounder: Larvae that consumed toxic cells exhibited signs of paralysis (swam erratically and sank); red sea bream lost equilibrium and swam on their side, upside down, and/or in circles prior to paralysis. "Such behavioral dysfunction poses a fundamental constraint on searching behavior of fish larvae for food via chemical, acoustical, and/or tactile stimuli" (Smayda, 1991, p. 284, and references therein). Adult fish exhibited mouth gaping (White, 1977).

Mussels:

- Atlantic surfclam: Reduced clearance rate (Lesser and Shumway, 1993).
- Bay scallop: Reduced clearance rate (Lesser and Shumway, 1993).
- Blue mussel: Exhibited shell valve closure, increased mucus production (Shumway and Cucci, 1987); reduced clearance rates (Lesser and Shumway, 1993); inhibited byssus production (Shumway *et al.*, 1987); and altered heart rate (Gainey and Shumway, 1988).
- Butter clam: Were larger and more abundant in high-toxicity areas, and were avoided by sea otters despite the fact that butter clams are usually the otters' preferred prey (explained above), lending support to the premise that STX toxicity provides a refuge for the butter clams from sea otter predation (Kvitek and Bretz, 2004). STXs appear to function as an effective chemical defense for butter clams in areas of high *A. tamarense* toxicity (Kvitek *et al.*, 1991; also see *Mammals* above).
- Farrer's scallop: Inhibited egg hatching and reduced larval survival (Yan *et al.*, 2001).
- Northern quahog: Exhibited shell valve closure (Shumway and Cucci, 1987) and reduced clearance rate (Lesser and Shumway, 1993).
- Pacific oyster: Reduced clearance rate (Lassus *et al.*, 1996).
- Ribbed mussel: Exhibited shell valve closure, reduced clearance rate, and increased mucus production (Shumway and Cucci, 1987); inhibited byssus production (Shumway *et al.*, 1987; note that the northern horsemussel was unaffected).
- Sea scallop: Exhibited shell valve closure, increased mucus production, and violent swimming activity (Shumway and Cucci, 1987); reduced oxygen consumption (Shumway *et al.*, 1985).
- Softshell clam: Exhibited shell valve closure (Shumway and Cucci, 1987), reduced clearance rate (Shumway and Cucci, 1987; Bricelj *et al.*, 1996), impaired burrowing response (Bricelj *et al.*, 1996), and decreased heart rate (Gainey and Shumway, 1988).

Zooplankton: Can accumulate and retain STXs and cause fish kills (White, 1981a).

Microcrustaceans:

- *Acartia hudsonica, Pseudocalanus* sp.: Depressed feeding and paralysis occurred with increasing levels of *A. tamarense* cellular toxicity (Ives, 1985, 1987).
- *Acartia tonsa, Eurytemora herdmanii*: Decreased feeding, and toxins bioaccumulated (Teegarden and Cembella, 1996).
- *Calanus finmarchicus*: Avoided feeding (Turriff *et al.*, 1995).
- *Calanus helgolandicus*: STX production in *A. tamarense* was positively correlated with both presence of the grazers and waterborne cues from them. This zooplankton species has high grazing impact on *A. tamarense*, whereas two other species tested (*Acartia clausi* and *Oithona similis*, with generally low grazing impact on *A. tamarense*) did not stimulate STX production (Wohirab *et al.*, 2010).
- *Calanus helgolandicus, Temora longicornis*: Reduced fecundity (Gill and Harris, 1987).
- *Calanus pacificus*: Depressed feeding (Huntley *et al.*, 1986).
- *Centropages hamatus*: Depressed feeding and fecundity (Turner *et al.*, 1998).

Ciliates:

- *Euplotes affinis*: Inhibited feeding (Johansson, 2000).
- *Favella ehrenbergii*: Reversed ciliary motion and caused abnormal backward swimming (Hansen, 1989).

Heliozoans: *Heterophrys marina* – Reduced growth (Tobiesen, 1991).

Phytoplankton: *A. tamarense* consumes various microalgal prey (see Table 7.1).

Cochlodinium polykrikoides (*Margalefidinium polykrikoides*)

Fish:

- Sheepshead minnow (age 1 week), Atlantic silverside (adults), striped killifish (adults): Gill function was impaired based on epithelial proliferation, with focal areas of fusion of gill lamellae (Gobler *et al.*, 2008).
- Spotted rose snapper: Liver catalase activity and lipid peroxidation decreased when exposed to toxic *C. polykrikoides* as a short-term effect. Fish developed an abnormal mucus secretion on the gills that was directly related to the dinoflagellate cell densities (2 to 4×10^3 cells mL^{-1}). The data suggested that oxidative stress contributes to the ichthyotoxic effect (Dorantes-Aranda *et al.*, 2010).

(*continued*)

Table 7.2 (*Continued*)

Organism(s) impacted and reported effect(s)

Molluscs:

- Bay scallop: Growth rates of juvenile survivors were significantly depressed. Gills exhibited hyperplasia; gill tissues near *C. polykrikoides* cells showed inflammation; both gills and the digestive tract hemorrhaged; and there were signs of starvation (Gobler *et al.*, 2008).
- Eastern oyster: Gills of juveniles hemorrhaged and apoptosis occurred, and digestive glands showed severe hemorrhaging and squamation (Gobler *et al.*, 2008).
- Eastern oyster: Larvae were deformed (Ho and Zubkoff, 1979).
- Pacific oyster: Metamorphosis of larvae slowed during blooms (Matsuyama *et al.*, 2001), and calcium uptake was depressed (Ho and Zubkoff, 1979).
- Mediterranean mussel: First feeding by larvae was delayed 12 days in comparison to feeding on control benign algae (Jeong *et al.*, 2004b).

Zooplankton:

- *Acartia tonsa*: Inhibited grazing, including significant reduction in ingestion rates; and impaired reproduction, with depressed egg production rates and smaller egg size when adult females were fed bloom densities of *C. polykrikoides* (Jiang *et al.*, 2009).
- *Acartia omorii*: Egg viability rapidly decreased when adults were fed *C. polykrikoides* (Shin *et al.*, 2003)

Phytoplankton: High grazing rates on cryptophytes (*Rhodomonas salina* and a second unidentified cryptophyte species) have supported significantly more growth than when in the same light regime without prey (maximum specific growth rates 0.324 day^{-1} versus 0.166 day^{-1}, respectively). *C. polykrikoides* has also consumed other small microalgae such as *Isochrysis galbana*, *Heterosigma akashiwo*, and *Amphidinium carterae*. The data suggest that *C. polykrikoides* can substantially affect some algal populations (Jeong *et al.*, 2004a). In addition, *C. polykrikoides* has consumed the cyanobacterium *Synechococcus* sp. (Jeong *et al.*, 2005b).

Bacteria: *C. polykrikoides* consumes bacteria (Seong *et al.*, 2006), and has shown resistance to algicidal bacteria (e.g., *Alteromonas, Pseudoalteromonas*; Imai and Kimura, 2008).

***Gambierdiscus toxicus*, other CTX producers**

General: CTXs accumulate in fish muscle tissue. "The potential adverse effects of carrying a high CTX body burden on the population dynamics of reef fishes are unknown" (Richlen *et al.*, 2012, p. 42).

Mammals: Hawaiian monk seal: Survey of free-ranging animals revealed CTXs in body tissues (liver, brain, and muscle) and blood (Dechraoui *et al.*, 2011).

Fish:

- Bluehead wrasse: Fish fed toxic *Gambierdiscus* cell pellets exhibited altered feeding behavior, erratic swimming, loss of equilibrium/orientation, respiratory distress, and inability to avoid capture, suggesting enhanced susceptibility to predation, which would also increase the rate of toxin concentration and bioaccumulation in coral reef food webs (Davin and Kohler, 1986).
- Coral reef fish: When exposed to a bloom of toxic *Gambierdiscus* in a zoo, several reef fish exhibited unusual "spinning" and "figure eight" swimming behaviors (Goodlet *et al.*, 1994).
- Coney, schoolmaster, mahogany snapper, largemouth bass (fed a ciguatoxic great barracuda as ether-soluble extracts or as ground flesh): Signs of intoxication included skin color variations, inactivity, loss of equilibrium, erratic swimming, jerky feeding movements, and loss of orientation. These signs were usually observed within 24 hours after consuming the toxic tissues, and were evident for up to 76 days. Largemouth bass fed freeze-dried toxic cells of *G. toxicus* exhibited similar signs (Davin *et al.*, 1988).
- Exposure adversely affected fish development during embryonic and larval stages, suggesting the potential for population-level impacts (Richlen *et al.*, 2012, and references therein). CTXs accumulated at highest concentrations in the viscera, including the gonads (Vernoux *et al.*, 1985), and also at relatively high concentrations in the eggs (Colman *et al.*, 2004). Lipophilic toxins in the ovaries were mobilized with fat stores during oogenesis and became available to developing embryos (Ungerer and Thomas, 1996).
- Medaka: CTXs were micro-injected into egg yolk of embryos so as to cause exposure over the course of yolk sac absorption. Exposure elicited dose-dependent hyperkinesis and tachycardia in embryos and, at highest toxin levels, prevented hatching. Larval fish that hatched had spinal curvature and symptoms of intoxication, exacerbated with increasing CTX levels (Edmunds *et al.*, 1999). Developing embryos exposed to purified CTX and toxic barracuda extracts sustained cardiac effects and spinal deformities (Colman *et al.*, 2004).

Karenia brevis

General:

- BTXs bioaccumulate; for example, various fish (e.g., menhaden, pigfish, pinfish, spot, striped mullet) can accumulate BTXs at sufficient levels to cause disease and death of seabird and mammal consumers both during *K. brevis* outbreaks and long afterward (Flewelling *et al.*, 2005; Fire *et al.*, 2008; van Deventer *et al.*, 2012). It can take several

Table 7.2 (*Continued*)

Organism(s) impacted and reported effect(s)
days to several weeks for BTXs to be completely depurated, based on laboratory experiments with mammals and fish (Cattet and Geraci, 1993; Hinton and Ramsdell, 2008). Toxins can cause hyperexcitability of affected neurons, in turn negatively influencing control and homeostasis of the target effector organs enervated by these neurons and leading to organ and locomotor dysfunction (Franz and LeClaire, 1989). - Adverse effects of *K. brevis* BTXs have occurred throughout the food web, in both pelagic areas and benthic habitats (Simon and Dauer, 1972, Roberts *et al.*, 1979, Summerson and Peterson, 1990) where BTX was documented (Mendoza *et al.*, 2008). - High toxic cell densities have suppressed clearance rates in benthic suspension-feeding invertebrates, creating a positive feedback for bloom formation. High BTX concentrations have accumulated in the tissues of benthic suspension-feeding invertebrates, which have then been transferred to higher-level consumers (Echevarria *et al.*, 2012). *Mammals*: - Manatee: Toxins have destroyed immune system functioning (Walsh *et al.*, 2015). BTXs and metabolites have persisted in seagrass beds long after blooms subsided and, thus, may have continued to affect grazing manatees (Flewelling *et al.*, 2005). - Bottlenose dolphin: Piscivorous bottlenose dolphins in a region of the west Florida coast that frequently sustains *K. brevis* outbreaks consumed several species of fish which can be BTX vectors (Flewelling *et al.*, 2005; Fire *et al.*, 2008). - Domestic dog: Signs of brevetoxicosis have occurred in areas near blooms; urine samples tested positive for BTXs and signs included heavy salivation, seizures, paralysis, and temporary blindness, but the dogs recovered in one to several weeks (Landsberg *et al.*, 2009 and references therein). - Coyote: Similar signs of brevetoxicosis have occurred as those described above for dogs, in areas near blooms (Castle *et al.*, 2013). *Birds*: At fish kills, sick cormorants, brown pelicans, and seagulls were observed. During prolonged blooms, most assessed piscivorous seabirds tested positive for BTXs and exhibited clinical signs of brevetoxicosis including severe cerebellar ataxis indicated by incoordination, hypermetric gait, inability to stand, slumping of the head, reluctance to fly, seizures, shaking, nasal and oral discharges, tachycardia, labored breathing, depressed reflexes, impaired motor control, atrophied musculature, dehydration, and disorientation (Landsberg *et al.*, 2009 and references therein). Many piscivorous seabirds taken to wildlife rehabilitation centers during a severe bloom tested positive for BTXs and exhibited impaired motor functioning, disorientation, and seizures (Fauquier *et al.*, 2013a), indicating that adverse impacts of BTXs have been vectored through the food web (van Deventer *et al.*, 2012). *Reptiles*: - Sea turtles: Exhibited clinical signs of neurointoxication, and tested positive for BTXs. Some of their stomach contents were finfish remains that were the likely toxin vectors (Fauquier *et al.*, 2013b). - Sea turtles: Exposure to BTXs resulted in swimming in circles, lack of coordination, head bobbing, muscle twitching, and odd, abrupt movements. In more extreme cases, animals exhibited extreme lethargy or coma (Foote *et al.*, 1998; Manire *et al.*, 2013). Sea turtles appeared to have slower BTX clearance rates than mammals (Landsberg *et al.*, 2009). *Fish*: - Impaired swimming behavior; fish also exhibited defecation, regurgitation, fin paralysis, and loss of equilibrium (Landsberg *et al.*, 2009 and references therein). - Killifish: Showed decreased schooling and shoaling behaviors (Salierno, 2005). - Goldfish: Sublethal BTX concentrations caused locomotor dysfunction and temporary hearing loss based on auditory evoked potentials (Lu and Tomchik, 2002). - Silver perch, spotted seatrout: Fish chorusing was significantly higher during years without *K. brevis* blooms than in years with blooms, suggested to be linked to ichthyotoxic effects of BTXs (Indeck *et al.*, 2015). - Sand seatrout: Exhibited altered spatial distribution of spawning aggregations (Walters *et al.*, 2013). *Molluscs*: - Bay scallop: Recruitment failed (Summerson and Peterson, 1990). - Banded tulip, crown conch, lettered olive: Lost muscle control (Roberts *et al.*, 1979). - Eastern oyster, northern quahog: Larval development was protracted by toxic *K. brevis* (10^3 cells mL^{-1}; Leverone *et al.*, 2006). *Zooplankton*: - *Acartia tonsa*: Sustained lethargy and paralysis. - *Calanus pacificus*: Avoided ingestion of *K. brevis* cells, or regurgitated the cells; exhibited rapid heart rate, lethargy, and paralysis (Huntley *et al.*, 1986; Sykes and Huntley, 1987).

(*continued*)

Table 7.2 (*Continued*)

Organism(s) impacted and reported effect(s)

Other invertebrates:

- Adverse effects have been documented on the abundance and distribution of a wide range of benthic fauna (e.g., brachiopods, echinoderms, gastropods, and polychaetes; Smith, 1975).
- Bryozoan *Bugula neritina*, sponge *Halicona tubifera*: Clearance rates on benign *Rhodomonas* sp. significantly decreased when *K. brevis* was present. Animals accumulated high levels of BTXs after 4 hours of exposure to *K. brevis*. When they were transferred to filtered seawater, BTX concentrations in their tissues decreased by ~80% (Echevarria *et al.*, 2012).
- Corals (see Appendix A): Apparently bleached during an extended toxic bloom, but recovered fairly quickly (Dupont and Coy, 2008).

Phytoplankton:

- Growth was inhibited from exposure to waterborne compounds from *K. brevis* (exudate from a natural bloom – uncharacterized allelochemical(s), 500–1000 Da, with aromatic functional groups) in co-occurring (competitor) species *Amphora* sp., *Asterionellopsis glacialis* (diatoms), and *Prorocentrum minimum*. These three taxa and a fourth, *Akashiwo* cf. *sanguinea*, were also growth-inhibited when exposed to exudate from a toxic culture of *K. brevis*. The exudates suppressed photosynthetic efficiency and damaged cell membranes of competing phytoplankton, but had no effect on competitor esterase activity and did not limit competitor access to iron (Prince *et al.*, 2008).
- In laboratory experiments, multiple lipophilic substances from *K. brevis* inhibited growth of *Akashiwo sanguinea*, *Asterionellopsis glacialis, Prorocentrum minimum,* and *Skeletonema grethe* (diatom). The two dinoflagellate competitors reacted to only 1 of 6 lipophilic substances, whereas the two diatom taxa reacted to 3 or 4 lipophilic substances. Thus, species varied in susceptibility; in addition, early-stage (lag phase) *S. grethae* cells were more susceptible to allelopathic effects than later growth stages (Poulson *et al.*, 2010).
- In a microcosm experiment with natural plankton assemblages, extracellular extracts from *K. brevis* (2 strains) inhibited growth of some diatom taxa but had no effect on others, suggesting that in a natural setting the importance of *K. brevis* allelochemicals may not be as great as predicted by laboratory studies (Poulson *et al.*, 2010).
- *K. brevis* exhibited high grazing rates on the cyanobacterium *Synechococcus* sp. (Jeong *et al.*, 2005b; Glibert *et al.*, 2009).
- *Amphora* sp., *Asterionellopsis glacialis, Rhizosolenia* sp., *Skeletonema costatum*, and *Thalassiosira pseudonana* (diatoms); and *Prorocentrum mexicanum* (toxigenic dinoflagellate): Allelopathic; inhibited growth (Kubanek *et al.*, 2005; Prince *et al.*, 2008, 2010; Poulson *et al.*, 2010; and Poulson-Ellestad *et al.*, 2014).

Karlodinium veneficum

Fish: In laboratory experiments with juvenile sheepshead minnows and zebra fish, gills showed epithelial necrosis and shortening or loss of secondary lamellae, or clubbing and bridging between secondary lamellae; and extensive cellular hypertrophy and lysis of epithelial and chloride cells (Deeds *et al.*, 2006).

Molluscs:

- Blue mussel: Reduced growth (Nielsen and Strømgren, 1991), reduced clearance rates, and reduced growth in juveniles (Glibert *et al.*, 2007 and references therein). Exposure to a toxic strain also caused increased infiltration of the percentage of phagocytic hemocytes into the digestive gland, and increased hemocyte production of ROSs (Galimany *et al.*, 2008a).
- Eastern oyster: When exposed to *K. veneficum* (10^4 cells in stationary phase mL^{-1}), adults developed mantle and gill lesions. Larvae (age 2 weeks) sustained a severe reduction in motility. Larvae (age several days) became deformed after embryos from freshly spawned animals were immediately exposed to *K. veneficum* (Glibert *et al.*, 2007).
- Eastern oyster, Suminoe oyster (former candidate species for introduction to Chesapeake Bay): Growth rates of spat (age 3–14 days) were severely depressed and organ development apparently was reduced when the spat were fed a toxic strain, in comparison to animals fed nontoxic *K. veneficum*. Effects were worse for Suminoe oyster spat. When exposed to bloom densities for 6 hours daily for 5 days, clearance rates of older juveniles (length 1–2 cm) were severely depressed for both species, again with worse effects for Suminoe oyster (Brownlee *et al.*, 2008).
- Great scallop: Inhibited feeding in post-larvae (Lassus and Berthome, 1988).
- Suminoe oyster: Exhibited severe reduction in motility of larvae; and larvae became deformed after embryos from freshly spawned animals were immediately exposed to toxic *K. veneficum* (10^4 cells mL^{-1}, stationary phase; Glibert *et al.*, 2007).

Zooplankton:

- KTXs depressed grazing capabilities of microzooplankton and copepods (Adolf *et al.*, 2007).

Table 7.2 (*Continued*)

Organism(s) impacted and reported effect(s)

- Microcrustaceans: *Acartia tonsa* females – Exhibited significantly higher clearance and ingestion rates when fed a nontoxic strain than when given a toxic strain or a mixed diet of toxic + nontoxic strains, suggesting that toxic strains can deter grazing by potential predators (Waggett *et al.*, 2008).
- Rotifers: *Brachionus plicatilis* – Exposure to field-equivalent densities of *K. veneficum* caused a reduction in all life history parameters measured, compared to parameters for control cultures with benign algal prey. Parameters included lifetime egg production, net reproductive rate, finite rate of increase, and intrinsic rate of population increase (Lin *et al.*, 2016).
- Ciliates: *Storeatula major* – KTXs served as a "predation instrument" to immobilize the ciliate prey prior to ingestion (Sheng *et al.*, 2010, 2082). Mixing ciliates with toxic *K. veneficum* caused prey immobilization at rates consistent with KTX potency and dosage. Ciliates that were able to continue swimming but decreased velocity. Swimming velocity of the toxic cells also slowed and they decreased vertical migration, likely to remain near the prey. In contrast, nontoxic cells did not alter their swimming and did not affect ciliate behavior. Separate exposure of ciliates to KTXs also caused ciliate immobilization at rates consistent with potency (Sheng *et al.*, 2010).
- Dinoflagellates: *Oxyrrhis marina* – Consumed mildly toxic *K. veneficum* as prey (Johnson *et al.*, 2003), but see Table 7.1 – the prey became the predator as the KTXs inhibited *O. marina*.

Phytoplankton: Abundant cryptophyte prey can trigger toxic blooms (Adolf *et al.*, 2008), and the prey population can be significantly affected. KTXs aid in prey capture and are used to stun cryptophytes prior to ingestion (Sheng *et al.*, 2010).

Lingulodinium polyedrum

Molluscs: Mediterranean mussel – First feeding by larvae was delayed 8 days in comparison to feeding on control benign algae (cells likely too large; Jeong *et al.*, 2004b).

Phytoplankton: *L. polyedrum* consumes various microalgal prey (see Table 7.1).

***Ostreopsis* spp. (*O. heptagona, O. labens, O. lenticularis, O. mascarenensis, O. siamensis, O. ovata*)** [T – palytoxin, PTX and analogs; ostreocin D; mascarenotoxins – Rhodes, 2011]

Arthropods: Impaired larval development in brine shrimp was thought to result from osmoregulatory dysfunction (Faimali *et al.*, 2011).

Echinoderms: Inhibited sperm motility in sea urchins (Morton *et al.*, 1982). Exposure also caused folding of spines and loss of spines after exposure for 3–4 days (*O. siamensis*), with recovery after 4 months (Shears and Ross, 2010).

Pfiesteria piscicida

Fish: Neurotoxic symptoms and behavior modification in juveniles and adults have occurred in the presence of actively toxic strains. Fish swam erratically, sank, became immobile, and then showed signs of panic, struggled to the water surface, and sank back down. They gulped air at the water surface and appeared to be suffocating, and also were lethargic and showed signs of narcosis (Burkholder and Glasgow, 1997 and references therein).

Molluscs:

- Bay scallop: Reduced ability to close shell valves, and reduced feeding (Springer *et al.*, 2002).
- Eastern oyster: Reduced swimming activity and reduced feeding when given actively toxic *P. piscicida*. Both juveniles and adults displayed significantly more feeding on nontoxic than on actively toxic *P. piscicida* (Springer *et al.*, 2002).

Zooplankton:

- "Semi-natural" microzooplankton assemblage (had been in the laboratory for 1 day to 2 weeks) – During 6-hour incubations, the zooplankton assemblage ingested both toxic and nontoxic *P. piscicida* (tested separately), but assemblage grazing coefficients were significantly lower when fed an actively toxic culture (Stoecker *et al.*, 2002).
- Microcrustaceans: *Acartia tonsa* – In short-term experiments there was no apparent effect on survival, but animals exhibited erratic behavior when fed an actively toxic strain of *P. piscicida*, alone or mixed with nontoxic algae, in comparison to behavior when fed only nontoxic algae (Mallin *et al.*, 1995).

Ciliates:

- *Euplotes vannus, Euplotes woodruffi*: Rapidly grazed nontoxic cultures, but showed no apparent grazing on actively toxic culture (Lewitus *et al.*, 2006).
- *Eutintinnus* sp., strobilidids (length < 20 μm): Abundance declined when exposed to actively toxic *P. piscicida* culture (Stoecker *et al.*, 2002). *Note*: Some ciliates showed comparable grazing activity when fed toxic or nontoxic *P. piscicida*.
- Dinoflagellates: *Oxyrrhis* sp., *Gyrodinium* spp. showed comparable grazing activity when fed toxic or nontoxic *P. piscicida* (Stoecker *et al.*, 2002).

(*continued*)

Table 7.2 (*Continued*)

Organism(s) impacted and reported effect(s)
Pfiesteria shumwayae
Fish:
- Have shown the same neurotoxic symptoms and behavior modification as for *P. piscicida* in the presence of actively toxic strains (Gordon *et al.*, 2002; Burkholder *et al.*, 2005).
- *P. shumwayae* consumed a sterile fish cell line (Parrow *et al.*, 2005) and in those monoxenic cultures, the dinoflagellates produced a small amount of PfTXs (Burkholder *et al.*, 2005).
Molluscs: Eastern oyster juveniles and adults reduced feeding when exposed to actively toxic culture (Shumway *et al.*, 2006).
Prorocentrum micans
Molluscs: *Mytilus galloprovincialis* – First feeding by larvae was delayed 8 days in comparison to feeding on control benign algae (Jeong *et al.*, 2004b).
Zooplankton: Microcrustacean *Centropages hamatus* – Eggs were not produced by adult females fed *P. micans* throughout their life history. In contrast, there was substantial egg production by control animals fed benign algae (Murry and Marcus, 2002).
Phytoplankton:
- *Asterionella japonica, Chaetoceros lauderi*: Inhibited pigment synthesis (Gauthier *et al.*, 1978).
- *Chaetoceros didymus, Skeletonema costatum*: Reduced population growth (Uchida, 1977).
- *Karenia mikimotoi, Skeletonema costatum*: Suppressed population growth (Xiaoqing *et al.*, 2011).
- *Karenia mikimotoi, Skeletonema costatum*: Tested strains of both species were strongly inhibited by *P. micans.* At size:density ratios of 1:1 or 1:10 of *P. micans: S. costatum*, *P. micans* outcompeted *S. costatum*. At a size:density ratio of 1:0.1 of *P. micans*: *K. mikimoitoi*, growth of *K. mikimoitoi* was significantly depressed. Enriched filtrates of *P. micans* exerted similar effects as the presence of *P. micans* cells without direct cell contact, suggesting an allelopathic effect of *P. micans* on the competing species (Ji *et al.*, 2011).
- *P. micans* consumes various microalgal prey (see Table 7.1).
Benthic *Prorocentrum* complex
Fish:
- Limited data suggest that fish in contact with toxic *P. lima* complex cells cease feeding and die from chronic exposure (Ajuzie, 2008).
- European sea bass: When juveniles were fed toxic *P. lima* complex cells, they exhibited stress-related behavior such as hyperactivities (jumps, fast left-right turns, surface swims), poor feeding reflexes, and cessation of feeding in juvenile fish that were exposed to either cell-free medium or whole-cell cultures. Adverse effects from ingesting *P. lima* complex cells along with a commercial fish diet did not manifest for 3 weeks; then fish ceased feeding and became progressively less active. Cultures of this *P. lima* complex strain exuded a strong, repugnant odor. Fish initially rejected clumps of the cells by spitting them out. Affected fish developed swollen gills with lifted epithelium, vacuolated tips, and ruptured lamellae. Secreted mucus "overwhelmingly covered" the respiratory epithelium of primary and secondary gill lamellae, causing the aorta blood to become hypoxic. The livers of affected fish were swollen and congested, with parachymal necrosis and erosion (Ajuzie, 2008).
Molluscs:
- Bay scallop: In laboratory experiments, within 24 hours of exposure, adults bioaccumulated DSP toxin concentrations to levels exceeding commonly accepted regulatory levels. Most toxins were in the viscera (76%) and gonadal tissue (12%). During depuration, rapid release of DSP toxins from scallops indicated that the toxins were poorly bound to all tissues except viscera. There was no scallop death, and feeding inhibition was not observed for juveniles or adults over a 2-week exposure, but absorption efficiency of organic matter was significantly lower when scallops were fed *P. lima* (complex) in comparison to nontoxic diatom *Thalassiosira weisflogii* (Bauder *et al.*, 2001).
- Intact *P. lima* complex cells found in scallop fecal ribbons vegetatively reproduced after gut passage and emergence from the fecal ribbons (Bauder and Cembella, 2000).
- Pacific oyster: Expression of six stress genes depended on *P. lima* (complex) cell density and exposure duration (Romero-Geraldo and Hernandez-Saavedra, 2014).
Zooplankton: Microcrustacean *Paracalanus parvus* reduced feeding (Huntley *et al.*, 1986).
Phytoplankton: Exudates from a clone of the *P. lima* complex inhibited growth of toxigenic dinoflagellates *Coolia monotis*, *Gambierdiscus toxicus*, and *Ostreopsis lenticularis*. In contrast, the clone maintained similar growth rates in response to exudates from the other dinoflagellate species, although it appeared to enter stationary phase earlier than without the exudates (Glibert *et al.*, 2012 and references therein).

Table 7.2 (*Continued*)

Organism(s) impacted and reported effect(s)
Prorocentrum minimum
Molluscs:
- Bay scallop: Poor growth occurred as well as intestinal pathology (necrosis or absorptive and some basil cells of the digestive diverticula). Hemocytes accumulated throughout the open vascular system, consistent with tissue damage and/or effects of a chemical toxin; and there were systemic immune responses (Hégaret and Wikfors, 2005a; Heil *et al.*, 2005; Wikfors, 2005).
- Bay scallop: Exposure to a toxic strain of *P. minimum* in either growth or senescent phases decreased the degree of shell opening, the amount of biodeposits produced, motility, and byssal-thread attachment. These effects were more severe as *P. minimum* neared senescence. Pathological effects included derangements of scallop digestive tubules and the adductor muscles, and abnormal hemocyte distributions, which were also more severe with senescent *P. minimum* (Li *et al.*, 2012a).
- Blue mussel: As an apparent immune response, mussels responded to *P. minimum* exposure via diapedesis of hemocytes into the intestine. Circulating hemocytes retained hematological and functional properties. Bacteria greatly increased in the intestines of mussels exposed to *P. minimum*; hemocytes appeared to be either overwhelmed by the large number of bacteria, or engaged in an encapsulating response to *P. minimum* cells. When hemocytes reached the intestinal lumina, they underwent apoptosis (Galimany *et al.*, 2008b).
- Eastern oyster: Mixed findings have been reported depending on the *P. minimum* strain and physiological status –
-- *P. minimum* (10^4 cells mL^{-1}) positively affected spat growth (Brownlee *et al.*, 2008).
-- Larvae had poorer survival and lower settling success with *P. minimum* (3×10^3 cells mL^{-1}) in their diet (Wikfors and Smolowitz, 1995).
-- Larvae did not metamorphose and exhibited various developmental and histopathological abnormalities, with transient digestive gland and systemic pathologies (digestive gland epithelial cells accumulated undigested food vacuoles). Juveniles sustained systemic immune responses and poor assimilation of consumed cells. Adult growth was depressed, and *P. minimum* cells were rejected as pseudofeces (Hégaret and Wikfors, 2005b; Wikfors, 2005 and references therein).
-- At high *P. minimum* densities (10^4 cells mL^{-1}), spawning did not occur; also, there was histological damage and reduced growth of larvae and juveniles (Luckenbach *et al.*, 1993). In stages of growth decline, *P. minimum* appeared to be more toxic than when rapidly growing (Wikfors, 2005 and references therein).
- Mediterranean mussel: First feeding by larvae was delayed 4 days in comparison to first feeding on control benign algae (Jeong *et al.*, 2004b).
- Northern quahog: *P. minimum* cells were rejected as pseudofaeces, and reduced feeding and growth were apparent (Wikfors, 2005; Glibert *et al.*, 2012 and references therein).
- Suminoe oyster: Severe reduction in motility occurred after exposure of larvae (age 2 weeks) to a toxic strain of *P. minimum* (Glibert *et al.*, 2007).
Zooplankton:
- *P. minimum* is consumed by various copepods, ciliates, and nanoflagellates (Glibert *et al.*, 2012 and references therein).
- Microcrustacean *Acartia tonsa*: Consumed *P. minimum* with high ingestion rates, but egg production was depressed unless prey were augmented with the benign diatom *Thalassiosira weisflogii*. The *P. minimum* strain apparently was not toxic but also was not nutritionally sufficient (Dam and Colin, 2005).
- Ciliate *Favella ehrenbergii*: *P. minimum* was a poor food source, and was avoided in cultures (Stoecker *et al.*, 1981).
Phytoplankton (also see Table 7.1): *P. minimum* inhibited growth of *P. micans* (Heil *et al.*, 2005).
- *Heterosigma akashiwo*, *Karlodinium veneficum*: Dominance when in mixed culture with *P. minimum* depended on the (molar) N:P ratio. Near or above Redfield proportions (16 or 25), *P. minimum* was dominant, whereas at a N:P ratio of 5, *P. minimum* and *K. veneficum* were co-dominants (Handy *et al.*, 2008).
- *K. veneficum*: *P. minimum* outcompeted *K. veneficum* under all treatments with various N:P ratios using different forms of N, and several light levels. When *Rhodomonas* prey were added, growth of *P. minimum* was inhibited relative to that of *K. veneficum*; when *Synechococcus* prey were added, the cyanobacteria became dominant. Thus, while allelopathy could be important in competitive outcomes involving *P. minimum*, the competitive advantage of *P. minimum* was overcome when *K. veneficum* was given suitable prey, or when a faster-growing species was added as prey (Li *et al.*, 2012b; Glibert *et al.*, 2012).
Bacteria: *P. minimum* consumes bacteria (see Table 7.1).

(*continued*)

Table 7.2 (*Continued*)

Organism(s) impacted and reported effect(s)
Pyrodinium bahamense* var. *compressum* and var. *bahamense [M; STX; toxins bioaccumulate in finfish and shellfish (Usup *et al.*, 2012 and references therein)]
Fish: Southern, checkered, and bandtail puffer fish bioaccumulated STXs from *P. bahamense* (in epidermis, muscle viscera, and ovary tissues) at lethal concentrations for human consumers (Landsberg *et al.*, 2006).
Zooplankton: During a major bloom of *P. bahamense*, numerical abundances of microcrustaceans *Oithona colcarva* and *Acartia tonsa* declined, whereas the abundance of tunicate *Oikopleura dioica* mimicked the pattern of *P. bahamense* var. *bahamense* abundance (Badylak and Phlips, 2008).
Phytoplankton: Picoplankton and diatom cell densities decreased during a major bloom of *P. bahamense* var. *bahamense* (Badylak and Phlips, 2008).
Scrippsiella trochoidea
Molluscs:
- Mediterranean mussel: First feeding by larvae was delayed 12 days in comparison to first feeding on control benign algae (Jeong *et al.*, 2004b).
- Pacific oyster: Clearance rates substantially declined when fed *S. trochoidea* (Bardouil *et al.*, 1993).
Zooplankton (Crustaceans):
- *Calanus helgolandicus*: Reduced feeding (Gill and Harris, 1987).
- *Calanus pacificus*: Females failed to maintain gut fullness when fed *S. trochoidea* (Sykes and Huntley, 1987), and fecundity decreased (Huntley *et al.*, 1986).
- *Centropages hamatus*: Eggs were not produced by adult females when fed *S. trochoidea* throughout their life history. In contrast, there was substantial egg production by control animals fed benign algae (Murray and Marcus, 2002).
Phytoplankton: *S. trochoidea* consumes various microalgal prey (see Table 7.1).
Euglenophytes
Euglena sanguinea
Phytoplankton: Growth of *Gomphonema parvulum* (diatom), *Oocystis polymorpha, Scenedesmus dimorphus* (chlorophytes), and *Microcystis aeruginosa and Planktothrix* sp. was inhibited by < 30 mg EUGL L^{-1} (Zimba *et al.*, 2010).
Haptophytes
Prymnesium parvum
General:
- Massive blooms have occurred in low-salinity coastal and inland waters, or freshwaters with high conductivity. The multiple toxins of *P. parvum* have cytotoxic, ichthyotoxic, neurotoxic, allelopathic, grazer deterrent, and antibacterial activities (Edvardsen and Imai, 2006). They commonly destroy the selective permeability of cell membranes and disrupt ion regulation in gills (Burkholder, 2009 and references therein).
- The toxins may function as defense compounds to prevent herbivory; some research suggests that they have allelopathic roles, but this has not been verified using purified toxins (Manning and La Claire, 2010).
Fish:
- Affected fish typically bleed from the gills and may develop a heavy mucus layer. They often swim slowly, lie on the bottom, gather nearshore or near a fresh source of water, or actively leap onto shore (Burkholder, 2009 and references therein).
- Long-term negative impacts on fish populations: In reservoirs repeatedly affected by *P. parvum* blooms, populations of white bass, white crappie, largemouth bass, bluegill sunfish, carpsucker, freshwater drum, channel catfish, flathead catfish, and blue catfish sustained declines in relative abundance, size structure, or both. The extent of the effect varied depending on the reservoir system and fish species (Van Landeghem *et al.*, 2013).
Zooplankton:
- Crustaceans:
-- *Acartia bifflosa, Eurytemora affinis*: Became inactive, without the zooplankton actually consuming toxic cells, and reproductive success decreased (Sopanen *et al.*, 2006).
-- *Acartia clausi*: Neither feeding nor reproduction occurred (Nejstgaard and Solberg, 1996).
-- *Daphnia magna*: Reduced survival and reproduction (laboratory and field studies; Ureña-Boeck, 2008).
-- *Eurytemora affinis*: Cell-free filtrates negatively affected survivorship (Sopanen *et al.*, 2008).
- Heliozoans: *Heterophrys marina*: Reduced growth (Tobiesen, 1991; Fisterol *et al.*, 2003).
- Ciliates: Cell-free filtrate from a *P. parvum* culture completely suppressed ciliates in a natural plankton community; no ciliates were present at the end of the 6- to 8-day experiments (Fisterol *et al.*, 2003).

Table 7.2 (*Continued*)

Organism(s) impacted and reported effect(s)

Phytoplankton:

- Natural plankton community: Cell-free filtrate from a *P. parvum* culture affected the entire phytoplankton assemblage. Growth of cyanobacteria and dinoflagellates was depressed, and growth of diatoms was completely inhibited so that no live diatom cells were left at the end of the 6- to 8-day experiments (Fisterol *et al.*, 2003).
- *Rhodomonas* cf. *baltica*, *Rhodomonas salina*, *Thalassiosira weissflogii*: Allelopathic; inhibited growth (Granéli and Johansson, 2003; Barreiro *et al.*, 2005; Uronen *et al.*, 2007).
- *Heterocapsa rotundata*: Allelopathic; inhibited motility (Skovgaard and Hansen, 2003).

Bacteria:

- Natural plankton community: Cell-free filtrate from a *P. parvum* culture reduced bacterial production (Fisterol *et al.*, 2003).
- *P. parvum* grazed bacteria during a bloom (3.4–5.8 bacteria cell^{-1} hour^{-1} depending on the depth of the water column; Nygaard and Tobiesen, 1993).

Pelagophyceans

Aureococcus anophagefferens

General: Massive blooms (~4×10^6 cells mL^{-1}); "Impaired physiological processes and trophic transfer as well as trophic dysfunction can accompany [brown tides] in natural communities" (Smayda, 1991, p. 278, and references therein).

Fish: Anchovy – Reduced fecundity; failed to spawn in the next annual cycle following a major bloom (Castro and Cowen, 1989).

Annelids: Polychaete *Streblospio benedicti* – Reduced growth and swimming velocity (Ward *et al.*, 2000).

Molluscs:

- General: Severe detrimental impacts were sustained by suspension-feeding bivalves (bay scallops, blue mussels in particular; Bricelj and Lonsdale, 1997).
- Bay scallop: Decreased feeding (larvae and adults) and feeding efficiency (Tracey, 1988; Bricelj and Kuenstner, 1989); reduced fecundity and larval shell growth (Gallager *et al.*, 1989); reduced adductor weight; and recruitment failed (Bricelj *et al.*, 1987). Mass mortality and recruitment failure resulted in the collapse of an economically valuable scallop industry on eastern Long Island (Bricelj and Kuenster, 1989; Sunda *et al.*, 2006 and references therein).
- Blue mussel: Decreased feeding (larvae and adults) and feeding efficiency, and reduced fecundity; filtering ceased, leading to starvation; reproductive failure occurred (Tracey, 1988; Bricelj and Kuenster, 1989); and inhibited ciliary activity (Gainey and Shumway, 1991). Impacts were strain-dependent (Bricelj *et al.*, 2001), as with other harmful algae.
- Carpet shell clam: Hemocyte function and viability were damaged (Prado-Alvarez *et al.*, 2013).
- Eastern oyster: Reduced clearance rates and size of larvae (Gobler *et al.*, 2013); inhibited gill ciliary activity (Gainey and Shumway, 1991).
- Northern quahog: Reduced growth (juveniles and adults) (Greenfield and Lonsdale, 1997; Greenfield *et al.*, 2004; Padilla *et al.*, 2006), reduced clearance rates (Gobler *et al.*, 2013), decreased feeding efficiency (Tracey, 1988), and inhibited gill ciliary activity (Gainey and Shumway, 1991). There may be subtle adverse, chronic effects on shellfish even when *A. anophagefferens* is present at background cell densities (Greenfield *et al.*, 2004).
- When fed bloom concentrations, larvae developed faster but growth was reduced. Larvae that were fed slowly growing or near-stationary phase cultures exhibited reduced growth and slower development. These impacts may reflect the poor nutritional quality of *A. anophagefferens*, "which could have a lasting legacy through ontogeny" (Padilla *et al.*, 2006, p. 736).
- European flat oyster: Inhibited gill ciliary activity (Gainey and Shumway, 1991).

Zooplankton:

- Microcrustaceans: *Acartia tonsa* – Reduced feeding, growth, fecundity development, and nauplii survival (Durbin and Durbin, 1989; Bricelj and Lonsdale, 1997; Marcoval *et al.*, 2013). Nauplii development was delayed (Smith *et al.*, 2008).
- Ciliates: Populations of ciliated protozoans declined following a major bloom (Marcoval *et al.*, 2013). Net growth rate was negatively correlated with brown tide abundance (field study; Lonsdale *et al.*, 1996).
- *Strombidium* sp.: Population growth did not occur when *Strombidium* was fed only *A. anophagefferens* (Smith *et al.*, 2008; but see Caron *et al.*, 2004). The data indicate that impacts were strain-dependent, as for other harmful algae.
- Dinoflagellates: *Noctiluca scintillans* – Reduced feeding and fecundity (Buskey and Hyatt, 1995).

Aureoumbra lagunensis

General: Massive continuous blooms have occurred for up to eight years in shallow saline lagoons (Buskey *et al.*, 2001).

Fish: Significantly reduced egg hatch rates of red drum and spotted seatrout; and reduced feeding of spotted seatrout larvae occurred at 5–7 days post-hatch (data of J. Holt in Buskey *et al.*, 1996).

(*continued*)

Table 7.2 (*Continued*)

Organism(s) impacted and reported effect(s)

Benthic Invertebrates:

- Blooms have been associated with a substantial decrease in biomass and diversity of benthic invertebrates (Smayda, 1991; Buskey *et al.*, 1996, and references therein).
- Polychaete larvae (*Streblospio benedicti*): Reduced growth rates and swimming speeds (Ward *et al.*, 2000).

Zooplankton:

- Blooms have been linked to large decreases in grazing activity, growth, and egg release rates of mesozooplankton such as *Acartia tonsa*, and decreases in the abundance and grazing rates of microzooplankton (Buskey *et al.*, 1996).
- *Acartia tonsa* reduced fecundity (poor food resource – Buskey and Stockwell 1993); and nauplii exhibited depressed development and lower development and survival of nauplii (Buskey and Hyatt, 1995).
- Reduced abundance of microzooplankton (Buskey and Stockwell, 1993; Buskey *et al.*, 1997).
- There was no growth of the ciliate *Stromibinopsis* sp., the heterotrophic dinoflagellate *Noctiluca scintillans,* or the rotifer *Brachionus plicatilus* when fed this brown tide species (Buskey *et al.*, 1996).

Raphidophyceans

Heterosigma akashiwo

General: "*H. akashiwo* is a remarkably broad-spectrum antagonist against microzooplankton, copepods, benthic larvae, fish, and a poor food source for many benthic invertebrates. . . . Ichthyotoxic flagellates ([such as] *H. akashiwo*) can also be allelopathic against copepods" (Smayda, 1997, p. 1147).

Fish:

- ROS production induced hypersecretion of mucus in fish gills, epithelial lifting, cell necrosis, and alteration of chloride balance (Basti *et al.*, 2016 and references therein).

Molluscs:

- Eastern oysters: Closed their shells partially or totally when exposed to toxic *H. akashiwo* (Hégaret *et al.*, 2007b).
- Exposure to laboratory cultures or blooms significantly increased hepatopancreas lysosomal destabilization rates, which continued to increase even after 7 days of recovery in clean seawater. The data suggest that *H. akashiwo* toxins or other metabolites continued to damage the hepatopancreas, and that even short-term exposures to high cell densities of *H. akashiwo* could have long-term adverse physiological effects. Oyster health may be compromised in areas with repeated *H. akashiwo* blooms (Keppler *et al.*, 2005).

Zooplankton:

- Microzooplankton: When there was high abundance of *H. akashiwo* (40–99% of the phytoplankton assemblage) along with other, larger toxigenic raphidophyceans (*Chattonella subsalsa, Chattonella* cf. *verruculosa*, *Fibrocapsa japonica*), microzooplankton grazed *H. akashiwo* (grazing rates 0.88 to 1.88 day^{-1}, depending on the specific zooplankter and the site) but not the other raphidophyceans. Grazing pressure on *H. akashiwo* may have afforded a competitive advantage for the other raphidophyceans, which were too large to be consumed at high rates by the microzooplankton (Demir *et al.*, 2008).
- Crustaceans:
  - -- *Acartia hudsonica*: Reduced feeding (Tomas and Deacon, 1981).
  - -- *Acartia omorii*: Rejected feed and reduced fecundity (Uye and Takamatsu, 1990).
  - -- *Acartia tonsa*: Reduced feeding (Tomas and Deacon, 1981).
  - -- *Pseudodiaptomus marinus*: Rejected feed, and reduced both fecundity and survival (Uye and Takamatsu, 1990).
- Ciliates:
  - -- Preferential feeding on other prey reduced impacts of *H. akashiwo* toxicity on microzooplankton. "Avoidance of *H. akashiwo* by a major group of grazers would promote bloom formation by reducing *H. akashiwo* mortality and focusing community grazing pressure on potential competitor species" (Graham and Strom, 2010, p. 111).
  - -- *Tintinnopsis tubulosoides, Favella* sp., *Synchaeta cecilia*: Reduced growth (Verity and Stoecker, 1982; Egloff, 1986).
  - -- Ciliates were unable to sustain growth when fed a monoculture of *H. akashiwo*, regardless of *H. akashiwo* cell density (Chang *et al.*, 1990; Black *et al.*, 1991).
- Rotifers:
  - -- *Brachionus plicatilis*: Reduced feeding when exposed to toxic *H. akashiwo*; and the population decreased while *H. akashiwo* grew comparably as controls without rotifers (Xie *et al.*, 2008).
  - -- *Synchaeta cecilia*: Did not consume *H. akashiwo. H. akashiwo* inhibited feeding on other, acceptable algal food at densities as low as 50 cells mL^{-1}, and decreased reproduction at densities > 10^3 cells mL^{-1} (Egloff, 1986).

Table 7.2 (*Continued*)

Organism(s) impacted and reported effect(s)
Phytoplankton:
- *Chaetoceros muelleri, Skeletonema costatum* (diatoms): Allelopathic; inhibited growth (Yamasaki *et al.*, 2007).
- *Skeletonema costatum* (competitor): Was inhibited by high concentrations of *H. akashiwo*-conditioned medium, but was stimulated by low concentrations. *H. akashiwo* may have achieved dominance by producing large amounts of an ectocrine (tannoid?) that inhibited *S. costatum* at high concentrations, but was stimulatory at low concentrations (Pratt, 1966).
- *Skeletonema costatum* (competitor): Allelopathic; an uncharacterized polysaccharide-protein complex from *H. akashiwo* inhibited growth (Yamasaki *et al.*, 2009).
- *Skeletonema costatum* (reversal of effects): Allelopathic against *H. akashiwo*; inhibited growth (Yamasaki *et al.*, 2007, 2009, 2012).
Bacteria:
- Consumes bacteria (see Table 7.1).
- Bacterial strain BBB25 significantly promoted growth of *H. akashiwo* and two other toxigenic raphidophycean species, as well as nontoxic algae including two diatoms, a cryptophyte, and a chlorophyte. This strain is a gram-positive, rod-shaped, spore-forming bacterium, closely related to *Bacillus*. The data demonstrate the potential for bacteria to influence *H. akashiwo* bloom formation (Liu *et al.*, 2008).

Note: T- toxigenic, and toxic effects of outbreaks [blooms]; B-other bioactive allelopathic compound(s); low DO-blooms commonly cause anoxia/hypoxia; Str-structural feature of the algal cell causes impacts, e.g., needle-like extensions; F-freshwater, Br-brackish, M-marine. Taxonomy basis, acronyms, and other terms are defined in Table 7.1. See Appendix A for scientific names if not included here. Information on habitats (fresh to marine), and on toxins (T) and other bioactive substances (B) is given for taxa not included in Table 7.1.

[a] The general phytoplankton group is indicated for each taxon when the genus name is first mentioned, unless the group for the genus was given in Table 7.1.

[b] Although there has been some question as to whether CYL from *Cylindrospermopsis raciborskii* bioaccumulates, bioaccumulation is now generally accepted (see http://nas.er.usgs.gov/queries/GreatLakes/FactSheet.aspx?SpeciesID=2651 and http://www.glerl.noaa.gov/res/HABs_and_Hypoxia/cylindro_factsheet.html from the U.S. Geological Survey [USGS] and the National Oceanic and Atmospheric Administration [NOAA], respectively; and Kinnear, 2010 – but see Sotton *et al.* 2015).

[c] After 2 weeks of accumulation, CYL distribution was as follows: haemolymph, 68.1%; viscera, 23.3%; foot and gonad, 7.7%; and mantle, 0.9%. CYL was not detected in gills or adductor muscle. Following 2 weeks of depuration, ~50% of the toxin still remained in the tissues (Metcalf *et al.*, 2004).

7.2 Approaches, Pitfalls, Progress, and Goals

Harmful algal toxins have been shown experimentally to be cytotoxic, genotoxic, mutagenic, teratogenic, pathogenic, and/or immunosuppressive, but there are many uncertainties about how these toxins actually operate in natural exposures at environmentally relevant field concentrations. Most research on the impacts of harmful algae in food webs consists of correlative fieldwork, or controlled experiments testing effects of a purified algal toxin or cell extracts on a representative species from one trophic level. The field approach is limited in interpretative power due to potentially confounding factors, while the laboratory experimental approach has limitations because of the highly artificial nature of the setting.

In general when biological effects from exposure to purified dissolved toxins are compared to effects from whole-cell extracts, the effects of whole-cell extracts can be much worse (Palikova *et al.*, 1998; Oberemm *et al.*, 1999), perhaps due to synergistic effects among multiple toxins and/or cofactors that are present (Burkholder and Glibert, 2006; Burkholder, 2009 and references therein). Thus, caution has been recommended in interpretations extrapolated from purified toxin tests to natural settings. In addition to how the toxin is administered, the exposure route is especially important in controlling organismal responses. In general, toxin exposure via the surrounding medium results in much less effect, or no mortality, than the same lethal dose applied orally (e.g., Tencalla *et al.*, 1994).

As a few of many examples of this phenomenon, toxic substances from ingested algae (intact cells)

are adsorbed through the zooplankton gut, whereas the animals typically are exposed to toxins experimentally via the water column and must absorb them through the carapace (DeMott and Dhawale, 1995). Toxins are often injected into mouse or rat models intraperitoneally (IP) or intracoelomically (IC), but such approaches do not simulate how terrestrial or aquatic fauna are exposed to toxins (e.g., STXs) under natural field conditions (Lefebvre *et al.*, 2007). Recent work has indicated that orally dosed Coho salmon, for example, do not exhibit behavioral changes such as those displayed by IC-dosed fish (Lefebvre *et al.*, 2007). Experiments to simulate more natural conditions are challenging because phytoplankton assemblages, including mostly monospecific blooms, commonly show high spatial/temporal variation in biomass, species composition, and the ratio of toxic to nontoxic strains (Zurawell *et al.*, 2005; Gobler *et al.*, 2016 and references therein). The lack of knowledge, even for acute levels of harmful algal toxins, is still so gaping that even the well-funded research area on Florida red tides involving *Karenia brevis* was described as lacking controlled experiments to assess the level of BTXs that is lethal to ecologically and commercially valuable species in the food web (Landsberg *et al.*, 2009).

Erroneous conclusions have resulted from use of one strain to generalize about toxicity of an entire species or genus (see Burkholder and Glibert, 2006; Burkholder and Marshall, 2012 and references therein; and see Glibert and Burkholder, 2018 – Chapter 1). In addition to variable toxicity, other strain-specific characteristics can dramatically alter results and conclusions. For example, differences in ingestibility of toxic *Microcystis* strains by zooplankton can influence the overall herbivore ingestion rate of cells and colonies, and the amount of toxin consumed (Rohrlack *et al.*, 1999). Many toxic algae harbor multiple toxins within a single cell, the proportion of which changes depending on the season and other environmental factors (Burkholder and Glibert, 2006 and references therein). The occurrence and proportion of other toxins in a strain at a given time can influence the outcome of an experiment focusing on only one type of toxin. For example, *Microcystis* strains differ in toxic oligopeptide content (Weckesser *et al.*, 1996), which can influence toxic effects attributed to MCs (Rohrlack *et al.*, 1999). Compounding intraspecific variation in harmful algae are differences among target populations such as zooplankton. Clonal differences have been shown in the sensitivity of a given zooplankton species to toxic algae (Zurawell *et al.*, 2005 and references therein). Some researchers do not verify that a strain is actually toxic, or use conditions that depress toxic activity, prior to conducting "toxicity experiments" using that strain(!) (see discussion in Burkholder and Marshall, 2012 and references therein). This problem can result in erroneous information about fundamental traits such as toxicity. Use of a strain that was verified to be toxic months or years ago is insufficient, considering that toxicity is commonly diminished or lost over time in culture (Burkholder *et al.*, 2005 and references therein).

Over the past few decades, bioassays have progressed from those that detected bioactivity with little concern about ecological relevance, to ecologically relevant tests about possible functions of phytoplankton toxins (Ianora *et al.*, 2011a and references therein). A major impediment in conducting such studies is that often the natural concentrations of a toxic substance and, thus, the concentration range that should be tested, are not known. In addition, concentrations of toxic substances from a given algal species can vary both within a region and geographically (Selander *et al.*, 2006). As an example of progress, Buttino *et al.* (2008) examined use of liposomes as a delivery system for assessment of toxic effects on copepods. Liposomes within the same size range as the food ingested by copepods were prepared and encapsulated with decadienal to assess the effects of polyunsaturated aldehydes (PUAs) on reproduction of two copepod species. Exposure via liposomes reduced egg hatching success and female survival, with concomitant appearance of apoptosis in both embryos and female tissues, and the concentrations of decadienal that induced blockage of cell divisions were tenfold lower than those used in classical feeding experiments (e.g., Ianora *et al.*, 2004). Use of liposomes for delivery of toxins in feeding trials is promising as a more realistic way of delivering a known quantity of toxin (Caldwell *et al.*, 2004). In further advancement, technologies are being developed to co-encapsulate nutritional substances such as amino acids and fatty acids together with the test substance to simulate algal cells more closely (Ianora *et al.*, 2011a).

Beyond improvements in fundamental experimental approaches, significant milestones in understanding food web- and ecosystem-level impacts of HAB will require much more information about chronic/sublethal effects, such as variations in toxin mixtures and concentrations during and after blooms; the presence and extent of *lag*

effects from exposure to blooms that cause disease or death; *indirect effects* of blooms in adversely affecting biota; *cascading effects*; and long-term impacts of repeated exposures on populations at different trophic levels. A major challenge in this research is that trophic transfer of algal toxins in food webs is complex – it can involve many biota at various trophic levels, affecting both pelagic and benthic habitats, and may differentially affect organisms in different life stages. Thus, it has been described as "far more complicated than originally conceived," even for longstanding areas of research such as BTXs in Florida red tides (Landsberg *et al.*, 2009, p. 598). Progress is beginning to be made in the area of toxin transfer through multiple levels in food webs. Lethal lag effects to fauna from higher trophic levels, for example, recently were demonstrated months after *K. brevis* bloom exposures, and significant mortalities from *K. brevis* blooms were revealed from cascading ("domino") effects (Landsberg *et al.*, 2009). Such information is still well beyond reach in the present status of research about most harmful algae, but it can be considered as a future goal.

7.3 High-Biomass Algal Blooms

There are hundreds of published examples of high-biomass, nontoxic microalgal and macroalgal blooms, some described throughout this compendium. Various species of microalgae and macroalgae respond to nutrient pollution (cultural eutrophication) by forming high-biomass blooms (Glibert and Burkholder, 2018a – Chapter 1; and Chapter 15). The most common in freshwaters are planktonic cyanobacteria outbreaks (e.g., *Microcystis aeruginosa* and various other toxic taxa, visible in satellite imagery as covering most of Lake Erie seasonally – Burkholder *et al.*, 2018; filamentous benthic macroalgal cyanobacteria blooms – e.g., *Lyngbya wollei*; and filamentous green macroalgal blooms – Chlorophyta; e.g., *Cladophora glomerata*). Brackish inland habitats have been affected by filamentous green macroalgal blooms (*Ulva* spp., now including species within the genus formerly known as *Enteromorpha* Hayden *et al.*, 2003). Estuarine and coastal marine habitats are characterized by much more diverse microalgal taxa such as dinoflagellates, haptophytes, and diatoms and pelagiophyceans (Dinophyta, Haptophyta, and Heterokontophyta, respectively; Graham *et al.*, 2016) (Burkholder, 1998). They also sustain often-massive, high-biomass blooms of a wide array of filamentous and thallose macroalgae, mostly various greens (e.g., *Ulva*, *Codium*), reds (Rhodophyta – e.g., *Laurencia* spp., *Gracilaria* spp.), and browns (Phaeophyceae, Heterokontophyta – e.g., *Sargassum muticum*, *Pilayella littoralis*) that can cause regime shifts whereby the entire ecosystem is disrupted and readjusts at an altered, more degraded stable state (see Chapter 15, and below). Algae mostly form high-biomass blooms in shallow, poorly flushed embayments and lagoons, but some, such as the microalgal haptophyte *Emiliana huxleyi*, can also be highly productive in open-ocean waters. This species can form massive (10^4 to 10^5 km^2), nearly monospecific seasonal blooms that are visible in satellite imagery, and it is estimated to produce nearly half of the Earth's atmospheric oxygen (Seyedsayamdost *et al.*, 2011a, 2011b and references therein).

High nutrient regimes, especially enrichment with inorganic nitrogen (N) and phosphorus (P), can select for noxious or "weedy," rapidly growing species that can tolerate associated adverse changes in environmental conditions. The excessive inputs of nutrients and high-biomass production can shift aquatic ecosystems out of balance within a season or over longer periods (Burkholder and Glibert, 2013 and references therein). Due to photosynthesis, the water commonly becomes supersaturated with dissolved oxygen (DO). *Supersaturation* develops from algal blooms when the temperature changes too rapidly to allow the oxygen being produced by abundant algae to equilibrate with the overlying air (YSI Environmental, Inc., 2009).

During the night, in contrast, the high respiration rate of the excessive biomass leads to *hypoxic or anoxic* conditions (low DO or no DO, respectively; Junk, 1973; Lyons *et al.*, 2014). The DO "sag" is usually most extreme for aquatic life just before dawn. Low-oxygen stress is described as "one of the most important consequences of high anthropogenic nutrient loadings" because it decreases the amount and suitability of habitat for many beneficial organisms (Breitburg *et al.*, 2003, p. 280). Low DO commonly reduces the abundance and distribution of fish and invertebrates (Kramer, 1987; Diaz and Rosenberg, 1995; Breitburg, 2002 and references therein), with an overall impact of completely altering trophic pathways within food webs – that is, a shift in the fundamental functions and energy dynamics of the food web (Breitburg *et al.*, 1999; Diaz,

2001). Unlike many animal species that cannot withstand repeated periods of hypoxia without severe physiological stress and death (Burkholder and Glibert, 2013), many algae can survive such conditions if alleviated during daylight hours (Lewin, 1962; Wetzel, 2001). The extreme changes over a diel cycle, from supersaturation to hypoxia/anoxia, result in large diel variations or "swings" that have also been shown to be detrimental to sensitive aquatic life (Breitburg, 2002; Morgan *et al.*, 2006; Izagirre *et al.*, 2007; Wilcock *et al.*, 2010). These diel DO swings are accompanied by diel variation in pH, with daytime increases from photosynthetic CO_2 consumption (see Chapter 1).

Degradation of high-biomass algal blooms can also deplete DO (Valiela *et al.*, 1992; Duarte, 1995) and lead to multiple-species kills (Zingone and Enevoldsen, 2000; Dodds, 2006). Most microbial decomposers use oxygen to decompose the remains of dead organisms and release dissolved nutrients back into the system. Increased respiration from stimulation of bacteria, fungi, and protozoans can result in, or contribute to, increased diel DO flux (Dodds, 2006). Algal bloom-dominated systems additionally sustain inputs of large amounts of labile organic matter when the algae die and decompose periodically due to self-shading, other stressors, and seasonal growth patterns (e.g., Havens *et al.*, 2001 and references therein). The algae generally release labile (readily biologically available) nutrients rapidly during decomposition, which promote additional outbreaks when conditions become favorable (Buchsbaum *et al.*, 1991; Havens *et al.*, 2001; Gao *et al.*, 2013).

Thus, high-biomass algal blooms stimulated by nutrient pollution typically cause major hypoxia/anoxia due to their respiration, death, and decomposition (Chorus and Bartram, 1990; Valiela *et al.*, 1992, 1997; Sfriso and Marcomini, 1997; Howarth *et al.*, 2001; Teichberg *et al.*, 2010 and references therein). Many adverse effects have been documented from overgrowth of both microalgae and macroalgae, and their subsequent death and decay:

- Noxious algal overgrowth (micro- or macro-) imparts substantial biotic turbidity, decreasing or virtually eliminating the light needed by beneficial benthic flora (Valiela *et al.*, 1997; Hauxwell *et al.*, 2001). If the growth blankets the water surface, it can block oxygen diffusion into the water from the overlying air and exacerbate low-oxygen stress for aquatic life in the water below. The noxious algae can form thick, slimy masses over beneficial plants, leading to their death (Havens *et al.*, 2001). Loss of beneficial plants such as freshwater eelgrass or seagrass meadows translates, in turn, into disappearance of the critical habitat they provided for fish and other aquatic organisms (Lembi, 2003; Burkholder *et al.*, 2007). Habitat quality becomes compromised, as the algal overgrowth can otherwise alter bottom habitat so that beneficial fauna can no longer use it for spawning and recruitment (Wennhage and Pihl, 1994; Pihl *et al.*, 1995; Thomsen *et al.*, 2006).
- Massive dead/dying algal biomass sinks down to the bottom of the aquatic system at the end of a bloom, depleting the oxygen in the lower water column via respiration and decomposition processes. Living filamentous algal biomass, for example, can increase sulfide concentrations by creating hypoxic/anoxic areas at night from respiration, leading to loss of beneficial seagrass (Holmer and Nielsen, 2007). Dying and rotting algal masses emit strong, foul odors from hydrogen sulfide (H_2S; e.g., Pryor *et al.*, 2007; Green, 2011 and references therein). As Bagarinao (1992, p. 22) wrote, "sulfide is more than just a disagreeable odor from a stagnant marsh; it is a serious menace to all aerobic organisms" because it is toxic to many biota. High sulfide concentrations in the water column have been implicated in mass mortalities of fish and other aquatic life (Shumway *et al.*, 1983; Bagarinao, 1992 and references therein). Sulfide acts as a neurotoxin in mammals: it inhibits cytochrome c oxidase and oxidative phosphorylation, leading to histotoxic hypoxia and loss of energy; it inhibits many other enzymes, causing various metabolic impairments; and it generates reactive radicals and alters membrane permeability, causing edema and organ-specific dysfunction (Bagarinao, 1992; Reiffenstein *et al.*, 1992 and references therein).
- Hypoxia and anoxia promote increased solubility of toxic metals and their release from bottom sediments into the overlying water (Stumm and Morgan, 1996; Mitch and Gosselink, 2007), where beneficial aquatic life can be more easily exposed to them.
- Nutrient transformation processes are dramatically altered by low-oxygen conditions. For example, the capacity of sediments to bind and hold phosphorus (P) is greatly reduced under hypoxia/anoxia (Wetzel, 2001), so that much more P is released to the overlying water

to fuel more blooms. These feedbacks create persistent internal P loading even if external nutrient loads are reduced (Glibert *et al.*, 2011 and references therein). In addition, denitrification, and loss of nitrogen (N) from aquatic systems as N_2 (g), cannot occur in anoxic areas, and decomposition of organic remains becomes much slower (Wetzel, 2001).

- Many invertebrate species (D'Avanzo and Kremer, 1994; Diaz and Rosenberg, 1995; Gray *et al.*, 2002), as well as fish species and their young (Diaz and Rosenberg, 1995; Breitburg, 2002), are killed by hypoxia;
- Fish and macroinvertebrate foraging efficiencies are impaired, and prey abundance is reduced or otherwise adversely altered due to declines in available DO (Pihl *et al.*, 1991, 1995; Osterling and Pihl, 2001; Gray *et al.*, 2002);
- Foraging by some bird species declines because algal mats and scums are avoided (Cabral *et al.*, 1999; Green *et al.*, 2013);
- Fish recruitment and growth are reduced (Wennhage and Pihl, 1994, Shimps *et al.*, 2005) and fish are physiologically stressed, becoming prone to disease (Gray *et al.*, 2002; Stouder and McMullin, 2006 and references therein);
- Decaying blooms provide a substantial source of organic carbon and nutrients to the microbial loop (Karjalainen *et al.*, 2007); and
- Species diversity declines: Subsequent major, adverse changes occur in the trophic structure of invertebrates, birds, and fishes (Raffaelli *et al.*, 1989, 1991; Bolam *et al.*, 2000; Diaz, 2001). The habitat becomes unsuitable for many fish and benthic fauna, which move out of the area or die and exacerbate the hypoxic/anoxic conditions through their decomposition. Over time, the ecosystem becomes characterized by high abundance of relatively few, pollution-tolerant species, including various exotic/invasive taxa, while many beneficial species are lost (Burkholder and Glibert, 2013; Lyons *et al.*, 2014 and references therein). The loss of biodiversity has cascading effects on many ecological processes, such as bioturbation, nutrient generation, and invasion resistance (Solan *et al.*, 2004; Ieno *et al.*, 2006; Stachowicz *et al.*, 2007; Viaroli *et al.*, 2008).

Attempts at ecosystem-level restoration can be impeded by high-biomass algal blooms through diverse mechanisms. For example, blooms of the toxigenic dinoflagellate *Prorocentrum minimum* overlap the period of oyster spawning (Glibert *et al.*, 2007), and can reduce survival of early life history stages of oysters and decrease recruitment. The dense blooms can also decrease survival of beneficial submersed aquatic vegetation through lower light availability that, in turn, affects habitat suitability for many species (Glibert *et al.*, 2011). Thus, impaired physiological processes, impaired trophic transfer, and trophic dysfunction have occurred even in the absence of mortality for some biota.

High-biomass noxious algal blooms can severely alter or reduce ecosystem function by changing the energy flow within food webs. They tend to recur in affected ecosystems, such as blooms of various planktonic, toxigenic cyanobacteria (lower Great Lakes; e.g., Gobler *et al.*, 2016 and references therein); the toxigenic haptophyte *Prymnesium parvum* (certain rivers of Texas, U.S.; Van Landeghem *et al.*, 2013); the toxigenic dinoflagellates *Karlodinium veneficum* (Chesapeake Bay, U.S.; Li *et al.*, 2015) and *Karenia brevis* (west Florida coast, U.S.; Landsberg *et al.*, 2009); the nontoxic, benthic, filamentous freshwater macroalgae *Lyngbya wollei* (cyanobacterium; various freshwaters as described by Bridgeman and Penamon, 2010 and references therein) and *Cladophora glomerata* (filamentous green macroalga; lower Great Lakes – Higgins *et al.*, 2008); and the thallose marine macroalgae *Gracilaria tikvahiae* (e.g., Hawaiian Islands; Anonymous, 2001), *Codium fragile* ssp. *tomentosoides* (e.g., New England coastal waters; Provan *et al.*, 2007), and *Sargassum muticum* (e.g., northwestern U.S.; Britton-Simmons, 2004 and references therein). Blooms can remain or recur in a given environment for days to years to decades depending on the species and conditions (e.g., Valiela *et al.*, 1997; Buskey *et al.*, 2001; Landsberg *et al.*, 2009; Van Landeghem *et al.*, 2013).

In extreme cases, *regime shifts* can occur which involve high-biomass blooms in ecosystems that have sustained high nutrient pollution (also see Chapter 15). A regime shift is an abrupt shift in the biota of an ecosystem in response to a physical/chemical driver (Collie *et al.*, 2004; deYoung *et al.*, 2008; Kraberg *et al.*, 2011). Ecosystems can have more than one state with a self-stabilizing mechanism; a shift between states does not occur frequently and is not readily reversible (Genkai-Kato, 2011). Most often in regime shifts, abrupt changes in ecosystem structure are determined by the responses of biota such as algae to abiotic stressors (Collie *et al.*, 2004; Viaroli *et al.*, 2008). Increased intensity of the stressor or perturbation can induce persistent, dramatic changes in the abundance of one or more components of the community, leading to a major change in dominance, energy

pathways, and overall trophic structure (Viaroli *et al.*, 2008; Hershner, 2011). Basically, the ecosystem crosses an "ecological threshold" that causes an abrupt state shift which is difficult to reverse (Carpenter *et al.*, 1999).

Early research on regime shifts involved lakes driven by major nutrient pollution into chronic, high-biomass toxic cyanobacteria blooms (Scheffer *et al.*, 1993; Carpenter *et al.*, 1999 and references therein). Regime shifts in estuarine/marine coastal lagoons and embayments commonly involve high-biomass blooms of macroalgae (Valiella *et al.*, 1997; Viaroli *et al.*, 2008; Osman *et al.*, 2010). Benthic macroalgae often replace rooted vascular plants (macrophytes; Burkholder *et al.*, 2007; Hastings, 2013 and references therein). Shallow macrophyte-free aquatic ecosystems can also undergo regime shifts to undesirable, high-biomass blooms of benthic and/or floating macroalgae (Genkai-Kato *et al.*, 2012). Macroalgal blooms pervasively and fundamentally alter estuarine ecosystems (Valiella *et al.*, 1997). They dominate DO profiles in the water column of shallow estuaries, and thereby strongly control the biogeochemistry of the sediments and benthic community structure (Hauxwell *et al.*, 2001; Havens *et al.*, 2001; Viaroli *et al.*, 2008). In addition, macroalgal blooms tend to last, and can remain in an environment for years to decades (Valiella *et al.*, 1997).

7.4 Emerging Recognition of the Roles of Allelochemicals

Chemical ecology is the study of the production and interaction of bioactive molecules affecting organism behavior and function (Ianora *et al.*, 2011a). Harmful algae are prominent in this field. Algal allelochemicals other than characterized toxins are considered here because the available evidence, although piecemeal and generally sparse, indicates that they are important in aquatic chemical ecology and food web functioning. Although there are literally hundreds of algal *allelochemicals*, with exception of microalgal toxins (below), they are often overlooked in considerations about the ecology and impacts of harmful algae. Because of methodological impediments (e.g., purification of the substances involved, and use of allelochemicals at environmentally relevant concentrations and environmentally relevant routes of exposure), there historically was a controversy about the ecological relevance of allelopathy (Inderjit and Duke, 2003). Nevertheless, many ecological interactions in aquatic ecosystems have been shown to be mediated by secondary metabolites. As examples, they can deter or accelerate feeding by predators, act as settlement cues for larvae, prevent fouling by epiphytes, serve as pheromones in mate-searching behavior, assist in nutrient acquisition, and influence many other functions (Van Alstyne and Paul, 1989; Leão *et al.*, 2009 and references therein).

General groupings of algal secondary metabolites include terpenoids, tannins, phloroglucinol, phenolics (brominated phenols and polyphenolics), fatty acids (simple fatty acids and derivatives), highly oxygenated polyketides, polyethers, unusual amino acids, peptides, alkaloids, various reactive oxygen species (ROSs), and others (Van Alstyne and Paul, 1989; Cabrita *et al.*, 2010 and references therein). The reactive groups in these substances are often aldehydes, acetate, alcohols, or halogens (Cabrita *et al.*, 2010 and references therein). Other, less frequently occurring substances include acrylic acid, lanosol, and laurinterol (Stein and Borden, 1984). In Antarctic marine waters, for example, the bloom-forming haptophyte *Phaeocystis pouchetii* produces acrylic acid, which can comprise up to 8% of its dry weight (Sieburth, 1961). The sodium salt is an allelochemical that inhibits both gram-positive and gram-negative bacteria, with potential effects suggested at higher trophic levels (Sieburth, 1961). Overall, the hundreds of known algal bioactive substances are extremely diverse both structurally and (based on more limited knowledge) functionally, but they share one trait in common: they have no known role in the primary metabolism and other critical metabolic processes of the organisms that produce them (Van Alstyne and Paul, 1989).

Algal bioactive metabolites can have one highly specific function or multiple, simultaneous functions, including roles in chemical defense (antipredator, antibacterial) and/or cell-to-cell signaling (e.g., PUAs of diatoms) (Ianora *et al.*, 2011a and references therein). In describing inducible responses of marine bloom-forming diatoms, haptophytes, and dinoflagellates to grazers, high species-specific variation has been found in the impacts from these inducible chemical defenses,

> ranging from severe physical incapacitation and/or death to no apparent physiological response, depending on predator susceptibility and detoxification capability. Most bioactive compounds are present in very low concentrations, in both the producing organism and the surrounding aqueous medium. . . .

> Bioactivity may be subject to synergistic interactions with other natural and anthropogenic environmental toxicants. Most, if not all phycotoxins are classic secondary metabolites, but many other bioactive metabolites are simple molecules derived from primary metabolism (e.g. PUAs in [benthic and planktonic] diatoms, dimethylsulfoniopropionate (DMSP) in prymnesiophytes [haptophytes]. Producing cells do not seem to suffer physiological impact due to their synthesis. . . . Understanding chemical ecological responses to environmental triggers and chemically mediated species interactions [eventually] will help define crucial chemical and molecular processes that help maintain biodiversity and ecosystem functionality. (Ianora *et al.*, 2011a, p. 1616)

In most cases, it has not yet been possible to identify the specific compounds involved because of their extremely low effective concentrations. Moreover, extrapolation from allelopathic effects in cultures to natural habitats must ensure that the population densities of the organisms and the concentrations of the allelopathic substances are representative of the natural situation, which can be a considerable challenge; and that the possible effect of bacteria and other contaminating microbes has been eliminated. Thus, verification requires axenic cultures, which usually are not maintained in research on harmful algae (Burkholder *et al.*, 2005 and references therein).

7.4.1 Microalgae

Food web impacts of bloom-forming algae fundamentally begin at the microscopic level, but the chemical ecology of the species involved is only beginning to be understood (Cabrita *et al.*, 2010; Ianora *et al.*, 2011a). It involves the production of allelochemicals, which are natural products produced by one species that elicit physiological or behavioral response(s) in another species (Dicke and Sabelis, 1988). These substances generally are secreted secondary metabolites (i.e., not involved in major metabolic pathways) with growth-inhibiting properties, although some are simple molecules derived from primary metabolism (e.g., PUAs and DMSP) (Cabrita *et al.*, 2010). The most potent allelochemicals are the toxins produced by harmful algae, some of which have been chemically characterized. They are the emphasis of most research to date in this subject area, and their impacts are mostly considered in Section 7.5. Many other, as-yet-uncharacterized allelochemicals are also produced by harmful algae (Legrand *et al.*, 2003; Cabrita *et al.*, 2010). Based on a sparse knowledge base (e.g., Granéli *et al.*, 2008), these substances are important in food web interactions because they contribute to growth, reproduction, and chemical defense. The latter function, involving herbivore–prey interactions, is the most commonly invoked but extremely variable depending on the species involved. In the category of inducible algal chemical defenses, high species-specific variation is known in allelochemical effects on grazers, ranging from disease and/or death to no apparent physiological response, depending on predator susceptibility (Ianora *et al.*, 2011a).

Allelopathic properties have been reported for many phytoplankton species, but few studies have obtained data indicating that allelopathic substances at field-relevant concentrations are used by some harmful algae to achieve dominance in natural habitats. In early work, Keating (1977, 1978) isolated seven species of eukaryotic algae and tested them for their effect on cyanobacteria species that were dominant in earlier or later blooms. Culture filtrates (unialgal or axenic) of each tested species had an inhibitory or neutral effect on the growth of species immediately preceding it in the bloom sequence, but also had a stimulatory or neutral effect on species that immediately followed it in the bloom sequence. Similar results were obtained using lake water filtrates obtained when the various species were dominant. Keating (1978) hypothesized that these algal substances are important in phytoplankton assemblage succession. Also in early research, Mason *et al.* (1982) reported that the filamentous freshwater cyanobacterium *Scytonema hofmanni* produces secondary metabolites that inhibit growth of other cyanobacteria. The antibiotic, a halogenated bioactive substance called cyanobacterin ($C_{23}H_{23}O_6Cl$), was isolated and characterized. It has a low molecular weight (430 daltons) and contains γ-lactone and a chlorinated aromatic nucleus. While it inhibited growth of many cyanobacteria, it only minimally affected tested eubacteria and protozoans.

Since that early work, cyanobacteria have been found to produce many bioactive substances (e.g., Borowitzka, 2016 and references therein). Exudates from *Microcystis aeruginosa* were reported to have much higher estrogenic potency than exudates from other tested species of cyanobacteria and green algae (Sychrová *et al.*, 2012). Cyanobacteria can produce dioxins (Haglund *et al.*, 2007), and they are a natural source of polybrominated diphenyl ethers (PBDEs) as well

(Malmvärn *et al.*, 2005), which are known from other sources used by humans as flame retardants (de Wit, 2002). These substances can act as endocrine disruptors, suppress the immune system, and be neurotoxic (Legler, 2008), and they can adversely affect shellfish, finfish, and wildlife (Darnerud, 2003). They are also widespread contaminants in marine mammals (Weijs *et al.*, 2009; Desforges *et al.*, 2012).

Various marine and freshwater diatoms as well as certain other algae, long considered as benign, and some harmful algae such as the haptophyte *Phaeocystis pouchetti*, produce PUAs (Ianora *et al.*, 2011a and references therein). For example, the highly reactive PUA, decadienal, appears to be involved in grazer defense (Miralto *et al.*, 1999; Ianora *et al.*, 2004), other allelopathy (Ribalet *et al.*, 2007), cell-to-cell signaling (Vardi *et al.*, 2006), antibacterial activity (Ribalet *et al.*, 2008; Balestra *et al.*, 2011), and onset of bloom termination (Vardi *et al.*, 2006; Vidoudez and Pohnert, 2008; d'Ippolito *et al.*, 2009; Vidoudez *et al.*, 2011). Laboratory and field observations have indicated that copepod species feeding exclusively on diatoms, or in diatom-dominated blooms, can be heavily compromised, with only a small percentage of their eggs hatching compared to ~90% hatching in post-bloom conditions (Miralto *et al.*, 1999 and references therein). Three aldehydes (all decatrienals) isolated from the predominant diatoms in the blooms (*Skeletonema costatum*, *Thalassiosira rotula*, and *Pseudo-nitzschia delicatissima* [the latter species, also toxigenic]) were found to be responsible for the poor hatching activity. These substances arrested embryonic development in both copepod and sea urchin bioassays (e.g., Miralto *et al.*, 1999). Some PUAs have teratogenic activity, which causes structural deformities in larval stages of organisms exposed to them during gestation, such as fetal growth, retardation, embryo and fetal mortality, and functional impairment due to malformed limbs or organs. As noted by Ianora *et al.* (2011a), these insidious effects would reduce predators' and grazers' overall fitness, which would help to facilitate bloom development by harmful algal species. The PUAs can also adversely affect the growth and physiological performance of other phytoplankton (Ribalet *et al.*, 2007). Another allelochemical, DMSP, can adversely affect some grazers and is especially common among marine flagellates including haptophytes such as the high-biomass bloom former *Emiliania huxleyi*, and dinoflagellates such as *Alexandrium*, *Amphidinium*, *Gonyaulax*, and *Gymnodinium* (Wolfe, 2000; Steinke *et al.*, 2006). This substance was shown to stimulate search behavior for algal prey in the copepod *Temora longicornis* (Steinke *et al.*, 2006). It apparently acts as a chemical cue, indicating inferior or weakened algal prey (Ianora *et al.*, 2011a).

The diatom *Pseudo-nitzschia delicatissima* is renowned for its production of the potent neurotoxin domoic acid (see Section 7.5), but it also can produce hydroxyl-fatty acid, epoxy-fatty acid, and oxoacid allelochemicals that cause reduced hatching, teratogenic effects, growth inhibition, and/or anti-mitotic apoptosis in copepods (e.g., Miralto *et al.*, 1999, described above, and Ianora *et al.*, 2011b). These substances can reduce grazer overall fitness through induced abortions, birth defects, and reduced larval survivorship (Ianora *et al.*, 2011a). Uncharacterized lytic compounds produced by the toxigenic dinoflagellate *Alexandrium tamarense* have caused cell membrane lysis in the microalga *Rhodomonas baltica* (Ma *et al.*, 2009). These lytic substances have been described as large molecules (> 5 kilodaltons), stable over broad temperature and pH ranges, and refractory to bacterial degradation (Ma *et al.*, 2009). Their lytic activity targets both competitor phytoplankton and grazers (Tillmann and Hansen, 2009).

Recent work by Seyedsayamdost *et al.* (2011a, 2011b) exemplifies the progress needed to strengthen understanding about the potentially widespread importance of algal-produced chemicals other than established toxins in food web interactions. The focus was the dynamic relationship between *E. huxleyi* and its bacterial symbiont, *Phaeobacter gallaeciensis*. The bacterium is a member of the roseobacter clade of α-proteobacteria, a large group that comprises up to ~25% of all marine coastal bacteria (Seyedsayamdost *et al.*, 2011b). The symbiosis is mutualistic when the alga is healthy; the bacterial population apparently promotes algal growth by synthesizing and secreting antibiotics and growth stimulants (auxins; Seyedsayamdost *et al.*, 2011a). When *E. huxleyi* senesces, however, it produces a lignin breakdown product, p-coumeric acid (pCA). In what has been described as "Jekyll-and-Hyde" chemistry (Seyedsayamdost *et al.*, 2011a), the bacterium responds to pCA by switching to become an opportunistic pathogen that produces roseobacticides (novel bacterial troponoids) which cause cell lysis and death of *E. huxleyi* (Figure 7.3). Assays with various other estuarine/marine microalgae (haptophyte *Isochrysis* sp., prasinophyte *Tetraselmis suecica*, diatom *Chaetoceros muelleri*, and cryptophyte *Rhodomonas salina*) have shown that the roseobacticides also have specific and

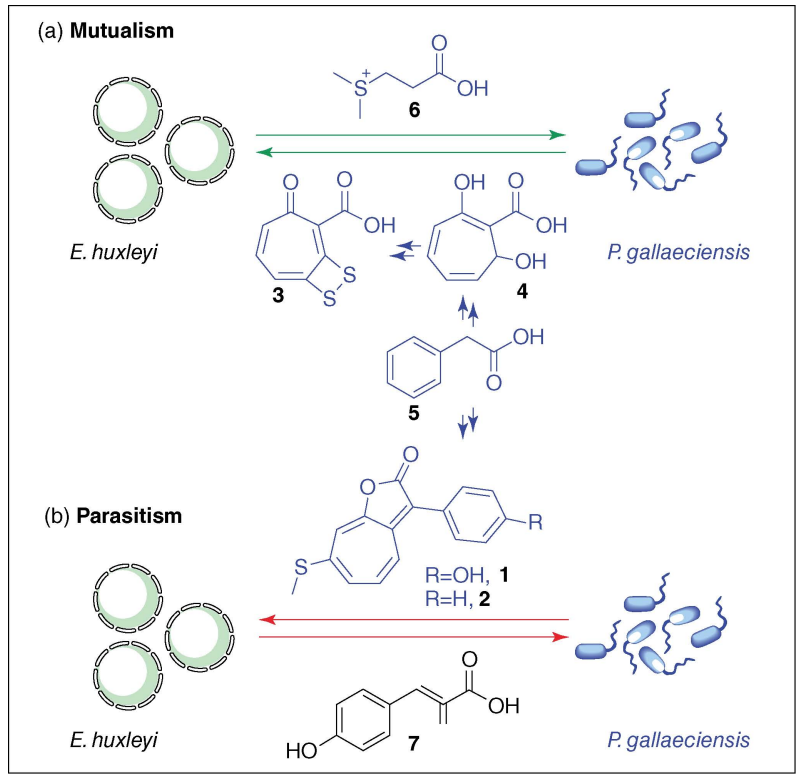

Figure 7.3 Proposed conceptual model from Seyedsayamdost *et al.* (2011a, 2011b) for the dynamic interaction between *P. gallaeciensis* B107 and *Emiliania huxleyi*. The two phases of the interaction are shown by green (mutualistic phase) and red (parasitic phase) arrows. Compounds produced by *P. gallaeciensis* BS107 and *E. huxleyi* are shown in blue and black, respectively.
(a) *Mutualistic phase* of the symbiosis. Under these conditions, the healthy algal host provides DMSP (**6**); DMSP attracts roseobacter (and other) bacteria, which use DMSP as a carbon and sulfur source, and as an attachment surface. Note that the bacteria metabolize DMSP to volatile DMS, which is converted in the atmosphere to condensation nuclei for water droplets. The bacterial symbiont provides the antibiotic tropodithietic acid (TDA, **3**), its precursor (**4**), and the plant growth promoter phenylacetic acid (**5**).
(b) *Parasitic phase* of the symbiosis. When the algal host senesces, it releases p-coumaric acid (pCA, **7**, a lignin component), which elicits the production of the anti-algal roseobacticides (**1, 2**), likely derived from **5**. Roseobacticides **1** and **2** contain a 1-oxaazulan-2-one core, and they can adversely affect marine phytoplankton with nanomolar potency. The roseobacticides arise from variable substituents at the C3 and C7 positions of the roseobacticide core, suggesting that they are produced via modifications and combinations of aromatic amino acids. Note that **5** is likely a precursor to metabolites that are health-promoting in the mutualistic phase (A) and toxic in the parasitic phase (B). Thus, **5** may be a critical player in the switch from mutualism to parasitism. *Source*: Reprinted with permission of the *Journal of the American Chemical Society*.

potent (nanomolar-level) algicidal activity against those algae, suggesting that the mechanism may be common among marine microalgae and roseobacteria (Seyedsayamdost *et al.*, 2011a). Terrestrial plant-associated bacteria respond to lignin components that are released into the surrounding soil when the plants senesce (Schaefer *et al.*, 2008). Considering that point, and the fact that lignin component substances have been found in green, red, and brown macroalgae, Seyedsayamdost *et al.* (2011b) suggested that a similar response may be widespread in marine macroalgal-bacterial interactions.

7.4.2 Thalloid Macroalgae

Allelopathy in thalloid estuarine/marine macroalgae is well known but poorly understood from an ecological perspective except for relatively few compounds, largely because many of the biologically active substances that are produced appear to have

multiple, interactive ecological roles which mostly remain to be elucidated (see review by Van Alstyne and Paul, 1989; and see Chapter 15). Nevertheless, some of the most fascinating information about the roles of allelochemicals has been published about thalloid macroalgae (e.g., Hay, 2009; Van Alstyne *et al.*, 2015 and references therein).

Various brown macroalgae release an array of phlorotannins that have been shown to inhibit growth of epiphytic bacteria and invertebrates (e.g., Sieburth and Conover, 1965), and anti-grazing polyphenolics that are induced by herbivores (e.g., *Fucus distichus*; Van Alstyne, 1988). Perhaps the most "extreme" allelochemical known among brown algae is concentrated sulfuric acid, produced by several species of *Desmarestia* within specially constructed, subcellular vacuoles (Meeuse, 1956; Eppley and Bovell, 1958). If these mostly subtidal species are exposed to the air during low tide, the acid is released, killing both the macroalga and other organisms in the surrounding area (O'Clair and Lindstrom, 2000). Sulfuric acid-laden *Desmarestia* is avoided by grazers, thus serving an important ecological role. For example, sulfuric acid in *Desmarestia* has inhibited grazing by the sea urchin *Strongylocentrotus droebachiensis* (Pelletreau and Muller-Parker, 2002).

Many halogenated (bromine, iodine) aliphatic haloketones and brominated phenols are produced by red macroalgae, with antimicrobial and anti-herbivore functions (Paul *et al.*, 2006), as well as more complex monoterpenes, sesquiterpenes, and diterpenes (up to 5% of the thallus dry mass; Hay and Fenical, 1988). For example, *Laurencia* spp. produce elatol, which can act as a cytotoxin, ichthyotoxin, insecticide, and herbivore deterrent (Hay and Fenical, 1988 and references therein). Species within this genus are known high-biomass bloom formers in response to anthropogenic nutrient over-enrichment (Lapointe *et al.*, 2002). In general, however, as noted by Cabrita *et al.* (2010, p. 2301), "The ecological role of marine algal halogenated metabolites has somehow been overlooked."

Insights about the roles of other macroalgal allelochemicals in food webs have been derived from experiments wherein at least one of the substances involved has been chemically characterized. These experiments indicate that *macroalgal allelochemicals affect marine food webs in many far-reaching ways* (Duffy and Hay, 1990; Hay, 2009; Van Alstyne *et al.*, 2015 and references therein). Species of ulvoid green algae (Chlorophyta, Ulvophyceae), for instance, have been a research focus for decades. Many of them produce allelochemicals with effects that have included reducing barnacle densities in tidepools; causing mortality of larval crabs, oysters, and juvenile abalones; inhibiting growth of planktonic microalgae and other benthic macroalgae; and reducing epiphytism by bacteria, algae, and invertebrates (Van Alstyne *et al.*, 2015 and references therein).

An example is provided here from recent research on the green macroalga *Ulvaria obscura* (e.g., Van Alstyne *et al.*, 2006, 2008, 2011, 2014, 2015). This species is abundant in subtidal "green tide" blooms, especially in urbanized areas of the northwestern U.S. The blooms can adversely affect marine communities, fisheries, and aquaculture. They can result in fragmented seagrass meadows, produce noxious odors, and release allelochemicals that detrimentally affect other algae and invertebrate larvae (Nelson and Lee, 2001; Nelson *et al.*, 2003; Van Alstyne *et al.*, 2011, 2015 and references therein). Among the metabolites produced by *U. obscura* are dopamine (averaging 4.4% of the thallus dry mass in some studies), quinones resulting from dopamine oxidation in seawater, ROSs (van Hees and Van Alstyne, 2013), and dimethyl sulfide (Van Alstyne and Houser, 2003). The dopamine and quinones have reduced growth and germination rates of other marine macroalgae, increased mortality rates of crab zoeae, and depressed feeding by sea urchins (*Strongylocentrotus droebachiensis*), snails (*Littorina sitkana*), and isopods (*Idotea wosnesenskii*) (Van Alstyne *et al.*, 2015 and references therein). Various herbivores avoid or minimize *U. obscura*, which may contribute to its ability to form persistent blooms (Van Alstyne *et al.*, 2006). Ulvoid algae tested thus far also produce DMSP (Van Alstyne *et al.*, 2007; Van Alstyne, 2008), which inhibits growth of epiphytic bacteria (Saha *et al.*, 2014) and can have other allelopathic effects as mentioned.

Recent research has indicated that competition among marine macroalgae can induce allelopathy while also suppressing growth and anti-herbivore defense. After eight days of competition with the coral *Porites cylindrica*, the red macroalga *Galaxaura filamentosa* (described as chemically rich) induced allelochemical release and became nearly twice as damaging to the coral, while also decreasing in growth and increasing in palatability to herbivores (likely due to reduced chemical defenses; Rasher and Hay, 2014). Under the same conditions, the brown macroalga *Sargassum polycystum* did not induce allelopathy, and maintained the same level of growth and palatability. The authors (Rasher and Hay, 2014, p. 1) described their observations on *G. filamentosa* as "the first

demonstration of induced allelopathy in a seaweed, or of competitors reducing seaweed chemical defences against herbivores." They concluded that the nuanced, complex chemical ecology of coral-seaweed-herbivore interactions underscores the need to consider more ecological complexity in studies of chemical defense.

Although some of the earliest research on allelopathy in thalloid macroalgae with substance identification was conducted on freshwater/brackish species, much less is known about allelopathy in those habitats. In classic work by Anthoni *et al.* (1980) and Wium-Andersen *et al.* (1982), the streptophyte *Chara* (Streptophyta, Charales) was shown to produce several low-molecular-weight sulfur compounds with important ecological roles as scent markers, insecticides, and inducers of feeding behavior. This macroalga can be a noxious benthic bloom former in some freshwaters of the western U.S. (Lembi, 2003 and references therein). Two sulfur-containing allelopathic compounds, 4-methylthio-1,2-ditholane and 5-methylthio-1,2,3-trithaine, were isolated and characterized from both freshwater and estuarine charaleans. The purified substances inhibited microalgal photosynthesis (of the benthic, co-occurring diatom *Nitzschia palea*) at a 3 micromolar (μM) concentration. The data suggested that these substances from *Chara* can reduce growth of epiphytic algae, which may explain why *Chara* is seldom found with epiphytes (Wium-Andersen *et al.*, 1982).

7.4.3 Filamentous Mat-Forming Macroalgae

Filamentous marine cyanobacteria were first evaluated for natural products (bioactive secondary metabolites) about 40 years ago, and they have become well known as rich sources of a wide array of bioactive substances (Tan, 2007; Tidgewell *et al.*, 2010). These substances have antibacterial, antifungal, antiviral, anticancer, antiplasmodial, algicidic, antiplatelet aggregation, and immumosuppressive properties (Ramamurthy *et al.*, 2014 and references therein). Allelochemicals from freshwater cyanobacteria are also well known (Chorus and Bartram, 1999), although not as intensively examined as those from marine species.

The cyanobacterial genus *Lyngbya* consists of species that are "prolific producers of secondary metabolites, primarily lipopeptides, cyclic peptides, and depsipeptides" (Sharp *et al.*, 2009, p. 2879). Foremost among them is *Lyngbya majuscula*, which occurs worldwide in tropical and subtropical environments (Tidgewell *et al.*, 2010). This species negatively affects coral larvae recruitment (Kuffner and Paul, 2004) and inhibits potential herbivores using chemical defenses (Paul *et al.*, 2005). More than 180 bioactive substances have been reported from *L. majuscula*, including curacin A (anticancer – Chang *et al.*, 2004) and jamaicamides (neurotoxic – Edwards *et al.*, 2004). Although the number of bioactive substances from *L. majuscula* likely has been overestimated due to contamination by co-occurring microbes (Jones *et al.*, 2011), even so, many bioactive secondary metabolites from this species have been confirmed (Tidgewell *et al.*, 2010). Most have been identified in efforts to find substances with pharmacological or other beneficial health applications (Dixit and Suseela, 2013 and references therein). Their roles in the survival and ecology of *L. majuscula* are poorly understood. Many of the bioactive substances from this cyanobacterium and other mat-forming species in fresh as well as marine waters (e.g., *Oscillatoria* spp., *Phormidium* spp., and freshwater *Lyngbya wollei*) have diverse chemical structures and often include bactericidal and algicidal activities (Priyadarshani and Rath, 2012). These activities are believed to afford an advantage for the cyanobacteria in competition for resources against co-occurring algal and bacterial populations.

Little is known about allelochemical production by other filamentous macroalgae. The noxious, widespread chlorophyte, *Cladophora glomerata* (see Chapter 15), produces various fatty acids (e.g., antibacterial steroids), polyphenols (antioxidants), terpenoids (regenerative), and other bioactive substances, but the ecological roles of these substances are poorly understood (Fabrowska *et al.*, 2015).

7.5 Toxigenic Algae in Aquatic Food Webs

Toxins are regarded as the "ultimate" in bioactive substance potency, and have been best expressed in selected microalgae including species of cyanobacteria, dinoflagellates, haptophytes, diatoms, and raphidophyceans. The term *toxin* (Greek: τοξικόν *toxikon*, first used by organic chemist Ludwig Brieger in the late 1800s) is loosely defined as a poisonous substance produced within living cells or organisms. Toxins can be small molecules (e.g., peptides or proteins) or much larger substances, which can cause disease and/or death upon contact or from absorption by other cells

or organisms. These substances vary greatly in potency, making their distinction from other allelochemicals a "gray area," not well defined. Some researchers use the term when there is clear biological activity toward organisms not found in the same habitat as the toxin producer (Leflaive and Ten-Hage, 2007; Zimba *et al.*, 2010).

The effects of chemically characterized, partially characterized, and uncharacterized (putative) toxins of harmful algae are considered here. Although much information is available on toxigenic algae and their impacts on various organisms (examples are given in Tables 7.1 and 7.2; see also reviews by Shumway, 1990; Landsberg, 2002; and see Chapter 4, and Broadwater *et al.*, 2018 – Chapter 5), few publications have attempted to consider their effects at the level of food webs or ecosystems. Those works are highlighted below. The term toxigenic is best applied to these algae because (1) within a given species known to be capable of producing toxin(s), strains commonly range from benign (with no toxin production), to producers of very small amounts of toxin, to highly toxic strains; and (2) toxic strains often do not consistently express toxicity – they may only express toxicity under certain environmental conditions (Burkholder *et al.*, 2005; Burkholder and Glibert, 2006 and references therein). These characteristics have led to differences and apparent contradictions in the published literature (Ibelings and Havens, 2008). Emphasis here is on findings from research with known toxic strains. Tables 7.1 and 7.2 reflect the highly variable knowledge base, depending on the species; for example, there are well over 100 published studies on effects of the cyanobacterium *Microcystis aeruginosa* and of the dinoflagellate *Karenia brevis* on other biota, but relatively few studies on impacts of most toxigenic raphidophyceans.

Many effects from toxic algae have been described. Their sublethal and chronic impacts – while often subtle or insidious and, therefore, much more challenging to detect and characterize under realistic conditions – are considered more important overall influences on affected populations than obvious, more easily detected, acute effects (Landsberg, 2002; Shumway *et al.*, 2003; Karjalainen *et al.*, 2007). Toxic strains of harmful algae adversely affected species within every trophic level of aquatic ecosystems, ranging from other phytoplankton, benthic algae, and seagrasses to apex predators including carnivorous fish and aquatic birds and mammals (also humans – which, although not aquatic and so not considered here, are surely top predators).

Some interesting generalizations emerge from the studies synthesized in Tables 7.1 and 7.2. Algae produce some of the most potent toxins known (e.g., Oshima *et al.*, 1989). Among the most common of their effects are:

- Damage of the liver or hepatopancreas, kidneys, nervous system, and/or gills, the latter often involving osmoregulatory dysfunction. Some algal toxins cause increased ion permeability and inhibit ATPase activity of sodium and potassium pumps in gills; e.g., Ulitzer and Shilo, 1966; Zambrano and Canelo, 1996);
- Reduced or inhibited growth;
- Reproductive impairment (reduced fitness) – lower fecundity, reduced spawning success, reduced embryo development and survival, depressed larval survival and settlement, deformed young, and lower recruitment; and
- Reduced or inhibited grazing, including avoidance of the toxic algae (but note: a complicating factor in zooplankton studies is that nontoxic cyanobacteria, which are generally considered low-quality food, can induce similar effects as toxic cyanobacteria; Laurén-Määttä *et al.*, 1997); and
- In phototrophs, reduced or inhibited photosynthesis.

Importantly, the adverse impacts of algal toxins are generally worse for young life stages of the affected organisms (Tables 7.1 and 7.2), attributed to factors such as a thin epithelial layer and a relatively large body surface, a high metabolic rate, and limited motility. Damage from the toxins to key developmental processes often leads to death, and the young stages of many organisms are often restricted to nearshore littoral areas where the toxic algae can accumulate (e.g., Oberemm, 1999).

Each of these effects alone could cause significant damage at the population level in a bloom area, and some blooms can cover many square kilometers. Collectively, the effects can be devastating at the population level. Food web-level impacts most commonly reported are the loss of biomass and diversity of phytoplankton assemblages, and of zooplankton or benthic invertebrate grazers. These effects, in turn, modify the structure of higher trophic levels, especially the organisms that had depended on those phytoplankton or grazers as food resources. At the ecosystem level, the most severe adverse effects are caused by toxic algae that are also high-biomass bloom formers (see Tables 7.1 and 7.2 – all planktonic cyanobacterial species listed, as well as the benthic *Lyngbya* spp.; dinoflagellates *Akashiwo sanguinea*,

Ceratium tripos, *Karenia brevis*, *K. veneficum*, *Prorocentrum micans*, and *Prorocentrum minimum*; the haptophyte *Prymnesium parvum*; the raphidophycean *Heterosigma akashiwo*; the euglenophyte *Euglena sanguinea*; and the two brown tide species). Their effects can impair ecosystem structure as well as overall trophic structure, as the toxic effects occur in combination with the loss of critical habitat such as submersed aquatic vegetation from algal overgrowth as explained above.

Food web impacts of harmful algae that *have* been studied are believed to be underestimated. For example, Shumway *et al.* (2010) noted that seabirds are among the most common members of marine food webs, and most likely to consume toxins that have bioaccumulated in other organisms. Sublethal toxin impacts may render birds more vulnerable to other environmental stressors, resulting in mortalities. This potential danger could especially affect migratory species that have spent their energy reserves, and arrive emaciated at toxin-contaminated shellfish beds or encounter schools of toxin-laden fish. In their weakened condition, even a small dose of toxin likely would impair the birds' feeding ability and lead to starvation. Research to verify these effects, however, is sparse. Few experimental studies exist because of difficulties in holding birds in captivity, the logistics of field studies, the unpredictable nature of toxic outbreaks, the short period of some outbreaks, and the lack of human awareness that seabirds might be affected. Also, cause-and-effect is difficult to establish because bird deaths often occur offshore; the carcasses drift into shore and are detected well after the toxic outbreak (see Gibble and Hoover, 2018 – Chapter 6).

While many studies have examined the effects of consumption of toxin-laden live prey, a special case of exposure via food is coprophagy or exposure to toxin-contaminated wastes (feces and pseudofeces), which can provide a mechanism for further transport of the toxins especially to benthic communities (Lehtiniemi *et al.*, 2002; Jang *et al.*, 2004; Svensen *et al.*, 2005). For example, pseudofeces of zebra mussels in Great Lake Erie are rich in cyanobacteria, and they can transfer cyanotoxins to benthic communities (Babcock-Jackson *et al.*, 2002). The same bivalve mollusc can be contaminated by its own toxic feces (e.g., MCs in Mediterranean mussels; Amorim and Vasconcelos, 1999). During and after a toxic bloom, there is no clear pattern in the detoxification, dilution, and dissipation of accumulated toxins (Doucette *et al.*, 2006b). Detoxification rates vary greatly among toxins, species, and tissues. Bacteria contribute to pathways of algal toxin transfer by enhancing or inhibiting toxin production, lysing toxic algal cells, and transforming or degrading toxins (Doucette *et al.*, 2006a). Bacteria can also affect the production of some algal toxins (e.g., DA, STXs, PfTXs), either positively or negatively, and some bacteria can modify certain algal toxins into more, or less, potent substances. In addition, bacterially mediated lysis of toxic algal cells often results in the release of dissolved toxins that can adversely affect other organisms (Doucette *et al.*, 1999, 2006a).

Many direct effects of algal toxins on aquatic biota have been documented in laboratory experiments. The effects in natural habitats are more often modulated by environmental factors or the physiological status of the biota (Ibelings and Havens, 2008). The following five examples illustrate the many indirect as well as direct effects of toxic algal blooms, and the fact that the impacts are largely controlled by environmental conditions and the characteristics of the affected species. It should be noted that, with the exception of *K. brevis*, the species involved occur in other regions as well as those considered in the examples.

7.5.1 Toxic *Microcystis aeruginosa* Blooms across North America

Blooms of *M. aeruginosa* affect eutrophic lakes, rivers, and estuaries that have high nitrogen (N) supplies, and *M. aeruginosa* cells are adept at scavenging low concentrations of inorganic phosphorus (P) above P-rich sediments (Burkholder, 2002, 2009; Davies *et al.*, 2010; O'Neill *et al.*, 2012; Gobler *et al.*, 2016). This species (as well as various other cyanobacteria) produces MCs, of which there are more than 100 congeners (Meriluoto and Spoof, 2008). Depending on the strain, *M. aeruginosa* can synthesize an array of other toxins and other bioactive substances as well (Table 7.1). Changes in MCs and other cyanotoxin concentrations often vary tenfold or more during *M. aeruginosa* blooms (Chorus and Bartram, 1999; Zurawell *et al.*, 2005) as inorganic N concentrations decrease and nontoxic strains gain predominance over toxic strains (reported as *Microcystis* or as *M. aeruginosa*; Briand *et al.*, 2009; Davis *et al.*, 2009, 2010).

The blooms typically are high biomass, although small blooms can be much more toxic (e.g., Boyer, 2007). Blooms of *M. aeruginosa* are known historically dating back to the 1800s (Chorus and Bartram, 1999 and references therein). Present-day massive

blooms in some U.S. waterbodies can often be viewed in satellite imagery (Sims, 2013; and see Burkholder *et al.*, 2018).

Havens (2008) designed a simple conceptual model summarizing ecological effects of high-biomass cyanobacteria blooms and their potential adverse impacts, applicable to blooms of *M. aeruginosa*. Bloom formation leads to reduced light availability for submersed plants, benthic algae, and other phytoplankton; elevated pH, which can adversely affect fish populations; reduced CO_2 which alters competitive interactions with other phytoplankton, as *M. aeruginosa* is adept at sequestering carbon; and production of toxins and other bioactive substances (e.g., Sychrová *et al.*, 2012) which can cause sublethal and lethal impacts for zooplankton, macroinvertebrates, fish, wading birds, and other aquatic vertebrates. The developed bloom also adversely affects zooplankton and other grazers, and food web efficiency. Reduced grazing, or avoidance of *Microcystis* by grazers (e.g., Vanderploeg *et al.*, 2001), can act as a positive feedback, promoting further bloom development until environmental conditions become unfavorable. At night, the respiring bloom can cause hypoxia/anoxia, leading to fish kills and sublethal as well as lethal effects on other biota. As the bloom senesces and dies, hypoxia/anoxia and high ammonia concentrations can stress and kill biota as well.

Blooms of toxic *M. aeruginosa* have been lethal to various zooplankton, fish, waterfowl, and mammals, including domestic animals that have orally ingested the toxins in cells and water (Table 7.1). MCs can take various routes in moving through the food web (Figure 7.4), and at relatively low concentrations they can adversely affect biota across trophic levels (Table 7.2). The effects tend to be more severe at higher temperatures (Zurawell *et al.*, 2005). Bioaccumulation commonly occurs depending on the MC congeners, which would exacerbate toxin effects (e.g., Prepas *et al.*, 1997; Lehman *et al.*, 2010; Ibelings and Havens, 2008 and references therein). As Lehman *et al.* (2010, p. 229) noted, the data suggest that "even at low abundance, *Microcystis* spp. may impact estuarine fishery production through toxic and food web impacts at multiple trophic levels." There additionally is well-known benthic-pelagic coupling in the life history of *M. aeruginosa* and other *Microcystis* spp., wherein viable populations containing MCs reside in benthic habitats until conditions are conducive for another planktonic bloom (Latour *et al.*, 2004; Schöne *et al.*, 2010; Misson *et al.*, 2012). These toxins are also found in fish feces in substantial amounts (Jang *et al.*, 2004; Xie *et al.*, 2005), and in pseudofeces of dreissenid mussels (Pires *et al.*, 2004). Thus, MCs are readily moved between the water column and benthic habitats to affect benthic as well as pelagic communities (Figure 7.4).

7.5.2 Toxic *Prymnesium parvum* Blooms and Fish Communities in Two Texas Rivers

Van Landeghem *et al.* (2013) used multiple before-after, control-impact analyses to assess whether repeated toxic *P. parvum* blooms have led to long-term declines in the relative abundance and size structure of fish populations (21-year database) in two river systems in Texas. In the upper Colorado River drainage, about 3 million fish died in 37 fish kills from toxic *P. parvum* blooms during ~2001–2007, whereas about 20 million fish died in 28 *P. parvum*-linked kills in the Brazos River drainage (Van Landeghem *et al.*, 2013 and references therein). Maximal bloom densities were fairly similar in the two rivers (40,000 to 170,000 cells mL^{-1} and 30,000 to 100,000 cells mL^{-1} in the Brazos and Colorado, respectively), but bloom duration and frequency varied substantially. In the Colorado, toxic blooms had occurred each year since 2001 and had usually lasted for six months or more. By contrast, in the Brazos, blooms had been irregular and usually had lasted for two months or less, perhaps affording more refuge availability (temporally and spatially) to allow for better recovery of fish populations. Hydrologic differences would also have influenced bloom impacts – inflows into the Colorado reservoirs were extremely low for the past few decades, in contrast with substantial flows into Brazos reservoirs. High inflows would have helped to terminate blooms by providing nutrient pulses that alleviated nutrient-imbalanced conditions which can promote *P. parvum* toxicity (Granéli and Johansson, 2003b; Granéli *et al.*, 2012). In addition, regular inflows may have reduced salinity levels in the Brazos to below thresholds for *P. parvum* growth, diluted toxin levels, and created low *P. parvum* refuge areas for fish.

The analysis indicated sustained declines in relative abundance and/or size structure, related to toxic *P. parvum* blooms, for 9 of 12 fish species in the upper Colorado River (white bass, white crappie, largemouth bass, bluegill, river carpsucker, freshwater drum, channel catfish, flathead catfish, and blue catfish), but only for 1 of 8 species (blue catfish) in the Brazos River. The authors were

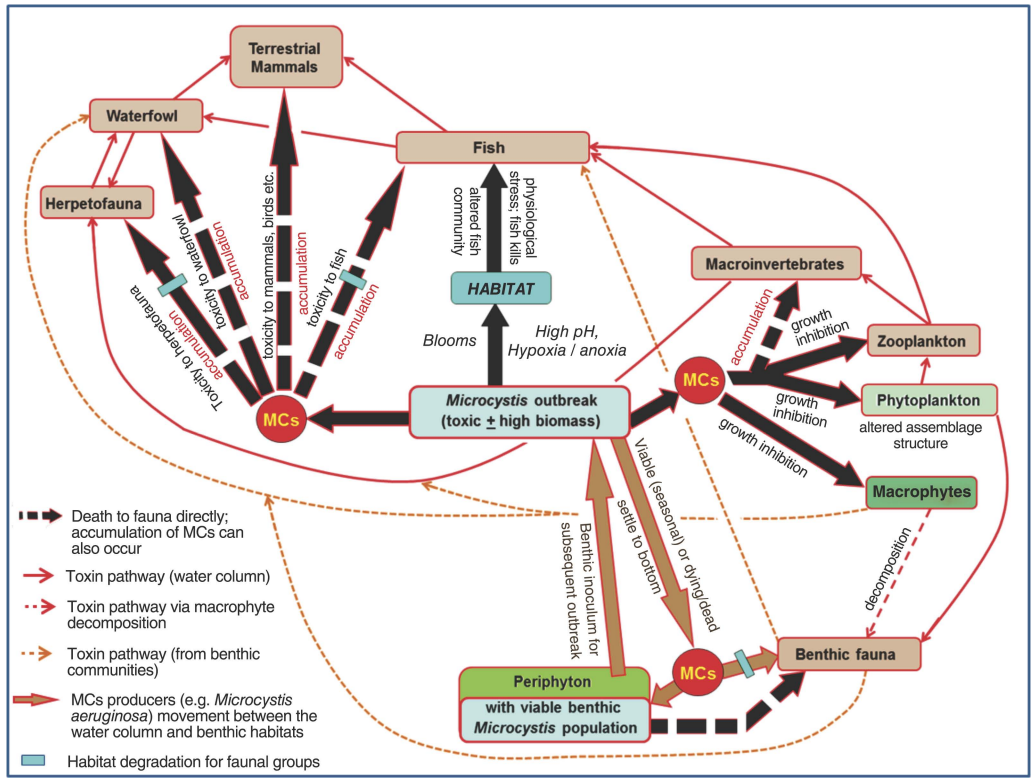

Figure 7.4 A conceptual model of microcystin (MC) pathways in aquatic ecosystems. These toxins are known to bioaccumulate in various fauna (e.g., Ibelings *et al.*, 2005; Xie *et al.*, 2005), and they are contained within cyanobacteria cells but can also be released into the surrounding medium. Not represented in this diagram are other cyanotoxins and other bioactive substances from *M. aeruginosa*, and toxins and bioactive substances from other sources that may be present. Also not shown, other than for macrophytes, are pathways for MC distribution via dead/dying flora and fauna that sink out of the water column to benthic habitats.

able to relate the varying patterns of *P. parvum* impacts on fish populations to several environmental factors, and to differences in the fish species. Previous research on aquatic disturbances had indicated that populations of species with high fecundity or high mobility recovered better and faster following a fish kill than species with low fecundity or low mobility. The much lower water levels characteristic of the Colorado likely created conditions that limited fish dispersal into smaller streams with fresh, nontoxic water, so that population recovery would have depended primarily on reproductive success or supplemental fish stocking rather than immigration from other systems. In addition, the extended duration of the toxic blooms in the Colorado overlapped with spawning periods of affected fish species, thereby severely impeding the ability of these species to recover.

For example, channel catfish in the Colorado have sustained a long-term decline despite major restocking efforts, whereas in the Brazos, a combination of stocking and natural recruitment likely was able to restore or maintain channel catfish populations. Channel catfish spawning in shallow areas (initiating in March–April, with maximal activity in June–July) considerably overlapped with toxic *P. parvum* blooms in the Colorado (from mid- to late fall through late May or June of the following year). This species resides in deeper waters before spawning, which may have provided refuge from exposure to the algal toxins. In the Brazos, by contrast, toxic blooms generally occur from January to March, overlapping only narrowly with fish spawning activity, which may allow higher natural recruitment and better recovery of catfish populations in that system after fish kills. As other examples, river carpsucker and freshwater drum usually mature at age four or later, which may have contributed to their relative inability to recover from toxic

blooms in the Colorado. Overall, a combination of ecological and physiological characteristics influenced the ability of fish species to recover from toxic *P. parvum*-related fish kills. In the upper Colorado River, fish populations have been severely affected by *P. parvum*, whereas most fish populations in the Brazos have remained stable despite toxic *P. parvum* blooms.

7.5.3 Toxic *Pseudo-nitzschia* Blooms in Coastal Upwelling Areas

Although DA is a potent water-soluble neurotoxin, it does not cause fish kills or abnormal behavior in fish during or after toxic *Pseudo-nitzschia* blooms (Lefebvre *et al.*, 2012). The toxin is usually depurated rapidly, so that the residence time in fish tissues is only 2–3 days (Lefebvre *et al.*, 2007 and references therein). Herbivorous fish species such as Pacific sardines and northern anchovies track *Pseudo-nitzschia* blooms to feed (Lefebvre *et al.*, 2002). Stomach contents of anchovies sampled during toxic *Pseudo-nitzschia* blooms, for example, contained high densities of the diatom remains (siliceous cell walls), suggesting that *Pseudo-nitzschia* was a major food resource (Lefebvre *et al.*, 1999), but the fish usually contain DA only in bloom areas or for a short time after leaving a bloom (Lefebvre *et al.*, 2002). Non-planktivorous pelagic fish such as club mackerel and jack mackerel can contain DA during toxic blooms, suggesting toxin transfer from smaller planktivorous fishes (Busse *et al.*, 2006). Filter-feeding bivalve molluscs can also accumulate DA without apparent impact.

Despite the lack of DA impacts on finfish and shellfish during natural *Pseudo-nitzschia* blooms, these animals can act as vectors for DA bioaccumulation in higher trophic levels, especially affecting birds and mammals that rely upon the *Pseudo-nitzschia* consumers as major food resources. Consequently, DA intoxication has resulted in illness and death of human consumers of blue mussels contaminated with DA (Nova Scotia, Canada; Wright *et al.*, 1989); mass mortalities of seabirds such as Brandt's cormorants and brown pelicans that had eaten fish contaminated with DA (coastal California; Work *et al.*, 1993; Sierra-Beltran *et al.*, 1997); and mortality of cetaceans and pinnipeds such as sea lions from consumption of northern anchovies contaminated with DA (Lefebvre *et al.*, 1999; Scholin *et al.*, 2000; Fire *et al.*, 2010). Planktivorous fishes such as anchovies are eaten by many aquatic animals and seabirds in marine food webs (Smith *et al.*, 2011); thus, even one species can be a major vector of DA to higher trophic levels. Limited studies available for zooplankton and phytoplankton have shown reduced feeding and fecundity of a rotifer fed *P. multiseries* (Whyte *et al.*, 1996); and growth inhibition of phytoplankters including the toxigenic dinoflagellate species *Akashiwo sanguinea*, *P. parvum*, and *Chattonella marina*, and a benign cryptophyte exposed to toxic *Pseudo-nitzschia*, as an apparent allelopathic effect (Table 7.2) (Xu *et al.*, 2015).

Diverse benthic fauna (e.g., Pacific sanddabs, longspine combfish, and bottom-feeding fish such as soles, turbots, and halibut) can also accumulate high levels of DA in association with blooms (Kvitek *et al.*, 2008; Vigilant and Silver, 2007), from dying toxic *Pseudo-nitzschia* and contaminated feces that settle out of the water column as blooms subside (Sekula-Wood *et al.*, 2009). Benthic fauna can contain detectable levels of DA even during non-bloom periods (Vigilant and Silver, 2007). Thus, there is a strong pelagic-benthic coupling of DA contamination and bioaccumulation from *Pseudo-nitzschia* blooms.

7.5.4 Toxic *Alexandrium* Blooms in the Northeast

Kills of planktivorous fish (Atlantic herring, Atlantic menhaden, sand lance) have been associated with summer blooms of STX-producing *Alexandrium* spp. in New England and eastern Canada coastal waters for decades (e.g., Smayda, 1991 and references therein). Dying fish have manifested neurological signs at the water surface, such as erratic swimming and paralysis (White, 1977). Larvae of many fish species directly depend on dinoflagellates as food items for about the first week of feeding (Smayda, 1991). In experiments, larval fish (Atlantic cod, Atlantic herring, Atlantic mackerel, capelin, red sea bream, winter flounder) fed toxic *Alexandrium tamarense* swam erratically, lost equilibrium, and/or swam on their side, upside down, or in circles prior to paralysis (Robineau *et al.*, 1991a, 1991b; Samson *et al.*, 2008). Larval fish contaminated with smaller amounts of STXs can serve as vectors for biomagnification and food web transfer of the toxins (Smayda, 1991 and references therein). Although the major route of STX exposure is the diet, STXs are water-soluble and exposure to fish can occur in several ways.

Adult fish such as Atlantic mackerel can accumulate STXs via gill absorption of dissolved toxins, without ingesting toxic cells (Montoya *et al.*, 1996). Grazers such as zooplankton can lyse toxic cells, so that a major fraction of the STXs is dissolved. Planktivorous fish mainly consume zooplankton, but incidentally take in toxic *Alexandrium* cells as well (Turner, 2006; Turner and Granéli, 2006).

Atlantic mackerel can accumulate STXs as they age, suggesting that their health could be affected, and that they could be vectors in transferring STXs to higher trophic-level consumers (Castonguay *et al.*, 1997). As STXs bioaccumulate in the food web, top predators such as piscivorous seabirds (e.g., terns; Nesbet, 1983) and marine mammals such as humpback whales (Geraci *et al.*, 1989; Anderson and White, 1992) have died in association with toxic *Alexandrium* blooms. The whales had fed mostly on Atlantic mackerel, and had acted normally until about 30 minutes before death.

Based on experimental information, organisms from lower trophic levels, while serving as STX vectors, are themselves adversely affected by toxic *Alexandrium* blooms. Bivalve molluscs (e.g., Atlantic surfclams, bay scallops, blue mussels, northern quahogs, and/or ribbed mussels) have responded to toxic *A. tamarense* with reduced clearance rates (Lesser and Shumway, 1993), shell valve closure, and increased mucus production (Shumway and Cucci, 1987), inhibited byssus production (Shumway *et al.*, 1987), and altered heart rate (Gainey and Shumway, 1988). Sea scallops have exhibited shell valve closure, increased mucus production, violent swimming activity, and reduced oxygen consumption when exposed to toxic *A. tamarense* (Shumway *et al.*, 1985; Shumway and Cucci, 1987). Softshell clams have attempted to close their siphons and have shown reduced clearance rates, impaired burrowing response, and decreased heart rate (Shumway and Cucci, 1987; Gainey and Shumway, 1988; Bricelj *et al.*, 1996). Various species of ciliates and microcrustacean zooplankton have responded to toxic *A. tamarense* with depressed feeding, feeding avoidance, feeding inhibition, reduced fecundity, aberrant swimming behavior, and/or reduced growth (Table 7.2). Some *Alexandrium* spp. are mixotrophic and have been shown to consume microalgal prey (Table 7.1). Thus, overall, *A. tamarense* has been documented to adversely affect trophic levels ranging from phytoplankton at the base of the food web to apex predators.

7.5.5 Toxic *Karenia brevis* Blooms along the Florida Coast

The toxic microalga that has been studied the longest for food web impacts in marine ecosystems of U.S. waters is *K. brevis* in the Gulf of Mexico (see Landsberg *et al.*, 2009 and references therein, from which this information is taken unless otherwise indicated). Blooms (outbreaks or red tides) affect other regions of the Gulf Coast along the U.S. and Mexico shores, but the hardest hit area is Florida. Descriptions of *K. brevis* blooms have appeared in various accounts and records since the mid-1800s; the blooms occur every year, can last for much of the year (four to nine months or longer), and annually can kill millions of marine flora and fauna, from phytoplankton to marine mammals such as dolphins and manatees, along more than 5000 km of coastline over a ~7000 km^2 area.

Water-soluble BTXs produced by delicate *K. brevis* cells are released into the surrounding medium when cells lyse, e.g., during grazing, when water carrying cells moves over fish gills, or when waves break onto shore. The toxins have killed hundreds of marine species (see examples in Table 7.1; also Broadwater *et al.*, 2018; and Gibble and Hoover, 2018 – Chapters 5 and 6, respectively). The high concentrations of dissolved BTXs can result in a time lag between a detected bloom and fish mortalities. For example, a bloom that ended late in 2005 was followed by a fish kill that lasted five months in the same area. Sampled fish had high levels of BTXs in their tissues, and the behavior of dying fish fit the symptoms expected from BTX exposure. The blooms are frequently associated with hypoxia/anoxia, likely from the oxygen demand (especially at night) created by the decomposition of dead fauna in shallow waters. Aerosolized BTXs also have affected land animals such as dogs and coyotes (Table 7.2; and see Landsberg *et al.*, 2009 and references therein for effects on humans). High levels of BTXs persist among seagrass leaves, epiphytes, and epiphytic biofilm debris for weeks, and at lower levels for months in the absence of *K. brevis* (Flewelling, 2008).

The persistent, highly toxic blooms each year and the long fish kills can have lingering effects on fish populations. During surveys conducted after these events, up to nearly 60 resident finfish species have been absent. In bloom years, declines have been reported in the annual recruitment of juvenile spotted seatrout, sand

seatrout, and red drum in some affected areas, which would alter the overall fish community structure (Flaherty and Landsberg, 2010). The prolonged blooms have led to changes in fish community structure and ecology in benthic habitats as well. Gannon *et al.* (2009) reported that during *K. brevis* outbreaks in Sarasota Bay, Florida, in 2003–2007, pelagic filter feeders became more abundant while demersal, invertebrate-feeding fish species declined by as much as 88% in comparison to their abundance in periods without blooms. The data also suggest that pelagic filter-feeding fish are more tolerant of bloom conditions or better able to avoid dense bloom areas than other fish, which would also make filter-feeding fish more effective vectors of BTXs to higher predators (Gannon *et al.*, 2009).

An outbreak and associated hypoxia/anoxia in 2005 resulted not only in massive fish kills but also in a collapse of epibenthic communities along the central west Florida shelf (DuPont and Coy, 2008). Divers provided a photographic time series (2005–2007) of the natural hard-bottom/ledge community succession after the bloom. Corals apparently bleached during the toxic bloom, but recovered fairly quickly (Table 7.2). Successional stages of fish communities generally followed a predictable progression and reverted to a pre-bloom state, but within the context that this is a disturbed area ecologically. It was hypothesized that the fluctuating environmental conditions in the shallow eastern Gulf of Mexico (major toxic blooms with extended hypoxia/anoxia, varying temperatures, turbidity, and hurricanes) limit the colonizing species and prevent communities from reaching dynamic equilibrium (DuPont and Coy, 2008).

7.6 Ecosystem-Disruptive Algal Blooms

A subcategory of HAB is sometimes referred to as *ecosystem-disruptive algal blooms* (EDAB; term from Sunda *et al.*, 2006). These EDAB are high-biomass blooms and can be either toxic or nontoxic (see Sunda *et al.*, 2006). Their impacts have been distinguished from impacts of other HAB due to direct toxicity, because EDAB effects are considered to be the result of complex feedback interactions among nutrient regulation of algal growth, population losses to grazing, and grazer-mediated recycling of nutrients (Sunda *et al.*, 2006; Gobler and Sunda, 2012). Candidate EDAB species listed by Sunda *et al.* (2006) were based on three criteria: (1) they cause massive, often-monospecific blooms that negatively affect ecosystem structure and function; (2) they adversely affect grazing rates and grazer populations through toxicity, unpalatability, or physically impeding the grazing mechanisms; and (3) positive feedbacks from reduced grazer-mediated nutrient recycling and/or shading of benthic habitats likely contribute to bloom maintenance.

Most of the candidate EDAB species suggested by Sunda *et al.* (2006) have very small cells (maximum dimension 1–5 [8] μm). They included the brown tide organisms *Aureococcus anophagefferens* and *Aureoumbra lagunensis* (Heterokontophyta, Pelagophyceae), the chlorophyte *Nannochloris atomus* (Trebouxiophyceae), the heterokontophyte *Nannochloropsis qaditana* (Eustigmatophyceae), and the cyanobacterium *Synechococcus elongatus* (Ryther, 1954, 1989; Sunda and Guillard, 1976; Phlips *et al.*, 1999; Glibert *et al.*, 2004; Gobler *et al.*, 2005; Buskey, 2008; taxonomy as in Graham *et al.*, 2016). Unlike various high-nutrient-adapted harmful algal taxa, these organisms were hypothesized to be low-nutrient-adapted, K-selected species that compete well with other algae only at low concentrations of available nutrients (Sunda *et al.*, 2006; Gobler and Sunda, 2012). Importantly, Sunda *et al.* (2006) also proposed that EDAB usually require a pre-bloom of *high*-nutrient-adapted species which reduce inorganic nutrients to low levels, stimulate population growth of grazers, and increase organic N and P availability through grazer-mediated recycling. The nutrient conditions promoting EDAB species are likely far more complex than this hypothesis, as is the diversity of nutrient strategies of EDAB species.

Like other HAB, EDAB can severely alter or degrade ecosystem functioning by disrupting nutrient and energy transfer to higher trophic levels. As their blooms develop, the resulting reduction in light penetration can shade the bottom of the typically shallow systems where they occur. The low light availability, in turn, reduces nutrient competition by benthic phototrophs, allowing sediment release of nutrients to further fuel these blooms. Thus, once the blooms become established, they can often be maintained for considerable periods of time – years as in the case of a brown tide (*Aureoumbra lagunensis*) bloom in Texas (8 years; Buskey *et al.*, 2001).

Typically, the small cells forming EDAB are not well grazed (e.g, Gobler *et al.*, 2002, 2005; Caron *et al.*, 2004), so there is little transfer of organic matter from these primary producers through the food web. As another consequence, because of the low rate of grazing, there is a reduction in water-column nutrient recycling. In Narragansett Bay, Rhode Island, U.S., during a brown tide EDAB, the

microzooplankton community of ciliates and heterotrophic flagellates was unusually sparse, suggestive of reduced grazing (Table 7.2) (Smayda and Villareal, 1989; Smayda, 2008). In Laguna Madre, Texas, the density of protozoan grazers was greatly reduced during brown tide blooms, and a thick polysaccharide layer around the cells may have made it difficult for the protozoa to feed (Buskey and Stockwell, 1993; Buskey *et al.*, 2001). The elevated pH resulting from the high algal biomass accumulation likely inhibited grazers as well (Buskey, 2008). It has also been suggested that allelopathic chemicals and toxin(s) may be important in maintaining EDAB (Sunda *et al.*, 2006; Granéli *et al.*, 2008; Robbins *et al.*, 2010).

In addition to not being well grazed by zooplankton, these picoplankton EDAB species generally are poor-quality food for bivalve molluscs and other macroinvertebrate grazers. Brown tide blooms (*Aureococcus anoffagefferens*) in Narragansett Bay led to extensive mortality and recruitment failure of scallops and mussels, die-offs of macroalgae and seagrasses, failure of the cladoceran zooplankton community to appear, failure of bay anchovies (*Anchoa mitchelli*) to spawn, and cessation of mussels to filter, which led to their starvation and death (Bricelj *et al.*, 1989; Smayda, 1991 and references therein). Brown tides (*Aureoumbra lagunensis*) in Texas lagoons (mentioned above) caused the dominant clams to virtually disappear (Montagna *et al.*, 1993). In the Coastal Bays of Maryland, U.S., growth of northern quahogs ceased during a brown tide, but recovered once the bloom subsided (Wazniak and Glibert, 2004). In all, reduced rates of grazing by both pelagic (micro- and macrozooplankton) and benthic macroinvertebrate grazers are thought to have contributed substantially to the development and persistence of brown tides in New England (Gobler *et al.*, 2002, 2004; Caron *et al.*, 2004), the mid-Atlantic U.S. coast, and the Gulf of Mexico (Buskey and Stockwell, 1993; Buskey *et al.*, 1997).

Another classic example of a small EDAB species (following Sunda *et al.*, 2006) is provided by the massive, longstanding blooms of the unicellular cyanobacteria *Synechococcus/Synechocystis*, now mostly referred to as *Synechococcus elongatus*. This organism has damaged the coastal ecosystems of south Florida (Lapointe *et al.*, 1994; Glibert *et al.*, 2004), partly by causing widespread mass mortality of various sponge species (Porifera). Sponges are the primary benthic filter feeders in these systems. When high biomass accumulations occur, the pico-cyanobacteria can release copious external polysaccharides that can obstruct the internal canal system of sponges and/or impair feeding. Thus, declines in sponge populations are thought to have been caused by *S. elongatus* via impaired grazing by sponges (Kuffner and Paul, 2004; Charpy *et al.*, 2012 and references therein). Sponges once provided the primary habitat for juvenile spiny lobsters, which also have significantly declined (Butler *et al.*, 1995). Longstanding, dense *S. elongatus* blooms have exacerbated loss of seagrasses as well (turtlegrass, shoalgrass, and manateegrass) via light reduction (Phlips *et al.*, 1995, 1999; Hall *et al.*, 1999). As described by Butler *et al.* (1995, p. 119), "This cascade of disturbances has dramatically altered the community structure of affected hard bottom areas and demonstrates the coupled dynamics of . . . shallow marine ecosystem[s]." Some strains of these unicellular cyanobacteria have been reported to be toxic (Tables 7.1 and 7.2). Toxicity would exacerbate the impacts of these high-biomass blooms (see Section 7.5).

In considerations about EDAB, Sunda *et al.* (2006) made careful interpretations based on data available at that time, and indicated that the term *EDAB* likely would be applicable to various other HAB as additional data on their ecology and impacts became available. At present, there is increasing recognition that many HAB, including both microalgae and macroalgae, can be ecosystem-disruptive.

7.7 Future Directions

The broad overview presented in this chapter provides examples, among many, of the adverse impacts of harmful algae on food webs and ecosystems. Much is known about the effects of some major algal toxins on certain animal species but, as stated as "goals" in Section 7.2, studies are needed that assess impacts on *natural communities*, rather than on one or a few species at a time. Even for one of the best studied harmful algae to date, *Karenia brevis*, as Pierce and Henry (2008, p. 629) wrote, "an ecosystem-based approach is needed to investigate the acute and subacute impacts resulting from *K. brevis* blooms."

The fate of many algal toxins, both in the ecosystem and post-ingestion at the organism level, is not well understood. Depuration of some toxins is thought to be rapid, but depuration is seldom complete, and low concentrations can even be carried through to the next growing season (e.g., cyanotoxins; see Ibelings and Havens 2008, and references therein). Additional research should include assessment of toxin impacts in benthic

communities and benthic processes (Palmer, 2000), which have been underemphasized (e.g., Glibert *et al.*, 2012) despite the fact that benthic processes are important influences on harmful blooms (Vargo *et al.*, 1996; Vanderploeg *et al.*, 2001; Wikfors, 2005; Schöne *et al.*, 2010). From that improved knowledge base about the fate of algal toxins, models can finally be developed that reliably predict toxin concentrations of a given species/strain in natural settings, and impacts from blooms of a given HAB population on specific trophic levels and species.

Much more information is needed on the degree of bioaccumulation that occurs in aquatic communities affected by some harmful algae such as toxic cyanobacteria. There is a pressing related need to develop improved techniques to measure algal toxins in biota, and to develop biomarkers for toxin exposure. For example, all but a few published studies on toxic cyanobacteria have not accounted for covalently bound cyanotoxins in biota. Microcystins are routinely extracted using aqueous methanol, which does not extract the MCs that are covalently bound to protein phosphatases in cells of the affected organism (Ibelings and Havens, 2008). Studies that have compared the data from standard aqueous methanol extraction to extraction after Lemieux oxidation, which *does* account for covalently bound MCs, have shown that a major proportion of the total MCs in biota is covalently bound (e.g., Smith *et al.*, 2010). Thus, most published research may have significantly underestimated MC concentrations in biota (Ibelings and Havens, 2008 and references therein). Moreover, the transfer and accumulation of MCs depend on the toxin profile of the bloom (Issam *et al.*, 2010), yet most present research is still at the level of examining only one MC (usually MC-LR) or total MCs. Such research should also be extended to many other harmful algae that produce multiple toxins in varying profiles (Burkholder and Glibert, 2006 and references therein). Minor structural changes in toxin profiles produced by the dominant strain(s) in a harmful bloom may have major effects on toxin uptake, organ distribution, and excretion (Dietrich and Hoeger, 2005; Ibelings and Havens, 2008).

The chemical ecology of harmful algae should extend beyond toxins to assess much more about the roles of other potent allelochemicals that strongly influence food web functioning. As Ianora *et al.* (2011a, p. 1616) wrote, "Understanding chemical ecological responses to environmental triggers and chemically mediated species interactions [eventually] will help define crucial chemical and molecular processes that help maintain biodiversity and ecosystem functionality."

This lengthy chapter only briefly mentions certain aspects of harmful algal interactions with food webs. For example, HAB (both high-biomass and toxic) can stress and physiologically weaken aquatic fauna, which would make them more susceptible to diseases from pathogenic viruses, bacteria, fungi, and protozoans. Research is needed to unravel the complex direct and indirect effects of harmful algae on pathogenic organisms and disease in fish, macroinvertebrates, and other aquatic life.

Finally, among the most important areas for future research are the impacts to food webs from exposure to multiple algal toxins, and from simultaneous and sequential exposure to algal toxins and toxins from other sources (Codd *et al.*, 2005). Such conditions are the reality in aquatic ecosystems. Toxic cyanobacteria, dinoflagellates, haptophytes, and raphidophyceans are known to produce more than one toxin within an algal cell, and surely within a bloom (Burkholder and Glibert, 2006 and references therein). Cyanobacteria blooms commonly have been documented to contain multiple cyanotoxins (Graham *et al.*, 2010; de la Cruz *et al.*, 2013; Loftin *et al.*, 2016 and references therein). Analysis of marine mammal tissues during mortality events has shown that exposure to multiple toxins occurs, such as the presence of DA and BTXs in tissues of bottlenose dolphins, and the presence of DA and STXs in feces from North Atlantic right whales (Landsberg *et al.*, 2014 and references therein). Evidence suggests that MCs and CYLs can act additively and synergistically, both with each other and with other toxic substances (Prieto *et al.*, 2011; Rymuszka and Sierosławska, 2013; Freitas *et al.*, 2014; Pinheiro *et al.*, 2016). Algal toxins can act synergistically with heavy metals, for example (Traoré *et al.*, 1999). In addition, harmful algae excrete substances with metal-complexing properties, which can render toxic heavy metals more bioavailable (Moffett *et al.*, 1996; Krishnan *et al.*, 2007). High-biomass blooms that cause anoxic conditions also make toxic substances such as heavy metals and organohalogens much more soluble, and thereby more bioavailable to adversely affect aquatic communities (e.g., Garcia-Hernández *et al.*, 2005).

HAB caused by many high-biomass and/or toxic species are increasing in frequency and extent, due to a combination of nutrient pollution, warming trends and associated effects of climate change, and overfishing (Dale *et al.*, 2006; Casini *et al.*,

2008; Heisler *et al.*, 2008; Hallegraeff 2010; Glibert *et al.*, 2012; O'Neil *et al.*, 2012; see also Chapter 1). Evidence for range expansion of some warmwater harmful algal species is being reported (Hallegraeff, 2010 and references therein; Wells *et al.*, 2015). Research to strengthen understanding about both the obvious and the insidious major food web-level and ecosystem-level impacts of HAB will be valuable in designing reliable predictive models and more effective management strategies to mitigate their impacts and improve protection of aquatic ecosystems.

Appendix A: Scientific Names for Organisms Listed by Common Name in This Chapter, Also Indicating Species Affected by *Karenia brevis* (*Kb*)

I. Vertebrates (Phylum Chordata)

Mammals

Baleen whale (*Balaenoptera* sp.)
Bottlenose dolphin (*Tursiops truncatus*)[Kb]
California sea lion (*Zalophus californianus*)
Coyote (*Canis latrans*)[Kb]
Domestic dog (*Canis familiaris*)[Kb]
Hawaiian monk seal (*Monachus schauinslandi*)
Humpback whale (*Megaptera novaeangliae*)
Manatee (*Trichechus manatus latirostris*)[Kb]
North Atlantic right whale (*Eubalaena glacialis*)
Sea otter (southern sea otter) (*Enhydra lutris nereis*)

Birds

American coot (*Fulica americana*)
Bald eagle (*Haliaeetus leucocephalus*)
Black-crowned night heron (*Nycticorax nycticorax*)
Black oystercatcher (*Haematopus bachmani*)
Brandt's cormorant (*Phalacrocorax penicillatus*)
Brown pelican (*Pelecanus occidentalis*)
Clark's grebe (*Aechmorphorus clarkii*)
Common eider (*Somateria mollissima*)
Common murre (*Uria aalge*)
Common tern (*Sterna hirundo*)
Double-crested cormorant (*Palacrocorax auritus*)[Kb]
Glaucous-winged gull (*Larus glaucescens*)
Lesser scaup (*Aythya affinis*)[Kb]
Mallard (*Anas platyrhynchos*)
Northern fulman (*Fulmarus glacialis*)
Pacific loon (*Gavia pacifica*)
Red-breasted merganser (*Mergus merganser*)[Kb]
Red-throated loon (*Gavia stellata*)
Shag (*Phalacrocorax aristotelis*)
Surf scoter (*Melanitta perspicillata*)
Western grebe (*Aechmorphorus occidentalis*)
White wing scoter (*Melanitta deglandi*)

Reptiles

European pond turtle (*Emys orbicularis*)
Green sea turtle (*Chelonia mydas*)[Kb]
Kemp's ridley sea turtle (*Lepidochelys kempii*)[Kb]
Loggerhead sea turtle (*Caretta caretta*)[Kb]
Mediterranean turtle (*Mauremys leprosa*)[Kb]

Amphibians

Cane toad (*Bufo marinus*)

Fish

American eel (*Anguilla rostrata*)
American pollock (*Pollachius virens*)
Anchovy (*Anchoa mitchilli*)
Atlantic bumper (*Chloroscombrus chrysurus*)[Kb]
Atlantic croaker (*Micropogonais undulatus*)
Atlantic herring (*Clupea harengus harengus*)
Atlantic menhaden (*Brevoortia tyrannus*)[Kb]
Atlantic midshipman (*Porichthys porosissimus*)[Kb]
Atlantic moonfish (*Selene setapinnis*)[Kb]
Atlantic needlefish (*Strongylura marina*)[Kb]
Atlantic salmon (*Salmo salar*)
Atlantic silverside (*Menidia menidia*)
Atlantic spadefish (spadefish) (*Chaetodipterus faber*)[Kb]
Balloonfish (*Diodon holocanthus*)[Kb]
Bandtail puffer fish (*Sphoeroides spengleri*)
Bank cusk-eel (*Ophiodon holbrooki*)[Kb]
Barracuda (*Sphyraena barracuda*)[Kb]
Bay anchovy (*Anchoa mitchelli*)[Kb]
Belted sandfish (*Serranus subligarius*)[Kb]
Black bullhead (*Ictalurus melas* [*Amierus melas*])
Black crappie (*Pomoxis nigromaculatus*)
Black drum (*Pogonias chromis*)[Kb]
Black grouper (*Mycteroperca bonaci*)[Kb]
Black tip shark (*Carcharinus limbatus*)[Kb]
Blackcheek tonguefish (*Symphurus plagiusa*)[Kb]
Blindfish (*Typhlogobius californicus*)
Blue catfish (*Ictalurus furcatus*)
Blue tilapia (*Oreochromis aureus*)
Bluefish (*Pomatomos saltatrix*)
Bluegill (bluegill sunfish) (*Lepomis macrochirus*)
Bluehead wrasse (*Thalassoma bifasciatum*)
Bluerunner (*Caranx chrysos*)[Kb]

Bluestriped grunt (*Haemulon sciurus*)[Kb]
Brown trout (*Salmo trutta*)
Buffalo (*Megastomatobus cyprinella*)
Catfish (*Arius felis*)[Kb]
Channel catfish (*Ictalurus punctatus*)
Checkered puffer (checkered puffer fish) (*Sphoeroides testudineus*)[Kb]
Chinook salmon (*Oncorhynchus tshawytscha*)
Chub mackerel (*Scomber japonicus*)
Cobia (*Rachycentron canadus*)[Kb]
Cod (*Gadus morhua, Gadus* sp.)
Coho salmon (*Oncorhynchus kisutch*)
Common carp (carp) (*Cyprinus carpio*)
Common snook (*Centropomus undecimalis*)
Coney (*Epinephelus fulvus*)
Cowfish (*Lactophrys quadricornis*)[Kb]
Crested blenny (*Hypleurochilus geminatus*)
Crevalle jack (*Caranx chrysos*)[Kb]
Damselfish (genus, species not given)[Kb]
Dogfish (*Galeus californicus*)
Eel (*Ophichthus* sp.)[Kb]
European sea bass (*Dicentrarchus labrax*)
Flathead catfish (*Pylodictis olivaris*)
Freshwater drum (*Aplodinotus grunniens*)
Gafftopsail catfish (*Bagrus marinus*)[Kb]
Gag (*Mycteroperca microlepis*)[Kb]
Gar (*Lepisosteus* sp.)[Kb]
Goldfish (*Carrasius auratus*)
Goldspotted killifish (*Floridichthys carpio*)[Kb]
Grass carp (*Ctenopharyngodon idella*)[Kb]
Gray angelfish (*Pomacanthus arcuatus*)[Kb]
Gray snapper (*Lutjanus griseus*)[Kb]
Gray triggerfish (*Balistes capriscus*)[Kb]
Graysby (*Epinephelus cruentatus*)[Kb]
Great barracuda (*Sphyraena barracuda*)
Groupers (genera, species not given)[Kb]
Grunts (genera, species not given)[Kb]
Guitarfish (*Rhinobatus productus*)
Gulf flounder (*Paralichthys albiguttus*)[Kb]
Gulf kingfish (*Menticirrhus littoralis*)[Kb]
Gulf menhaden (*Brevoortia patronus*)[Kb]
Halfbeak (*Hyporhamphus unifasciatus*)[Kb]
Haller's round ray (*Urolophus halleri*)
Hardhead catfish (*Arius felis*)[Kb]
Harvestfish (*Peprilus triacanthus*)[Kb]
Hogchoker (*Trinectes maculatus*)
Hogfish (*Lachnolaimus maximus*)[Kb]
Hogsucker (*Hypentelium* sp.)
Horn shark (*Heterodontus francisci* [*Gyropleurodus francisci*])
Hybrid striped bass (cultured) (*Morone saxatilis* × *Morone chrysops*)
Inland silverside (*Menidia beryllina*)
Inshore lizardfish (*Synodus foetens*)[Kb]
Jack crevalle (*Caranx hippos*)[Kb]
Jack mackerel (California or Pacific jack mackerel) (*Trachurus symmetricus*)
Jack smelt (*Atherinopsis californiensis*)
Jacks (*Caranx* spp.)[Kb]
Jewfish (*Epinephelus itajara*)[Kb]
Ladyfish (*Elops saurus*)[Kb]
Lancet (*Branchiostoma caribbaeum*)[Kb]
Largemouth bass (*Micropterus salmoides*)
Leatherjacket (*Oligoplites saurus*)[Kb]
Leopard searobin (*Prionotus scitulus*)[Kb]
Lined sole (*Achirus lineatus*)[Kb]
Loach (*Misguruns mizolepis*)
Longnose batfish (*Ogcocephalus vespertilio*)[Kb]
Longnose gar (*Lepisosteus osseus*)
Longnose killifish (*Fundulus similis*)[Kb]
Mahogany snapper (*Lutjanus mahogoni*)
Medaka (*Oryzias latipes*)
Mississippi silversides (*Menidia audens*)
Monkfish (*Lophias americanus*)
Mosquitofish (*Gambusia affinis*)
Mummichog (*Fundulus heteroclitus*)
Needlefish (*Strongylura marina*)
Northern anchovy (*Engraulis mordax*)
Northern pike (*Esox lucius*)
Northern puffer (*Sphoeroides maculatus*)[Kb]
Orange filefish (*Aluterus schoepfi*)[Kb]
Oyster toadfish (*Opsanus tau*)[Kb]
Pacific herring (*Clupea harengus pallasi*)
Pacific sanddab (*Citharichthys sordidus*)
Pacific sardine (*Sardinops sagax*)
Palespotted eel (*Ophichthus ocellatus*)[Kb]
Planehead filefish (*Monocanthus hispidus*)[Kb]
Perch (yellow perch) (*Perca flavescens*)
Perch (*Diplectrum formosum*)[Kb]
Pikeperch (*Stizostedion lucioperca*)[Kb]
Pinfish (*Lagodon rhomboides*)[Kb]
Pompano (*Trachinotus carolinus*)[Kb]
Porgy (*Calamus* sp.)[Kb]
Porcupine fish (*Diadon hystrix*)[Kb]
Puffer (*Sphoeroides* sp.) [Kb]
Purplemouth moray (?) (*Gymnothorax vicinus*)[Kb]
Queen triggerfish (*Balistes vetula*)[Kb]
Rays (genera, species not given)[Kb]
Red drum (*Sciaenops ocellatus*)[Kb]
Red grouper (*Epenephilus morio*)[Kb]
Red perch (rose fish) (*Sebastes norvegicus*)
Red sea bream (*Pagrus major*, *Pagellus bogaraveo*)
Red snapper (*Lutjanus campechanus*)[Kb]
Redfin needlefish (*Strongylura notata*)[Kb]
Redfish (*Sciaenops ocellatus*)[Kb]
Rio Grande darter (*Etheostoma grahami*)
River carpsucker (*Carpiodes carpio*)

Roach (*Rutilus rutilus*)
Round scad (*Decapturus punctatus*)[Kb]
Sailfish (*Istiophorus platypterus*)[Kb]
Sailors choice (*Haemulon parrai*)[Kb]
Sand lances (sand eels) (*Ammodytes* spp.)
Sand perch (*Diplectrum formosum*)[Kb]
Sand seatrout (*Cynoscion arenarius*)[Kb]
Scaled sardine (*Harengula pensacolae*)[Kb]
Scamp (*Mycteroperca phenax*)[Kb]
Schoolmaster (*Lutjanus apodus*)
Sharksucker (*Echeneis naucrates*)[Kb]
Sheepshead (*Archosargus probatocephalus*)[Kb]
Sheepshead minnow (*Cyprinodon variegatus*)
Shiners (genera, species not given)[Kb]
Short bigeye (*Pristigenys alta*)[Kb]
Shortnose batfish (*Ogcocephalus radiatus*)[Kb]
Shortnose sturgeon (*Acipenser brevirostrum*)
Silver carp (*Hypophthalmichthys molitrix*)
Silver jenny (*Eucinostomus gula*)[Kb]
Silver perch (*Bairdiella chrysoura*)[Kb]
Silver trout (silver seatrout) (*Cynoscion nothus*)[Kb]
Snook (common snook) (*Centropomus undecimalis*)[Kb]
Sole (common sole) (*Solea solea*)[Kb]
Sooty eel (*Bascanichthys bascanium*)[Kb]
Southern kingfish (*Menticirrhus americanus*)[Kb]
Southern puffer fish (*Sphoeroides nephelus*)
Southern seabass (black seabass) (*Centropristis striata*)[Kb]
Southern stargazer (*Astroscopus* y-*graecum*)[Kb]
Spadefish (Atlantic spadefish) (*Chaetodipterus faber*)[Kb]
Spanish mackerel (*Scomberomorus commerson*)[Kb]
Speckled worm eel (*Myrophis punctatus*)[Kb]
Spinner shark (*Carcharhinus brevipinna*)[Kb]
Spiny boxfish (butterfish) (*Lactoria diaphana*)[Kb]
Spot (*Lieostomus xanthurus*)[Kb]
Spotted moray (?) (*Gymnothorax moringa*)[Kb]
Spotted rose snapper (*Lutjanus guttatus*)
Spotted seatrout (speckled trout) (*Cynoscion nebulosus*)[Kb]
Steelhead trout (*Oncorhynchus mykiss*)
Stingray (*Mylobatis californicus*)
Stippled clingfish (*Gobiesox punctulatus*)
Striped bass (*Morone saxatilis*)
Striped bass – reciprocal cross-hybrid (*Morone saxatilis* male × *Morone chrysops* female)
Striped burrfish (*Chilomyterus shoepfi*)[Kb]
Striped killifish (*Fundulus majalis*)
Striped mullet (*Mugil cephalis*)[Kb]
Suckers (freshwater; genera, species not given)
Tarpon (*Tarpon atlanticus*)[Kb]
Thornback guitarfish (*Platyrhinoidis triseriata* [*P. triscriatus*])
Thread herring (*Opisthonema oglinum*)[Kb]
Threadfin (*Polydactylus octonemus*)
Tidewater silverside (*Menidia beryllina*)[Kb]
Tilapia (*Oreochromis niloticus*, *O. mossambicus*)
Toadfish (*Opsanus tau*) [Kb]
Tomtate (*Haemulon aurolineatum*)[Kb]
Tripletail (*Lobotes surinamensis*)[Kb]
Trunkfish (buffalo trunkfish) (*Lactophrys trigonus*)[Kb]
Warsaw grouper (*Epinephelus nigritis*)[Kb]
Whip eel (*Basanichthyes scuticaris*)
White bass (*Morone chrysops*)
White crappie (*Pomoxis annularis*)
White grunt (*Haemulon plumieri*)[Kb]
White perch (*Morone americana*)
Whiting (genus, species unspecified)
Winter flounder (*Pseudopleuronectes americanus*)
Yellow pike (walleye) (*Sander vitreus*)
Yellowtail amberjack (*Seriola lalandi*)[Kb]
Zebrafish (*Danio rerio*)

Ascidians[Kb]

II. Invertebrates

Annelids (Phylum Annelida)

Common clam worm (*Nereis succinea*)
Polychaetes – *Americonuphis magna, Diopatra cuprea, Clymenella mucosa, Glycera americana*[Kb], *Glycera capitata*[Kb], *Laeonereis culven*[Kb], *Onuphis magna,* mudworm (*Polydora websteri*), *Scoloplos fragilis*[Kb], *S. rubra* [Kb], *S. squamata*[Kb]
Ragworm (*Branchioasychus americanus*, *Neanthes succinea*[Kb], *Nereis* sp.)

Arthropods (Phylum Arthropoda)

Chelicerates

Horseshoe crab (*Limulus polyphemus*)[Kb]

Crustaceans

American lobster (*Homarus americanus*)
Barnacle (*Balanus* sp.)
Blue crab (*Callinectes sapidus*)[Kb]
Blue shrimp (*Penaeus stylirostris*)
Brine shrimp (*Artemia salina*)
Brown rock crab (*Cancer antennarius*)
Calico crab (Dolly Varden crab) (*Hepatus epheliticus*)

Daggerblade grass shrimp (*Palaemonetes pugio*)
Depressed mud crab (*Eurypanopeus depressus*)
Dungeness crab (*Cancer magister*)
Dwarf crab (lesser blue crab) (*Callinectes similis*)
Flat porcelain crab (*Petrolisthes cinctipes*)
Florida stone crab (*Menippe mercenaria*)
Green porcelain crab (*Petrolisthes armatus*)
Ivory barnacle (*Balanus eburneus*)
Lady crab (*Hepatus epheliticus*)[Kb]
Morbid sand crab (*Emerita analoga* [*Hippa analoga*])
Pacific oyster (*Ostrea lurido*)
Pandalid shrimp (*Pandalus platyceros*)
Pea crab (*Pinnixa* sp.)[Kb]
Porcelain crab (*Petrolisthes armatus*)
Razor clam (*Siliqua patula*)
Red king crab (*Paralithodes camtschatica*)
Red swamp crayfish (*Procambarus clarkii*)
Sand flea (mole crab, sand crab) (*Emerita benedicti*)
Shrimps (*Penaeus* spp.)
Signal crayfish (*Pacifastacus leniusculus*)
Southwestern Atlantic burrowing crab (South American estuarine crab) (*Neohelice granulata* or *Chasmagnathus granulata*)
Speckled swimming crab (*Arenaeus cribarius*)
Spiny lobster (*Panulirus argus*)
Spotted porcelain crab (saltwater porcelain crab) (*Porcellana sayana*)
Stone crab (*Menippe mercenaria*)
Surf hermit crab (surf hermit) (*Isocheles wurdemanni*)
Tanner crab (*Chionoecetes bairdi*)
Thinstripe hermit crab (*Clibanarius vittatus*)

Brachiopods (Phylum Brachiopoda)

Lampshell (*Glottidia pyramidatum*)[Kb]

Cnidarians (Phylum Cnidaria)

Corals (*Oculina diffusa, Slenastrea hyades, Stephanocoenia intersepta, Siderastrea* spp.) [Kb]
Warty sea anemone (*Bunodosoma cavernata*)

Echinoderms (Phylum Echinodermata)

Brittle star (*Micropholis atra*)
Six-keyhole sand dollar (*Mellita quinquesperforata*)
Holothurians (sea cucumbers; genera, species not given)[Kb]

Molluscs (Phylum Mollusca)

Bivalves (Class Bivalvia)

Asian clam (*Corbicula fluminea*)
Atlantic deep-sea scallop (*Placopecten magellanicus*)
Atlantic surfclam (*Spisula solidissima*)
Bay scallop (*Argopectens irradians*)[Kb]
Blood ark clam (blood ark) (*Andara ovalis*)
Blue mussel (*Mytilus edulis*)
Brazil ark (incongruous ark clam) (*Anadara brasiliana*)
Butter clam (*Saxidomus giganteus*)
California mussel (*Mytilus californianus*)
California semele (*Cumingia californica*)
California tagelus (*Tagelus californianus*)
California venus (*Chione californiensis*)
Californian beanclam (*Donax californicus*)
Carpet shell clam (*Ruditapes decussatus*)
Dwarf surfclam (coot clam) (*Mulinia lateralis*)[Kb]
Eastern oyster (*Crassostrea virginica* [*Ostrea virginicus*])
European flat oyster (*Ostrea edulis*)
Farmer's scallop (*Chlamys farreri*)
Florida coquina (coquina, coquina clam) (*Donax variabilis*)
Freshwater clam (*Anodonta grandis simpsoniana*)
Freshwater mussel (*Anodonta cygnea*)
Frilled venus (*Chione undatella*)
Gaper clam (horseneck clam) (*Tresus capax*)
Gould beanclam (*Donax gouldii*)
Great scallop (*Pecten maximus*)
Green mussel (*Perna viridis*)
Hooked mussel (*Ischadium recurvum* [*Brachidontes recurvus*])
Japanese littleneck clam (*Venerupis japonica*)
Kelp scallop (*Leptopecten* [*Pecten*] *latiauratus*)
Lamellibranch bivalve (*Lasaea rubra*)
Littleneck clam (*Protothaca staminea*)
Mediterranean mussel (*Mytilus galloprovincialis*)
Minor jacknife (*Ensis minor*)[Kb]
Northern horsemussel (*Modiolus modiolus*)
Northern quahog (*Mercenaria mercenaria*)
Nuttall's cockle (basket cockle, heart cockle) (*Clinocardiurn nuttali*)
Ocean quahog (*Arctica islandica*)
Olympia oyster (*Ostrea lurida*)
Pacific eggcockle (*Laevicardium substriatum*)
Pacific oyster (*Ostrea lurido*)
Quahog (*Mercenaria* sp.)
Ribbed mussel (*Geukensia demissa*)
Rock scallop (*Hinnites multirugosus*)
Sea scallop (*Placopecten magellanicus*)
Softshell clam (*Mya arenaria*)

Spiny scallop (swimming scallop) (*Chlamys hastata*)
Straight horsemussel (*Modiolus rectus*)
Surfclam (*Spisula solidissima*)
Swam mussel (*Anodonta cygnea*)
Weathervane scallop (*Pecten caurinus*)
Zebra mussel (*Dreissenia polymorpha*)

Cephalopods (Class Cephalopoda)

Octopus (*Tevila crassatelloidis*)

Gastropods

Abalone (*Haliotis discus, Haliotis midae*)
Auger snail (*Terebra cinerea*)
Banded pheasant (*Eulithidium comptum* [as *Tricolia compta*])
Banded tulip (*Fasciolaria lilium hunteria*)
Barrel-bubble (*Acteocina californica*)
Beatic dwarf olive (*Olivella baetica*)
Blister glassy-bubble (*Haminoea viscula*)
Bruised nassa (*Nassarius vibex*)[Kb]
California cone (*Conus californicus*)
Common whelk (*Buccinum undahtum*)
Crown conch (*Melongena corona*)
Double moon shell (*Polinices duplicatus*)
Florida dogwinkle (*Thais haemastoma*)
Gray augur (*Terebra cinerea*)
Great pond snail (*Lymnaea stagnalis*)
Lettered olive (*Oliva sayana*)
Limpets (genera, species not given)
Lottia (*Acmaea conus*)
Striped false limpet (*Siphonaria benedicti*)
Tectura (*Acmaea depicta*)
Long-tailed sea hare (sea slug) (*Stylocheilus longicauda*)
Malaysian trumpet snail (*Melanoides tuberculata*)
Marsh ramshorn (*Planorbella trivolvis* [*Helisoma trivolvis*])
Mexican pyramidella (*Pyramidella mexicana*)
Moonsnail (*Neverita reclusiana* [*Polinices recluzianus imperforatus*])
Onyx slippersnail (*Crepidula onyx*)
Purple dwarf olive (*Olivella biplicata*)
Shark eye (*Polinices duplicata*)
Tadpole physa (*Physa gyrina*)
Western mud nassa (*Nassarius tiarula* [*N. tegulus*])

Phoronids (Phylum Phoronida)

Horseshoe worm (*Phoronis architecta*)[Kb]

Sponges (Phylum Porifera)

Branch candle sponge (*Vergangid longissima*)
Gray-purple sponge (*Spinosella vaginalis*)
Ircinia sp.
Loggerhead sponge (*Spheciospongia vesparium*)
Sheepswool sponge (*Hippiospongia laehna*)
Stinker sponge (*Ircinia felix*)
Vase sponge (*Ircinia campana*)

Macrophytes (Vascular Plants)

Mosses (Phylum Bryophyta)

Java moss (*Vesicularia dubyana*)

Flowering plants (Phylum Anthophyta)

Coontail (*Ceratophyllum, Ceratophyllum demersum*)
Eelgrass, marine eelgrass (*Zostera marina*)
Elodea (*Elodea canadensis*)
Eurasian watermilfoil (*Myriophyllum spicatum*)
Freshwater eelgrass (tapegrass) (*Valisneria americana*)
Hydrilla (*Hydrilla verticillata*)
Manateegrass (*Syringodium filiforme*)
Phragmites (common reed) (*Phragmites australis*)
Round-leaf seagrass (*Syringodium isoetifolium*)
Shoalgrass (*Halodule wrightii*)
Turtlegrass (*Thalassia testudinum*)

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8

Assessing the Economic Consequences of Harmful Algal Blooms: A Summary of Existing Literature, Research Methods, Data, and Information Gaps

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8.1 Introduction

Harmful algal blooms (HAB) are marine or freshwater natural hazards that can lead to serious public health and socioeconomic consequences, depending upon their spatial distributions relative to: (1) human populations, (2) durations and frequencies of occurrence, (3) cell densities and toxicities, and (4) weather conditions. The focus of this chapter is mainly on the economic consequences of *marine* HAB. Several reports and studies have characterized the types and magnitudes of the adverse economic effects associated with marine HAB in the United States (Adams *et al*., 2000, 2002; Anderson *et al*., 2000; Hoagland *et al*., 2002; Hoagland and Scatasta, 2006; Hoagland, 2008; Ralston *et al*., 2011; Adams and Larkin, 2013). While the economic effects of marine HAB have received significant attention in the past, the economic effects of *freshwater* HAB now also are being assessed (Dodds *et al*., 2009; Bingham *et al*., 2015). Further evaluations of the impacts of HAB on the human uses of and values for both marine and freshwater resources could justify expanded public investments in natural and social scientific research, thereby supporting decisions to identify and implement appropriate prevention, mitigation, or control strategies.

Informed enquiry into the relevant economic methodologies, necessary data, and gaps in current understanding would help to delineate a national program of socioeconomic research, further clarifying the nature of the hazards and thereby guiding future scientific research efforts. In the United States, several science funding programs at the federal level could benefit from such guidance, including the interagency program on Ecology and Oceanography of Harmful Algal Blooms (ECOHAB) and the NOAA programs on Monitoring and Event Response for Harmful Algal Blooms (MERHAB) and Prevention, Control, and Mitigation of Harmful Algal Blooms (PCMHAB). The actions of state and municipal agencies to manage coastal and marine resources, especially shellfisheries, and to protect the public health also would likely benefit.

This chapter summarizes an extant literature that seeks to evaluate many of the economic consequences associated with marine HAB. The discussion utilizes a report by Adams and Larkin (2013) containing an annotated bibliography of both peer-reviewed and "gray" (no formal peer review) research papers. That bibliography is supplemented with publications from the more recent literature, focusing on the peer-reviewed literature and adding examples from the methodologically rigorous gray literature. Specifically, this chapter discusses methodologies that have been used to measure economic losses, review the types and sources of data, depict the spatial distribution of examples where economic impacts have been estimated in the United States, discuss the complexities of addressing the scopes of HAB events, and identify research gaps and areas for focusing future socioeconomic research efforts.

Harmful Algal Blooms: A Compendium Desk Reference, First Edition. Edited by Sandra E. Shumway, JoAnn M. Burkholder, and Steve L. Morton.

8.2 Overview

The following discussion summarizes 36 studies that estimated the economic losses of single or multiple HAB events over time. The summary is organized according to four criteria: (1) the methodologies used to assess the economic effects, (2) the sources and types of data, (3) the spatial or temporal scopes of the analyses (e.g., the accounting stances or time steps), and (4) the physical characteristics of the relevant hazard (e.g., the species and the harmful effects that are being measured). Each criterion is discussed with respect to the full collection of studies. Note that some overlaps among the criteria exist (e.g., methodologies and data often are logically linked). The individual studies are summarized and annotated briefly in Table 8.1.

8.3 Research Methodologies

The most basic distinction regarding approaches used to estimate economic consequences is whether the consequences are "market" or "non-market." Market-related losses are those that can be calculated from changes in prices or quantities of goods or services (as a result of a HAB) that are physically traded; market losses can be used to identify the tangible effects of HAB with data from either direct or surrogate markets. Non-market goods comprise those for which no formal market exists, and thus a theoretical equilibrium price and quantity cannot be observed. Non-market losses could relate to the adverse effects of HAB on recreational uses of coastal or ocean resources, such as sportfishing or beachgoing, or on passive uses, such as perceptions of well-being associated with healthy ecosystems.

In applying market-based methods, it is important to characterize the distribution of effects across producers (private firms or individuals), consumers, or both. *Direct market methods* are those that use data that reflect a change in market value, revenues, or expenditures, such as lost seafood market sales, lost reported sales by water-proximate businesses, or the costs of HAB monitoring or cleanup. *Surrogate market methods* use data from related or substitute markets to capture a change in value, such as depressed real estate values or the increased costs of travel to substitute recreational sites.

Ideally, using market data, one would want to develop models of supply and demand in the relevant product or service markets, and thereby calculate losses of economic surpluses to both producers and consumers when HAB negatively influence the use of coastal or ocean resources (Figure 8.1). Kahn and Rockel (1988) provide one of the few such analyses with respect to harmful blooms of the brown tide *Aureococcus anophagefferens*, which have led to the complete loss of the bay scallop *Argopecten irradians* fishery in the Peconic Estuary of Long Island, New York. Only rarely are such studies undertaken, however, because of data deficiencies or the expenses of undertaking the analyses.

Measuring losses to *producers* using direct market data is one of the most common – albeit often flawed in its application – methods of evaluating economic effects. In spite of its flaws, this approach is relatively easy to implement, and it has been adopted for use by policy makers in assessing the need for relief to producers affected adversely by HAB "disasters." Figure 8.2 and Table 8.2 depict the spatial distribution and scale of several examples of marine HAB for which economic impacts have been estimated. Typically, an estimate is made of reduced or lost output from a market, such as a seafood market, multiplied by a price for the lost output. Such estimates are known as lost gross revenues or *direct output impacts* (DOIs). Where a regional economic (input–output, or IO) model exists, indirect and induced impacts also can be evaluated by adjusting the estimate of DOIs with a "multiplier" (cf. Shumway *et al.*, 1988, for reference to such an application during HAB-related shellfish closures in Maine). *Indirect impacts* are changes in output in industry sectors that are linked to a sector experiencing DOIs, such as changes in the output of upstream suppliers as a consequence of reductions in shellfish digging and growing during a HAB. *Induced impacts* are changes in spending by workers in the affected direct and indirect sectors. In practice, the DOI approach has been used to determine the level of fishery disaster relief claims made pursuant to §312 (a) of the federal Sustainable Fisheries Act (P.L. 104-297). For example, the direct, indirect, and induced output impacts of shellfish closures in New England resulting from widespread blooms of *Alexandrium fundyense*, a HAB that can cause paralytic shellfish poisoning (PSP) in humans, were evaluated by state officials in Maine, New Hampshire, and Massachusetts to substantiate successful disaster relief claims in 2005 and 2008.

From the perspective of welfare economics, however, estimates of DOIs do not match true economic losses, and, without careful modeling of

Table 8.1 Summary of research evaluating the economic consequences of HAB or related events.

Authors	Agundez *et al.*	Athearn	Chadsey *et al.*	Diaby	Cummins
Title	Technical Adoption to Harmful Algal Blooms: Socioeconomic Consequences for the Shellfish Farming Sector in Bourgneuf Bay (France)	Economic Losses from Closure of Shellfish Harvesting Areas in Maine	Cooperation of Science and Management for Harmful Algal Blooms: Domoic Acid and the Washington Coast Razor Clam Fishery	Economic Impact of Neuse River Closure on Commercial Fishing	Potential Economic Loss to the Calhoun County Oystermen
Publisher (and type of publication)	*Aquaculture Economics & Management* (journal)	University of Maine (working paper)	*Coastal Management* (journal)	North Carolina Division of Marine Fisheries (agency report)	Dolphin Talk (online publication)
Year published	2013	2008	2012	1996	2012
Temporal scope	1997–2007	2001–2005	2005–2008	1995	2011–2012
Spatial scope	Bourgneuf Bay area (Pays de la Loire region, France)	Maine	Coast of Washington State	Neuse River, North Carolina	Calhoun County, Texas
Method	DOI: Estimated economic performance, cost of new technology, and economic viability	DOI: Estimated decline in landings, input–output modeling (IMPLAN)	RP: Institutional analysis and development (IAD) framework	DOI: Calculation of reduction in landings from previous year	DOI: Calculated decline in dockside value
Source or type of data	Survey of industry; small-scale fishery management (primary/secondary)	Maine Department of Marine Resources (secondary)	Survey of experts; other papers (primary/secondary)	Surveys of seafood dealers; North Carolina Division of Marine Fisheries (primary/secondary)	Texas Parks and Wildlife Department (secondary)
Algae or toxin	*Pseudo-nitzschia, Alexandrium*	*Alexandrium fundyense*/PSP	*Pseudo-nitzschia*	*Pfiesteria* spp.	*Karenia brevis*

Authors	Dodds *et al.*	Dyson and Huppert	Evans and Jones	Habas and Gilbert	Hoagland *et al.*
Title	Eutrophication of U.S. Freshwaters: Analysis of Potential Economic Damages	Regional Economic Impacts of Razor Clam Beach Closures due to (HAB) on the Pacific Coast of Washington	Economic Impact of the 2000 Red Tide on Galveston County, Texas: A Case Study	The Economic Effects of the 1971 Florida Red Tide and the Damage It Presages for Future Occurrences	The Economics Estimates of HAB in the U.S.: Estimates, Assessment Issues and Information Needs
Publisher	*Environmental Science & Technology* (journal)	*Harmful Algae* (journal)	Texas A&M University (agency report)	*Environmental Letters* (journal)	*Estuaries* (journal)
Year published	2008	2010	2001	1974	2002
Temporal scope	Unspecified	April 2008	2000 Red tide event	1971	1987–1992
Spatial scope	U.S. freshwaters	Washington State (Pacific and Grays Harbor counties)	Galveston County, Texas	Southwest Florida	United States

(*continued*)

Table 8.1 (*Continued*)

Authors	Dodds *et al.*	Dyson and Huppert	Evans and Jones	Habas and Gilbert	Hoagland *et al.*
Method	DOI: Calculated reductions multiplied by value	DOI: Input–output analysis (IMPLAN)	DOI: Input–output analysis (IMPLAN)	RP: Calculation of losses to tourism industry and commercial fishermen	DOI, RP: Calculation of losses per event, averaged annually
Source or type of data	Unspecified (secondary)	Survey of recreational clammers (primary)	Survey of agencies; Texas Parks and Wildlife; Texas Department of Health (primary/secondary)	Survey of industry; Florida Department of Revenue; accountant records (primary/secondary)	Survey of experts; literature review (primary/metadata)
Algae or toxin	*Cyanobacteria/Microcystins*	*Pseudo-nitzschia, Alexandrium*	*Karenia brevis*	*Karenia brevis*	Multiple species and toxins

Authors	Hoagland *et al.*	Hoagland *et al.*	Jin and Hoagland	Jin *et al.*	Kahn and Rockel
Title	The Human Health Effects of Florida Red Tides: An Expanded Analysis	The Costs of Respiratory Illnesses Arising from Florida Gulf Coast *Karenia brevis* Blooms	The Value of HAB Predictions to the Nearshore Commercial Shellfish Fishery in the Gulf of Maine	Economic Impact of the 2005 Red Tide Event on Commercial Shellfish Industries in New England	Measuring the Economic Effects of Brown Tides
Publisher	*Environment International* (journal)	*Environmental Health Perspectives* (journal)	*Harmful Algae* (journal)	*Ocean and Coastal Management* (journal)	*Journal of Shellfish Research* (journal)
Year published	2014	2009	2008	2008	1988
Temporal scope	1999–2009	2001–2006	Event specific	1990–2005	Unspecified
Spatial scope	Southwest Florida	Sarasota County, Florida	New England (Maine and Massachusetts)	New England (Maine and Massachusetts)	New York State
Method	DOI: Regression model that expresses hospital/ER admissions as a function of red tide events	DOI: Cost estimation: estimated number of cases multiplied by calculated cost of illness	DOI: Calculated value of using HAB prediction model	DOI: Estimated reduction in landings, imports, and prices with values extrapolated	RP: Regression analysis of bay scallop industry
Source or type of data	Florida Agency for Health Care Administration; STR SHARE tourism data (secondary)	Sarasota Memorial Hospital; Mote Marine Lab; CDC; Florida Agency for Health Care Administration (secondary)	NMFS; Massachusetts Division of Marine Fisheries (secondary)	NMFS; U.S. Census Bureau; New York Fulton Fish Market (secondary)	Unspecified
Algae or toxin	*Karenia brevis*	*Karenia brevis*	*Alexandrium fundyense*/PSP	*Alexandrium fundyense*/PSP	*Aureococcus anophagefferens*

Authors	Kirkpatrick *et al.*	Lankia and Huhtala	Larkin and Adams	Lipton	Lucas
Title	Florida Red Tide Knowledge and Risk Perception: Is There a Need for Tailored Messaging?	Valuation of Trips to Second Homes in the Country: Do Environmental Attributes Matter?	HAB and Coastal Business: Economic Consequences in Florida	*Pfiesteria*'s Economic Impact on Seafood Industry Sales and Recreational Fishing	Willingness-to-Pay for Red Tide Prevention, Control and Mitigation Strategies: A Case Study of Coastal Residents

Publisher	*Harmful Algae* (journal)	EAAE 2011 Congress (conference paper)	*Society and Natural Resources* (journal)	University of Maryland (working paper)	University of Florida (MS thesis)
Year published	2014	2011	2007	1998	2010
Temporal scope	2012	Jan 2009	1995 to 1999	January–December 1997	2009
Spatial scope	Sarasota, Florida	Finland	Northwest Florida	Maryland	Florida
Method	SP: Identify knowledge and perceptions of residents and need for tailored outreach efforts	RP/SP: Estimated decline in trips, reduction in CS/trip	RP: Estimated reduction in reported monthly earnings for tourism businesses	RP: Calculation of lost sales and recreational trips (multiplied by $/trip from Strand)	SP: Estimated preference for proposed strategy, WTP
Source or type of data	Survey of residents; other papers (primary/metadata)	Survey of owners of second homes (primary)	Florida Department of Revenue; National Climatic Data Center; Florida Marine Research Institute (secondary)	Survey of seafood industry members; NMFS/MRFSS (primary/secondary)	Survey of coastal residents (primary)
Algae or toxin	*Karenia brevis*	Unspecified	*Karenia brevis*	*Pfiesteria* spp.	*Karenia brevis*

Authors	**Morgan *et al.***	**Morgan *et al.***	**Morgan *et al.***	**Morgan *et al.***	**Nierenberg *et al.***
Title	Empirical Analysis of Media versus Environmental Impact of Park Attendance	Red Tides and Participation in Marine-Based Activities: Estimating the Response of Southwest Florida Residents	Firm-Level Economic Effects of HABS: A Tool for Business Loss Assessment	Public Costs of Florida Red Tides: A Survey of Coastal Managers	Changes in Work Habits of Lifeguards in Relation to Florida Red Tide
Publisher	*Tourism Management* (journal)	*Harmful Algae* (journal)	*Harmful Algae* (journal)	University of Florida (university extension)	*Harmful Algae* (journal)
Year published	2011	2010	2009	2008	2010
Temporal scope	2002–2004	January 2008–December 2008	1998–2005	2004–2007	2004, 2005
Spatial scope	Florida Gulf Coast	Sarasota and Manatee Counties, Florida	Southwest Florida	Florida Gulf coast	Sarasota County, Florida
Method	RP: Estimated the environmental impact of park attendance	RP: Estimated probability of behavioral change by activity	DOI/RP: Estimated reduction in daily restaurant revenues multiplied by affected days	RP: Calculated costs to cities and counties associated with HAB	RP: Test of reduced attendance multiplied by salary
Source or type of data	National Oceanic and Atmospheric Administration (NOAA); other papers (secondary and metadata)	Survey of county residents (primary)	Proprietary business data; National Climatic Data Center (primary)	Survey of coastal managers (primary)	Survey of lifeguards (primary)

(*continued*)

Table 8.1 (*Continued*)

Authors	Morgan *et al.*	Morgan *et al.*	Morgan *et al.*	Morgan *et al.*	Nierenberg *et al.*
Algae or toxin	*Karenia brevis*	*Karenia brevis*	*Karenia brevis*	*Karenia brevis*	*Karenia brevis*

Authors	Nunes and van den Bergh	Oh and Ditton	Parsons *et al.*	Ralston *et al.*	Rodriguez *et al.*
Title	Can People Value Protection against Invasive Marine Species? Evidence from a Joint TC-CV Survey in the Netherlands	A Time Series Approach to Estimating the Economic Impacts of Exogenous Events on Recreational Fishing	The Welfare Effects of *Pfiesteria*-Related Fish Kills in Seafood Markets: A Contingent Behavior Analysis	An Estimate of the Cost of Acute Health Effects from Food- and Water-Borne Marine Pathogens and Toxins in the U.S.	Are Red Tides Affecting Economically the Commercialization of the Galician (NW Spain) Mussel Farming?
Publisher	*Environmental and Resource Economics* (journal)	*Human Dimensions of Wildlife* (journal)	*Agricultural and Resource Economic Review* (journal)	*Journal of Water and Health* (journal)	*Marine Policy* (journal)
Year published	2004	2008	2006	2011	2011
Temporal scope	2001	2001–2003	2001	Annual	1990–2008
Spatial scope	Holland	Possum Kingdom Lake, Texas	Mid-Atlantic region, United States	U.S.	NW Spain
Method	RP/SP: Estimation of recreation demand and travel cost, comparison of WTP	DOI: Estimated reduction in visitors and IMPLAN	SP: Estimated demand functions and CS	DOI/RP: Estimated annual cost from lit review	RP: Correlation between industry metrics and HAB incidence
Source or type of data	Survey of beach visitors (primary)	Texas Comptroller of Public Accounts; Possum Kingdom Lake State Park (secondary)	Survey of seafood consumers (primary)	Other papers (secondary and metadata)	Official agency data (secondary)
Algae or toxin	Unspecified	*Prymnesium parvum*	*Pfiesteria* spp.	Multiple species, toxins	*Alexandrium* spp./PSP

Authors	Rongo and Woesik	Taylor and Longo	Todd	van Beukering and Cesar
Title	Socioeconomic Consequences of Ciguatera Poisoning in Rarotonga, Southern Cook Islands	Valuing Algal Blooms in the Black Sea Coast of Bulgaria: A Choice Experiments Approach	Estimated Costs of Paralytic Shellfish, Diarrhetic Shellfish and Ciguatera Poisoning in Canada	Ecological Economic Modeling of Coral Reefs: Evaluating Tourist Overuse at Hanauma Bay and Algae Blooms at the Kihei Coast, Hawaii
Publisher	*Harmful Algae* (journal)	*Environmental Management* (journal)	Book chapter	*Pacific Science* (journal)

Year published	2012	2010	1995	2004
Temporal scope	1989–2011	1983–2002	Unspecified	2001
Spatial scope	Cook Islands	Black Sea coast, Bulgaria	Canada	Hawaii
Method	SP: Extrapolation of health cost and consumption estimates	SP: Conjoint choice experiment and conditional logit model	DOI/RP: Estimates of # illness multiplied by cost of illness (society and individual, except pain and suffering)	RP: Calculated reductions in business using available dynamic econ-ecological simulation model and available economic values
Source or type of data	Survey of residents (primary and secondary)	Unspecified (primary)	Unspecified (secondary)	Previous studies including survey-based non-market valuation (metadata)
Algae or toxin	CFP	Various species, such as *Skeletonema*, *Cerataulina*, *Prorocentrum*, and *Gymnodinium*	Shellfish poisoning (paralytic, diarrhetic, and ciguatera)	Not specified

Authors	**Wessells *et al.***	**Whitehead *et al.***
Title	Toxic Algae Contamination and Demand for Shellfish: A Case Study of Demand for Mussels in Montreal	The Economic Effects of *Pfiesteria*
Publisher	*Marine Resource Economics* (journal)	*Ocean and Coastal Management* (journal)
Year published	1995	2003
Temporal scope	May 1987–March 1991	2001
Spatial scope	Montreal, Canada; Maine	Delaware, Maryland, North Carolina, Virginia
Method	DOI/RP: Estimation of shellfish demand and sales losses due to information	SP: Estimation of risk perceptions, seafood demand, and WTP for a safety program from CVM study
Source or type of data	Proprietary (weekly sales from one farm and one wholesaler); news articles from the *Montreal Gazette*; Agriculture Canada, Statistics Canada, and IMF for prices and income (primary/secondary)	Survey of seafood consumers (primary)
Algae or toxin	Domoic acid	*Pfiesteria*

Source: Adams and Larkin (2013). CDC, U.S. Centers for Disease Control and Prevention; CVM, contingent valuation method; DOI, direct output impacts; ER, emergency room; HAB, harmful algal bloom; MRFSS, Marine Recreational Fishing Statistical Survey; NMFS, National Marine Fisheries Service; RP, revealed preference; SP, stated preference; WTP, willingness-to-pay.

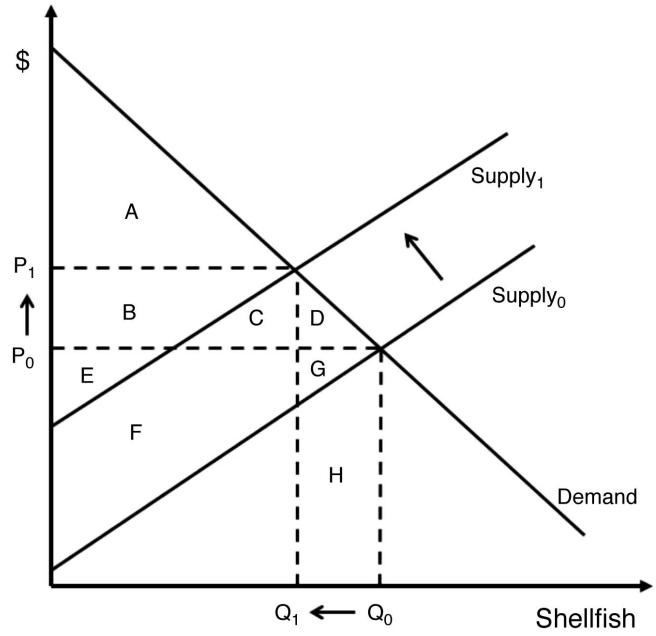

Figure 8.1 Economic welfare effects (value changes) from a hypothetical HAB leading to the closure of shellfish beds. The supply curve shifts in, causing output to the market from shellfish diggers and growers to decline from Q_0 to Q_1 (horizontal axis). Price rises from P_0 to P_1. Direct output impacts (DOIs) typically are calculated as the product of the reduction in output ($Q_0 - Q_1$) and P_0: the sum of areas G + H, where G is a producer surplus and H represents costs. True economic losses comprise changes in consumer and producer surpluses: the sum of areas C + D + F + G. The scale of DOI or value changes will depend upon the amount of shellfish output decline and the slopes of supply and demand in the shellfish market. There might also be changes in welfare associated with a contraction in demand (a *halo effect*), if consumers switch away from shellfish to other seafoods or other foods.

the relevant market, the relationship between the two measures of economic effects can be indeterminate (Figure 8.1). The reason for this is that DOIs comprise both producer surpluses (loosely, business "profits") and the costs of supply. When shellfisheries are closed, for example, diggers or growers relinquish producer surpluses – a true economic loss – but do not incur the costs of digging or growing, and therefore there are no losses associated with the latter. The use of IO multipliers would further compound these overstated losses in upstream supplier markets. Conversely, DOIs could understate economic losses in that they do not account for lost surpluses to consumers. Without an explicit model of the relevant market, it is not feasible to characterize the degree of over- or understating of economic value changes.

A further issue concerns the flexibility that individuals and firms have to avoid the adverse effects of marine HAB. For example, tourists may frequent other restaurants or hotels, fishermen may switch to other fisheries or other sources of employment, and beachgoers might choose to go to another beach (Lucas, 2010). In general, it is sensible to assume that there is an economic loss to producers or consumers when they switch from their preferred activities to alternatives, but the scale of such losses depends upon the availability of alternatives and the ease of switching. If the costs of switching are low, other sectors of the economy could benefit, thereby mitigating the economic impacts of the sectors that are directly affected by a bloom. There are few studies that measure such countervailing impacts. One example concerned the pecuniary effects of price increases in the New York Fulton Fish Market during a large-scale *Alexandrium* bloom restricting softshell clam *Mya arenaria* production in, and supply from, the Gulf of Maine in 2005 (Jin *et al.*, 2008). In the beginning of the bloom, consumers could still find softshell clams, albeit at higher prices. Prices declined as the bloom wore on, suggesting that consumers switched to other seafood or that supply to the market expanded from producers from regions that were unaffected by the bloom.

Notwithstanding this issue, the use of a regional economic impact framework to estimate DOIs and multipliers can give decision makers an

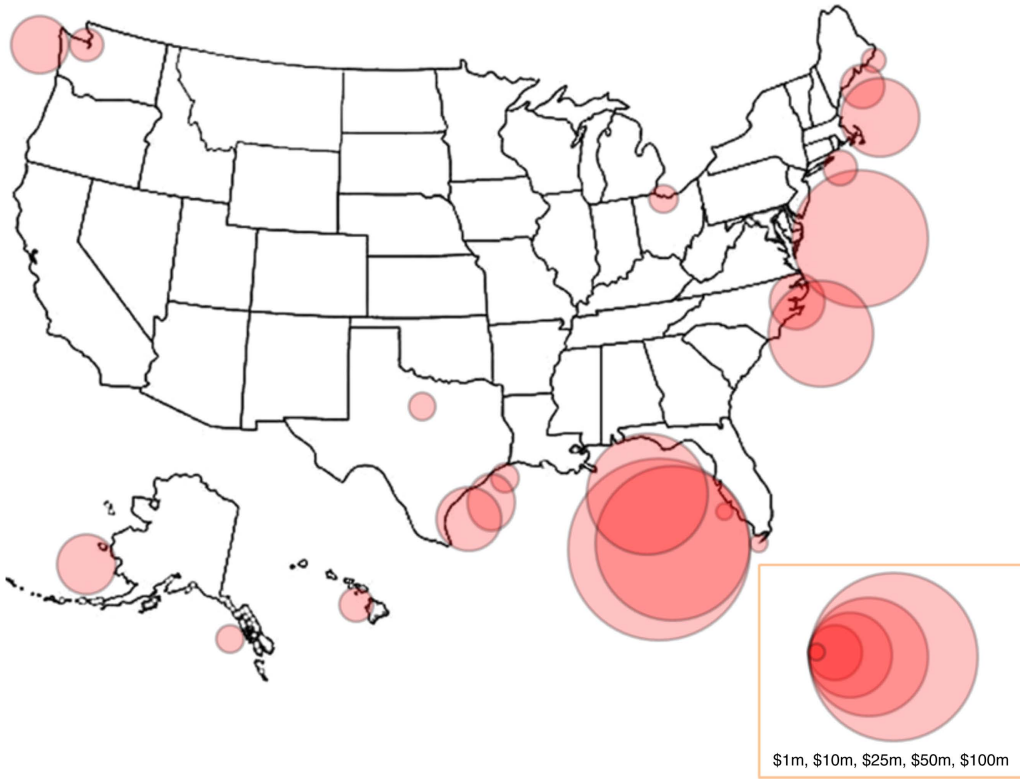

Figure 8.2 Some selected historical examples of HAB in the United States for which economic impacts (2015 dollars, in millions) have been estimated, showing the large range in scales of potential economic impacts. The circles comprise estimates of economic (not spatial) scales at different points in time, beginning in the 1970s; they have been constructed so that their areas are proportional to estimated economic impacts. The maps of Alaska and Hawaii are not drawn to scale. (Refer to the list in Table 8.2 for more detail on the scales and natures of impacts and the sources of the estimates.)

understanding of changes in the distribution of economic activity throughout an economy. For example, Dyson and Huppert (2011) employed an IO model to estimate changes in local income and employment from HAB closures of recreational Pacific razor clam *Siliqua patula* harvests at six beaches in two Washington State coastal counties, finding that a yearlong closure could lead to reduced local incomes of nearly $11 million with a loss of about 340 local jobs.

Measuring losses to *consumers* with either direct or surrogate market data is referred to as a *revealed preference* approach. Revealed preferences rely upon economic values that may be obtained, or revealed, through market data, such as through observations of consumer expenditures. These consumers could be restaurant patrons or recreational boaters, for example. Such market information can be obtained directly from businesses or from surveys that ask recreationists how their behavior changed during a HAB event. As one illustration, using a time-series approach, Larkin and Adams (2007) found that average monthly lodging and restaurant revenues declined by approximately one-third (losses of $3–4 million per month) during months in which Florida red tides (blooms of *Karenia brevis*) occurred in two northwest Florida coastal communities. Likewise, Morgan *et al.* (2009) found that daily sales declined at beachfront restaurants during a Florida red tide. The advantage of using revealed preference data is that such data reflect actual choices that users have made in response to the event.

Unfortunately, for some purposes, such as estimating the value of proposed programs designed to mitigate and control for the effects of HAB, revealed preference data may be unavailable. In those cases, "stated" preference approaches must be used. In contrast to revealed preference approaches, *stated preference* approaches ask individuals, through survey methods, to state their willingness-to-pay for certain goods or services. Survey questions might focus on attenuations in willingness-to-pay for visits to beaches where HAB

Table 8.2 Reference for the map in Figure 8.2 of selected examples of HAB in the United States for which economic impacts (especially direct output impacts [DOIs]) have been estimated within the identified year, 2015 (in millions of dollars).

State	Year	Estimated economic impacts ($m)	Type(s) of effect(s)	Species	Toxin	Source
Florida	1971	$117	Tourism industry; commercial fishing	*Karenia*	NSP	Habas and Gibert (1974)
Maine	1972	$5	Shellfish closures	*Alexandrium*	PSP	Jensen (1975)
Florida	1974	$72	Tourism industry; sport fishing; condo sales	*Karenia*	NSP	Habas and Gilbert (1975)
Maine	1980	$7	Shellfish closures	*Alexandrium*	PSP	Hoagland *et al.* (2002)
North Carolina	1987	$40	Hotel and restaurant businesses	*Karenia*	NSP	Tester *et al.* (1988)
Texas	1987	$8	Oyster bed closures	*Karenia*	NSP	Martin (1987)
New York	1988	$4	Bay scallop mortalities	*Aureococcus*	Brown tide	Kahn and Rockel (1988)
North Carolina	1988	$11	Shellfish closures	*Karenia*	NSP	Tester *et al.* (1991)
Washington	1990	$4	Salmon net pen mortalities	*Chaetoceros*	–	Hoagland *et al.* (2002)
Alaska	1992	13	Untapped Bering Sea surf clam fishery	*Alexandrium*	PSP	Anderson *et al.* (2000)
Hawaii	1992	$4	Commercial sales of sportfish	*Ciguatera*	CFP	Hokama (1992)
Florida	1992	$1	CFP morbidities in humans	*Ciguatera*	CFP	Hoagland *et al.* (2002)
Alaska	1996	$3	Closed geoduck commercial beds	*Alexandrium*	PSP	Ralonde (1998)
Maryland	1997	$66	Seafood sales	*Pfiesteria*	–	Lipton (1999)
Texas	2000	$15	Tourism industry; oyster landings	*Karenia*	NSP	Evans and Jones (2001)
Texas	2001	$3	Sport fishing	*Prymnesium*	Golden algae	Oh and Ditton (2005)
Florida	2005	$1	Human respiratory and GI morbidities	*Karenia*	NSP	Hoagland *et al.* (2014)
Maine	2005	$2	Shellfish closures	*Alexandrium*	PSP	Athearn (2008)
Massachusetts	2005	$22	Shellfish closures	*Alexandrium*	PSP	Jin *et al.* (2008)
Florida	2007	$51	Restaurant and hotel sectors	*Karenia*	NSP	Larkin and Adams (2009)
Washington	2009	$12	Recreational shellfish closures	*Pseudo-nitzschia*	ASP	Dyson and Huppert (2010)
Ohio	2014	$3	Drinking water decontamination	*Microcystis*	Microcystin	Bingham *et al.* (2015)

Note: These impacts are exemplary only; blooms do not necessarily recur at the same location and at the same scale, and actions taken to avoid bloom impacts may lead to their mitigation.
ASP, amnesic shellfish poisoning; CFP, ciguatera fish poisoning; GI, gastrointestinal NSP, neurotoxic shellfish poisoning; PSP, paralytic shellfish poisoning.

occur, for example. Stated preference approaches also could ask respondents how they would vote on a referendum to establish a program designed to prevent HAB-related losses. In one such application, Larkin *et al.* (2011) found that Florida coastal residents demonstrated willingness-to-pay – through alternative payment vehicles – for programs to prevent, control, or mitigate Florida red tides, with higher willingness-to-pay for prevention over either control or mitigation. Other examples of non-market goods as they relate to HAB also may include the values that residents are willing to pay for enhanced coastal water quality for marine-related recreation, the existence of marine mammals found in nearshore waters, or unpolluted coastal waters necessary for proper ecosystem functioning.

One advantage of stated preference approaches in general is that such studies are tailored to measure user preferences exactly, as opposed to being constrained by available market data that may not relate to the preference of interest. Stated preference approaches to measure non-market economic values generally are referred to as using *contingent valuation* methods (CVM) because the results are *contingent* upon the hypothetical scenario that respondents are being asked to evaluate. For example, Larkin *et al.* (2011) report on the results of a CVM study that found that almost 50% of respondents (Florida residents) would be willing to pay an increase in property taxes of $9–10 per $100,000 of assessed value on average for a program to fund pilot studies of either biological or chemical means for the mitigation of HAB.

Although stated preference surveys are most useful because they can be tailored to measure exactly what information is needed, this flexibility also is the most common criticism for the approach. In other words, respondents may be asked to value hypothetical programs or changes with which they may have little experience or expertise. For example, several researchers have focused on characterizing the value of reducing the economic consequences of a "halo" effect, which comprises the avoidance by consumers of goods, such as seafoods, for which the likelihood of being tainted by HAB toxins is perceived to be risky or uncertain (Jensen, 1975). Whitehead *et al.* (2003) use CVM to ask mid-Atlantic seafood consumers whether they would vote for a mandatory seafood inspection program that would involve an increase in seafood prices. The authors found that an inspection program would be effective at moderating halo-type welfare losses associated with fish kills caused by *Pfiesteria* spp. In a follow-up study, Parsons *et al.* (2006) affirm this finding but show that, if seafood prices increase due to inspections, then the costs of avoiding seafood are reduced but not fully mitigated by the inspection program. Interestingly, there is significant uncertainty regarding whether *Pfiesteria* leads to human health effects when infected seafood is consumed. Consequently, it is unclear whether a seafood inspection program actually may help seafood consumers avoid risk. Notwithstanding the issue of the lack of respondent expertise, a large and growing body of literature exists to guide the successful use of stated preference approaches in generating valid economic values (Mitchell and Carson, 1989; Freeman, 1993; Bateman and Willis, 1999; Hanemann, 1999; Carson, 2000; Haab and McConnell, 2002; Champ *et al.*, 2003).

Using the broad distinction between market and non-market methods, 28 of the 36 studies reviewed have employed DOIs or revealed preference approaches to estimate economic losses (Table 8.1). The majority of these papers have relied upon data as reported by government agencies or provided by businesses or individuals. Four papers used IO models to determine the economic impacts of HAB on local communities. An additional six papers used stated preferences, most by surveying residents or agency staff. In addition, two papers used both the revealed preference and stated preference approaches by surveying respondents for past behavioral choices (e.g., recreational visits before, during, and/or after a red tide event) before surveying their preferences concerning a hypothetical change in coastal water quality (i.e., preferences for a program that could affect HAB and coastal water quality at residential and recreational sites).

8.4 Sources and Types of Data

The data used to derive empirical results comprise both primary and secondary data. *Primary data* include that collected directly from businesses (e.g., firm-level sales data), coastal managers (e.g., costs incurred for cleanup or monitoring), or individuals (e.g., how previous HAB changed purchasing and recreational choices, or how individuals value proposed programs to reduce HAB losses). *Secondary data* (primarily collected by government agencies for use by others) include quantitative information that has been previously collected, such as a time series of commercial

fishery landings, recreational fishing trips, beach attendances, reported tourism revenues (restaurant and lodging industries), seafood sales, numbers of illnesses, environmental conditions, and HAB-related press releases, among others. The main sources of secondary data are government agencies. An additional source of secondary data is *metadata*, referring to data described and available through previous analyses and publications. Some HAB studies rely heavily on the findings of other analyses.

Of the 36 studies reviewed, 11 studies utilized primary data only, while 14 studies utilized secondary data only. Ten studies required the use of both primary and secondary data to achieve their research objectives. One study did not report the type of data used, and an additional study used metadata, or results (economic losses) reported from several previous studies.

Overall, the data used in these studies were obtained from a variety of sources. The secondary data were obtained mainly from municipal, state, and federal agencies, while the primary data were obtained from surveys administered by the research team. The surveys were administered to a wide range of users, depending on the particular nature of the study objectives.

8.5 Spatial and Temporal Scopes

Spatial scope refers to the geographic location of HAB events and the region of impacts. The spatial scope of studies focusing on economic effects of HAB has ranged from impacts on lakes, bays, or counties to national impacts (see Figure 8.3 for the distribution of the studies by jurisdiction). In between are impacts that encompass multiple counties (e.g., southwest Florida, northwest Florida, or Cape Cod) or multiple states (e.g., the U.S. mid-Atlantic region or New England). The relevant accounting stance depends on either the

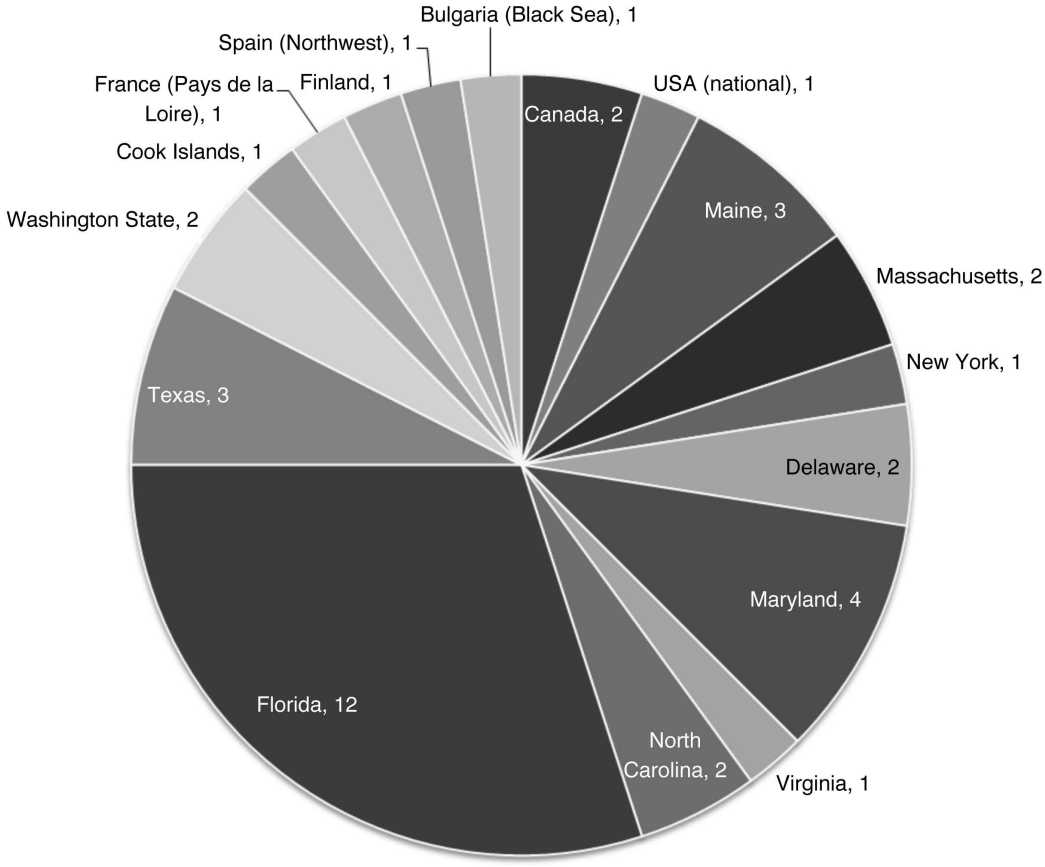

Figure 8.3 Jurisdictional distribution of the studies in Table 8.1.

geographic distribution of the HAB event or the impacted market area, such as local beaches or states where seafood consumers live or visit. In some studies, HAB impacts were assessed at a very fine spatial resolution. These studies often may be constrained by the available data on economic activity (e.g., issues of confidentiality may limit the availability of secondary data, or the spatial data simply do not exist on the level of resolution needed to assess very localized impacts). Thus, data constraints can impose limits on spatial resolution, increasing the difficulty of assessing the impacts of localized HAB events.

Temporal scope refers to the duration of HAB events and the time steps of measurement (i.e., days, weeks, months, and years). The temporal scope of studies focusing on the economic effects of HAB ranges from studies that attempt to quantify the impacts of a single bloom event, which could range from days to several months, to studies that span multiple years. Studies commonly analyze time series such as historic seafood landings or reported tourism revenues for tax purposes to identify changes in business activity or use patterns during HAB events. Assessing the impact of a HAB event at the appropriate level of temporal resolution sometimes may be difficult due to the lack of data. For example, secondary data often are collected periodically by state governments for tax or other reporting purposes. These data may be at a level of temporal resolution that does not match the time period needed to assess relatively short HAB. Or the data may not match the required spatial resolution for very brief, localized HAB events, such as zip-code-level tax data that are difficult to obtain due to issues of confidentiality. Data for shorter time periods or more localized coastal areas may need to be collected outside of existing agency data collection programs. Such primary data collection efforts can be time-consuming and costly for affected residents, businesses, government agencies, and researchers. Finally, the possible proprietary nature of high-resolution data may render access to such data problematic.

The studies reviewed exhibited an extensive range of temporal and spatial characteristics. The temporal and spatial scope of a study is obviously a function of the geographic range, duration, density, and toxicity of the HAB event being studied. The existing literature suggests that the economic effects of several major HAB have been investigated to date, and temporal and spatial data appear to be available to address the larger scale questions. Few economic analyses of smaller scale HAB events have been undertaken. Studies mainly used annual and monthly data, with weekly and daily data being extremely rare in terms of availability. On a spatial basis, of the 36 studies reviewed, six studies addressed HAB on a national scope, 18 on a regional or state level, six on a multi- or single-county level, and six on a local level.

8.6 Nature of the Hazard

The primary focus of this literature review effort was to summarize past economic research, which includes a range of algal species and toxins, and was initially focused on red tide events in southwest Florida. The term *red tide* often is used in a generic sense to refer to HAB, but only rarely do HAB, such as Florida red tide or *K. brevis*, lead to changes in water color due to blooms of localized high cell densities. Typically, analyses of the economic impacts of algal blooms are focused on the physical impacts (toxicity) to affected natural resources and humans.

There are six primary types of marine HAB toxins found in North America that may be taken up by shellfish or lead to morbidities or mortalities in finfish, marine mammals, sea turtles, seabirds, and humans. These toxins and their associated effects include:

1) Brevetoxins (neurotoxic shellfish poisoning [NSP])
2) Saxitoxins and their derivatives (paralytic shellfish poisoning [PSP])
3) Domoic acid (amnesic shellfish poisoning [ASP])
4) Okadaic acid (diarrheic shellfish poisoning [DSP])
5) Ciguatoxins (ciguatera fish poisoning [CFP])
6) Azaspiracids (azaspiracid poisoning [AZP]).

Red tides in the Gulf of Mexico have been dominated by *K. brevis*, which produces brevetoxins during a bloom. The economic effects of these HAB result from potential decreases in marine recreational activities, beachgoing, and human seafood consumption (red tides may also negatively affect those who passively value a healthy ecosystem and the services that resource provides). The red tide brevetoxins also can become airborne, affecting the respiratory system of humans residing and working in inland areas close to the bloom (Hoagland *et al.*, 2009, 2014). Of the 36 papers that were reviewed, 13 reported economic effects associated with blooms of *K. brevis*.

With respect to shellfish consumption, toxic algae typically are undetectable by sensory analysis, such that the potential exists for poisoning and illness due to ingestion. Some toxins are also heat resistant, which means the toxin (and its effect) cannot be destroyed by cooking. The toxins associated with PSP, ASP, DSP, and other illnesses are the subject of 12 papers; in these cases, the toxins are associated mainly with cold-water species, and the primary human health impacts are linked through impacts on recreational or commercial harvests for consumption.

CFP is produced by various species of algae from the *Gambierdiscus* genus, which occur naturally in coral reef ecosystems and which are not necessarily associated with high cell density blooms. The ciguatera toxin is bio-accumulated via the food chain associated with coral reef species assemblages, and it is encountered by humans when top trophic level fish containing the accumulated toxins find their way into seafood markets. Of the papers reviewed, two addressed the economic consequences of CFP. An additional nine papers did not clearly specify or focus on a specific toxin or algae species.

8.7 Current Research Gaps

While much research on the economic consequences of HAB has been conducted, this discussion identifies 15 gaps that provide the potential for additional and critically needed work. The issues identified may also provide guidance regarding data that may need to be gathered before, during, and after future bloom events, particularly if a more complete assessment of the economic consequences of HAB events is of primary interest.

1) Time-series analyses routinely utilize historic data on fishery landings, reported earnings, and recreational trips obtained from business or government sources that encompass red tide events. Few studies have utilized both time-series and cross-sectional data; one example is the cost-of-illness study relating to blooms of the red tide *K. brevis* off the southwest Florida coast (Hoagland *et al.*, 2014). The use of the NOAA Marine Recreational Information Program (MRIP), a national data collection program based on coastal intercept surveys of recreational fisheries, is one promising source of panel data for future studies of HAB economic effects.
2) Linking HAB impacts with community demographic data would allow for an assessment of the distribution of HAB impacts across segments of the population. Regional economic frameworks, such as IO models, are particularly well-suited to describing the extent to which changes in economic activity affect demographic layers.
3) HAB events and their corresponding impacts are known to be affected by weather conditions, and yet the incorporation of weather data has been sparse (e.g., Morgan *et al.*, 2011). While the challenges of using these data have been documented, the richness of weather data should ensure that future studies consider testing for use in identifying threshold cell counts above which humans are likely to be affected (to test, refine, and possibly extend the level estimated by Morgan *et al.*, 2011), and to provide further insight to help efforts to advise the public and mitigate losses.
4) Data useful for assessing the impact of HAB on water-related businesses have been mainly secondary. Secondary data may limit temporal and spatial characterizations of HAB impacts. Developing data collection programs in real time would allow for a more complete assessment of HAB events on businesses and communities at higher levels of temporal and spatial resolution. Real-time data may require ongoing data collection, and implementing primary data collection may require the participation of local businesses that are vulnerable to the effects of HAB.
5) HAB events may exhibit a lagged impact on local business communities, particularly those in the tourism sector. This potential dynamic element of HAB event impacts has not been fully explored.
6) HAB events are characterized by a wide range of intensity and duration levels. Few studies have investigated the role of intensity, and none appear to address the potential for a nonlinear relationship between economic losses and duration. Establishing a relationship between HAB intensity and duration and economic impacts would provide coastal planners with additional information useful in making decisions regarding prevention, mitigation, and control options for HAB.
7) HAB may impact coastal property values and regional planning efforts. How have local property markets and planning efforts been compromised and impacted by HAB events across communities and over time? While

some very preliminary research has been conducted in isolated areas (cf. Bingham *et al.*, 2015), there is room for additional work given that property market data are readily available.

8) HAB may generate significant indirect impacts, leading to declines in finfish and shellfish stocks. For example, recent impacts of red tides on the reef fish resource in the eastern Gulf of Mexico led to reductions in official grouper biomass estimates. Future research is needed on how such impacts could influence fishery yields and management.
9) The HAB "research" literature comprises studies exhibiting a range of scientific rigor. Some studies estimate statistical relationships, while others execute simplistic calculations using secondary data or metadata. Although the latter may be appropriate in some situations, future research proposals should be more explicit in terms of exactly what is being measured and how, so that resource managers and stakeholders can better assess the credibility of study results.
10) The results of economic studies are reported inconsistently. Absolute losses are important but difficult to assess in terms of magnitude without baselines or assessments of relative changes. To facilitate comparisons across studies, it would be useful for results to be reported as percentage changes.
11) Time horizons and geographic areas often are defined only vaguely. Studies should identify both spatial boundaries and time horizons. Furthermore, the extent to which data are able to capture the independent effects of a HAB event should be made clear.
12) Potential researchers should explore alternative ways to combine revealed and stated preference approaches following recent advances in the non-market valuation literature.
13) Stated preference studies would be strengthened with surveys of representative populations to lessen the biases typically associated with such surveys.
14) Few studies have utilized existing models to take full advantage of past and related modeling efforts. Ecosystem-type models in particular (e.g., ECOSIM and Atlantis) could be used to assess the relative importance of HAB events compared to other environmental and anthropogenic stressors.
15) Most of the published research on the economics of HAB focuses on the costs to coastal and ocean users of reductions in their activities. More research is needed on identifying feasible policies to lessen these costs, such as policies to prevent, control, or mitigate HAB; to insure against HAB risks; or to increase the set of alternative opportunities for users. Policy responses can themselves be costly, and it is the combined costs of the hazard and the response that are relevant in terms of selecting appropriate courses of action. Estimates of policy alternatives, their effectiveness in terms of reducing the hazard, and their costs are clear priorities.

8.8 Conclusion

This chapter focuses on surveying the literature describing or analyzing the economic consequences of HAB events. Although we focus mainly on peer-reviewed examples of HAB economic analysis, an extensive "gray" literature exists that provides further insight into the array of HAB economic impacts. Within the existing literature, we address briefly some key issues associated with the research methodologies employed, the sources and forms of data utilized, the spatial and temporal scopes of analyses, the types of HAB and associated toxins, and the current research gaps. Each of the 36 studies has been annotated in Table 8.1.This review leads to suggestions for future research concerning the economic consequences of HAB. With continued increases in the nation's coastal populations and ongoing expansion of development along its coastal corridors, the likelihood of negative economic effects resulting from HAB events grows. More carefully designed research efforts, coupled with improved data, would help guide coastal decision makers and resource managers as they seek to understand better the causes, dynamics, and consequences of HAB events, regardless of the species and locations involved.

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9

Public Health and Epidemiology

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9.1 Introduction

The management of harmful algal blooms (HAB) is a challenging task in a changing, and often unpredictable, coastal marine or freshwater environment. Faced with the responsibilities of environmental stewardship as well as the protection of human health, HAB managers must often engage in collaborative problem solving with public health officials, epidemiological scientists, surveillance teams, and clinicians. As many HAB managers would agree, this problem solving (and eventual policy making) does not occur in a vacuum. Typically, it takes place within the context of the unique history, culture, social resources, political capital, and economic structure of the impacted coastal community. The purpose of this chapter is to provide some guidance for HAB managers to facilitate this process from a public health perspective. Toward this end, this chapter is organized around four sections: (1) public health and epidemiology, (2) HAB-related clinical illnesses or syndromes, (3) exemplars for HAB management from the Quinault Indian Nation and the Florida Department of Health, and (4) individual and community resilience in vulnerable coastal regions. The ultimate goal of this chapter is to optimize engagement between HAB managers, public health professionals, and coastal communities responding to HAB problems.

9.2 What Is Public Health and Epidemiology?

Public health refers to the science and practice of preventing disease and improving the health of people, families, and communities. Given the broad range of potential health threats to a community or nation, public health is a multidisciplinary field. Accordingly, a diversity of health specialists and researchers typically work together toward identifying health threats, developing standard case definitions, searching for the causal agents, and devising interventions or solutions to the problem (Merrill, 2012; Aschengrau and Seage, 2014). The interventions typically include developing prevention, early intervention, education, and outreach programs for the affected community and their local healthcare providers. These target populations may represent a small coastal community, a state, or a larger region of the country or world. The leading public health agency in the United States is the Centers for Disease Control and Prevention (CDC). Working in collaboration with state and local health departments, academic institutions, community-based organizations, American Indian/Alaska Native communities, and worldwide partners, the CDC operates toward preventing and responding to a wide range of health threats. These include threats to fish and seafood safety as well as water-related illnesses or

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diseases as a result of exposure to marine toxins or other contaminants.

Public health *surveillance* refers to the ongoing systematic collection, analysis, and interpretation of illness or disease data that will facilitate control (Aschengrau and Seage, 2014). Surveillance systems gather data on the demographic characteristics of the individuals, exposure history, and date of diagnosis. Based upon carefully constructed case definitions, surveillance systems can serve as a potential warning of an emerging health problem. Surveillance data may also be used to inform public health policy, or document the efficacy of a policy or intervention. With HAB-related illnesses, oversight is typically provided by the environmental health division of state health departments, often in collaboration with the CDC. In an effort to coordinate the reporting systems of states and territories with federal partners, the CDC recently launched a national electronic reporting system for HAB-related illnesses, see the One Health Harmful Algal Bloom System (OHHABS) (www.cdc.gov/habs/ohhabs; CDC, 2016), for further information.

Unfortunately, with respect to HAB-related illnesses, there are many barriers to accurate surveillance. The symptom complex of some HAB-related illnesses and diseases is diverse and difficult to define, confirmatory biological markers or objective laboratory data are rarely available, and there is always the potential for new HAB-related illnesses or syndromes to emerge. A lack of clear case definitions, missed diagnoses, and underreporting also contribute to surveillance difficulties (Begier *et al*., 2006; Reich *et al*., 2015). For an example, the reader is referred to Klekamp and colleagues (2015) for an overview of both the complexity and efficacy of using surveillance systems in detecting an outbreak of ciguatera fish poisoning in Orange County, Florida (Radke *et al*., 2014). Many of the challenges to maintaining accurate and timely surveillance would be minimized with knowledge gained by epidemiological research of HAB-related illnesses and syndromes.

Epidemiology refers to the study of the occurrence, distribution, and determinants of disease in human populations and the application of this knowledge to prevent and control health problems (Merrill, 2012). It is both a subfield and foundation of public health. In epidemiological studies, the primary unit of interest is disease patterns in groups of people, not separate individuals. This includes investigations of the predisposing, exposure, and environmental factors that contribute to increased risk of illness onset or persistence. Epidemiological research involves rigorous sampling and data collection procedures, applies advanced statistical techniques, and incorporates biological principles and causal theory to increase understanding of the nature, extent, and cause of specific public health problems. The results of these investigations are ultimately used to drive interventions to improve the health of the community. With respect to HAB, the goal of epidemiological research is to define the range of symptoms associated with a specific HAB-related illness, then identify the source and exposure vector, the number of people exposed, the characteristics of people at greatest risk, and the potential threat to people outside of the target community. With this information, epidemiologists, public health officials, HAB managers, and community leaders may participate in the prevention, identification, early intervention, or community outreach and education related to the specific HAB problem. Accordingly, if the HAB manager is the first to become aware of a potential exposure risk, contact should be initiated with public health officials at the local/state health departments. Figure 9.1 represents a general flowchart for this activity, incorporating public health, academic, and community partners. Table 9.1 provides more specific guidance for the HAB manager, particularly with respect to illness prevention. It is noteworthy that each state/territory and/or Indian Nation may have different (or additional) reporting procedures based upon their specific HAB concerns and reportable disease/or condition (e.g., NSP, PSP, and ciguatera in Florida; see http://www.floridahealth.gov/diseases-and-conditions/disease-reporting-and-management/) requirements by health care professionals. In summary, the goal of public health activities such as surveillance, clinical and epidemiological research, policy development, outreach, and education is to provide the foundation for minimizing the risk of HAB-related illnesses.

9.3 HAB and Human Illness

There are eight recognized HAB-related illnesses. Most of them are due to consuming shellfish tainted by well-recognized algal toxins and include diarrhetic shellfish poisoning, amnesic shellfish poisoning, paralytic shellfish poisoning, neurotoxic shellfish poisoning, and azaspiracid shellfish poisoning. There is also a risk of ciguatera fish poisoning as the result of dietary exposure to toxic

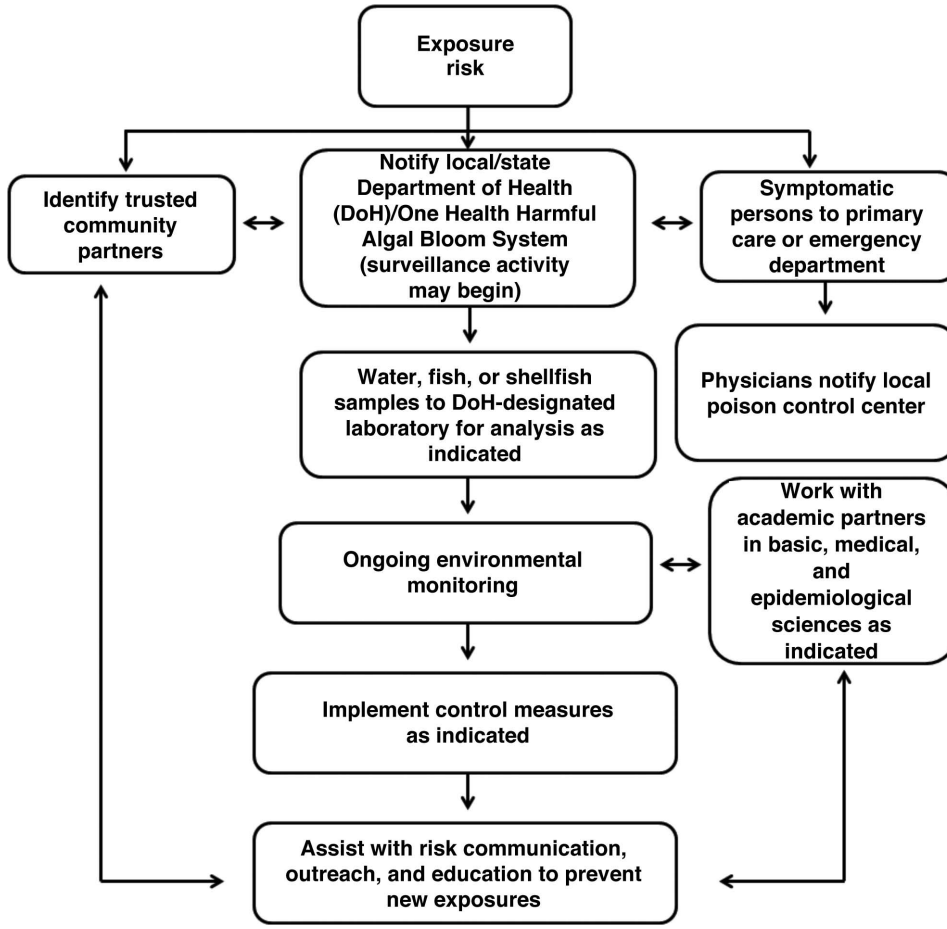

Figure 9.1 Flow chart of public health, surveillance, and epidemiological activity toward public education for HAB managers.

coral reef fish or finfish. None of these toxins are detectable in fish or shellfish by visual inspection, taste, or smell. These marine toxins are also heat-stable and unaffected by cooking, freezing, drying, or smoking. Finally, definitive diagnostic tests are not yet available to detect the presence of the toxins in people, and there are no known antidotes. Hence, the diagnosis and treatment of these seafood poisonings can be challenging. Exposure to toxic marine aerosols has been found to trigger a brevetoxin inhalation syndrome as well as cyanobacterial-related illnesses. As part as the latter complex of exposures, cyanotoxins may also trigger health problems as a result of exposure to contaminated water via dermal contact, drinking, and in rare events by consuming crops irrigated with contaminated water, and inadvertent use of contaminated water for kidney dialysis.

Since most HAB-related risks are regional in nature, HAB managers are typically well-informed about their local risks; however, vigilance needs to be maintained for the emergence of new harmful algal toxins and the biogeographic expansion of known HAB and potential HAB-related illnesses. The following is a review of the exposure risks, symptoms, and general clinical management of the known HAB-related human health syndromes (see Table 9.2 for a summary). Since illness prevention is such an important component of the HAB manager's activities, discussion of these important activities will be examined.

9.3.1 Paralytic Shellfish Poisoning (PSP)

9.3.1.1 Exposure

PSP is a potentially lethal clinical syndrome caused by consuming bivalve molluscs (mussels, scallops,

Table 9.1 Steps in response system for HAB managers.

Responsibility	Description	Responsible organization(s)
Coordination	Coordinate and oversee all public health response activities when HAB response activated; designate responsible organization for each primary responsibility; update key partners on progress; work closely with statewide DOH HAB Coordinator to activate and deactivate response effort.	State or county public health authorities (depends on location, capabilities, and number of counties affected); this role is often assumed by local health department HAB contact person or by statewide HAB Coordinator if requested by county health department.
Environmental investigation	Water sampling; animal carcass (in rare instances such as charismatic megafauna deaths, large-scale bird die-offs) testing according to recommended protocol; utilize information based on regular environmental monitoring as a complement to heightened activities. Carry out these activities in coordination with epidemiology outbreak investigation, if applicable.	County public health authorities in coordination with fish and wildlife entity, environmental epidemiologists, environmental regulatory agency, water management entity, laboratories, Universities, and private entities such as non-government organizations.
Outbreak investigation	Conduct activities in accordance with protocol for food and waterborne outbreak investigations; utilize information gathered from regular surveillance activities as a complement to heightened activities; epidemiological analysis.	County public health authorities in coordination with environmental epidemiologists, state food regulatory authority, laboratories (if multi-state then possible involvement by U.S. Centers for Disease Control and Protection (CDC)).
Interagency communication/ notification	Responsible for activation and deactivation notification to key partners (call-down list/email notification); maintain and update statewide HAB contact list.	Statewide HAB Coordinator
Risk communication	Carry out activities related to communicating status/risk to general public (i.e., press releases, signage).	County public health authorities together with other local entities; collaborations with other state entities to ensure consistent messaging.
Corrective action	Implement activities in support of decision to take action to remediate, contain and/or limit use of water body. It may include defining boundaries to establish areas that contain water that may be unsafe for use, limiting use of water body, etc.	County public health authorities together with other state, local and regional entities; collaborations with other state entities to ensure consistent messaging.

and clams) contaminated with a group of structurally related marine toxins collectively referred to as saxitoxins or STX (James *et al*., 2010; Shumway, 1990). PSP toxins are concentrated in the shellfish due to the filtration of toxic algae produced by several dinoflagellates (including *Alexandrium*, *Gymnodinium*, and *Pyrodinium*) during "red tide" blooms. Since predators of bivalve shellfish (scavenging shellfish, lobsters, crabs, and fish) may also be vectors for saxitoxins, the potential for human exposure is extended (Halstead and Schantz, 1984; Shumway, 1995; Deeds *et al*., 2008). Saxitoxins exert their effect by binding directly on the voltage-dependent sodium channels in nerve and muscle cell membranes, thus interrupting nerve signal transmission and initiating paralysis (Daranas *et al*., 2001; James *et al*., 2010). Geographically, the riskiest regions for PSP are cold water marine coasts. In North America, PSP concerns are greatest in the northeast, including the Gulf of Maine and New England as well as the St. Lawrence region of Canada. Some species of puffer fish have been found to accumulate saxitoxin in the Indian River Lagoon of Florida (Landsberg, *et al*., https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1626430/). PSP toxins and health risks have also been found in the Pacific Northwest, Alaska, and other regions. More recently, the biogeographical boundaries of PSP toxins have expanded with toxic shellfish cases reported in Mexico (Hernandez-Becerril *et al*., 2007) and Nicaragua (Callejas *et al*., 2015). Toxic shellfish have also been found in cold water regions of southern Chile, England, Japan, and the North Sea.

The first and gold standard for PSP monitoring programs in the United States has been attributed to John Hurst and the State of Maine (Shumway *et al*., 1988). As a result of these effective testing and control procedures, outbreaks of PSP are rare

Table 9.2 HAB-related intoxications in humans.

Syndrome (major toxin)	Vectors (known and potential)	Onset time and duration	Major symptoms	Treatment
Paralytic shellfish poisoning[a] (saxitoxins)	Scallops, mussels, clams, geoducks, cockles, puffer fish, some fish, gastropods, crustaceans[b]	30 minutes to 3 hours; a few hours to a few days	**p** (perioral, often spreading to neck and extremities), **n**, **v**, **r** (severe doses: respiratory paralysis and death). Muscular weakness, drowsiness, incoherent speech. No mortalities in recent U.S. and European outbreaks.	Supportive. Artificial ventilation in severe cases.
Amnesic shellfish poisoning[c] (domoic acid)	Razor clams, mussels, oysters, squid; viscera (not muscle) of scallops, sardines, anchovies, crab, and lobster[d]	Within 48 hours; months to years with permanent amnesia.	**ab**, **n**, **v**, **r**, disorientation, seizures, permanent short-term memory loss, possible neurodevelopmental delay. Excessive respiratory secretions.[e] Coma and death only among most severe cases or elderly.[d]	Supportive.
Neurotoxic shellfish poisoning[f] (brevetoxins)	Mussels, clams, whelks, conch, coquinas, oysters, scallops; liver and stomach contents of some planktivarous fish; inhalation of toxin aerosolized by coastal wind and waves[f,g]	*Consumption*: A few minutes up to 18 hours (often within 3–4 hours) *Inhalation*: Minutes to hours (<24 hours)	*Consumption*: **p** (perioral, face, extremities), **ab**, **t**, **d**, **b**, **r** (most severe cases). May appear disorientated or intoxicated (slurred speech, pupil dilation, overall fatigue, involuntary muscle spasms). *Inhalation*: **a**, **b**, **r**. Throat irritation, sneezing, coughing, itchy and watery eyes, burning of upper respiratory tract. No reported mortality for either pathways	*Consumption*: Supportive. *Inhalation*: Leave the beach and go to an air-conditioned area.
Diarrhetic shellfish poisoning[h] (okadaic acid)	Mussels, oysters, scallops, clams, cockles, some species of crab[i,j,k]	30 minutes to 15 hours; full recovery, within 3 days[i]	**d** (incapacitating), **n**, **v**, **ab**. Headache, fever. No reported mortality.	Supportive. Most people do not seek medical treatment.
Ciguatera fish poisoning[l] (ciguatoxin)	Large, predatory tropical reef fish (barracuda, grouper, red snapper, amberjack); some types of eels; farm-raised fish that feed on contaminated fish[m]	12 to 24 hours; neurological symptoms can last months to years	**n**, **v**, **d**, **ab**, **p** (especially hands and feet), **t**, **bp**. Also: metallic taste, itching, dizziness. Possible recurrence of neurological symptoms during times of stress, after ingesting alcohol or low-level fish. Low mortality in the United States.[n]	Supportive. Mannitol therapy is recommended for neurological symptoms.[o] Brevenal[p] and pregabalin[q] have also been indicated, although more research on their effectiveness is needed.
Azaspiracid shellfish poisoning (*Protoperidinium crasspie*)	Crabs, scallops, mussels[r,s]	Full recovery within 2–3 days	**n**, **v**, **ab**, **d** (severe).	Supportive.
				Supportive.

(*continued*)

Table 9.2 (*Continued*)

Syndrome (major toxin)	Vectors (known and potential)	Onset time and duration	Major symptoms	Treatment
Cyanobacterial HAB[t] (various cyanotoxins)	Ingestion of, inhalation of, or water during recreational or occupational contact; ingestion of contaminated drinking water; consumption of fish, shellfish, and possibly crops irrigated with contaminated water[u]	Within minutes to hours or days; days	*Ingestion*: Varies; **a**, **ab**, **b**, **d**, **n**, **v**, myalgia, headache, fever, blistering in the mouth. Chronic, low-dose via drinking water may be linked to liver disease, although more research is needed.[v] *Contact*: Allergy-like reactions, including skin irritation or rash, eye irritation, hay fever–like symptoms *Inhalation*: Cough, wheezing, and other respiratory symptoms	

a, allergic-like; **ab**, abdominal cramps; **b**, bronchoconstriction; **bp**, decrease in blood pressure; **d**, diarrhea; **n**, nausea; **p**, parathesias;.
r, respiratory distress; **t**, reversal of temperature sensation; **v**, vomiting.

[a] Etheridge (2009), Cusick and Sayler (2013), and Hurley *et al.* (2014).
[b] Deeds *et al.* (2010).
[c] Grant *et al.* (2010) and Pérez-Gómez and Tasker (2014).
[d] Lefebvre and Robertson (2010).
[e] Teitelbaum *et al.* (1990a, 1990b) and Perl *et al.* (1990a, 1990b).
[f] For examples, see [h,k] and Hinder *et al.* (2011).
[g] Hoagland *et al.* (2014).
[h] Hossen *et al.* (2011), Taylor *et al.* (2013), and Valdiglesias *et al.* (2013).
[i] James *et al.* (2010).
[j] Manerio *et al.* (2008).
[k] Vale and Sampayo (2008).
[l] Barbier and Diaz (2003).
[m] DiNubile and Hokama (1995).
[n] Morris *et al.* (1982), Hokama (1988), Kumar-Roiné *et al.* (2010), and Chan (2016).
[o] Dickey and Plakas (2010); see also for treatment for specific symptoms.
[p] Nguyen-Huu *et al.* (2010).
[q] Brett and Murnion (2015).
[r] Ofuji *et al.* (1999) and James *et al.* (2003).
[s] Lehane *et al.* (2002).
[t] Stewart *et al.* (2006), Zanchett and Oliveira-Filho (2013), Hillborn *et al.* (2014), and Otten and Paerl (2015).
[u] Corbel *et al.* (2014).
[v] Labine *et al.* (2015) and Zhang *et al.* (2015).

in commercial shellfish harvested from U.S. coastal regions, and these methods have been emulated globally. Extraordinary efforts are needed in educating the public as most PSP outbreaks involve the recreational harvesting of bivalves, often from quarantined areas. A series of well-documented cases by Hurley and colleagues provides one example of this public health challenge (2014). Despite the best efforts of the Washington State Department of Health to post health warnings, seven people suffered from PSP (at least four requiring treatment in an intensive care unit) after harvesting mussels outside of a resort hotel at about midnight (Hurley *et al*., 2014). Apparently, they could not read the health warning notice in the dark.

9.3.1.2 Clinical Symptoms

Initial symptoms of PSP are numbness or tingling around the mouth and lips from ten minutes to three hours or more after consumption. The onset and severity appear to be dose-dependent with the amount of ingested toxin (Gessner *et al*., 1997; McLaughlin *et al*., 2011; Callejas *et al*., 2015). In mild cases, this may be the only symptom; however, in more severe cases, the numbness and tingling may spread to the neck and face and be accompanied by headache, abdominal pain, nausea, vomiting, diarrhea, as well as a wide range of neurologic symptoms. Potential neurologic symptoms also include weakness, dizziness, dysarthria, paresthesias, double vision, loss of coordination, vertigo or dizziness, and/or a "floating" sensation. In the majority of cases, the symptoms resolve within 24 to 72 hours with a maximum duration of about two weeks (Rodrigue *et al*., 1990; Gessner *et al*., 1997; Hurley *et al*., 2014). In severe cases, however, the initial symptoms may rapidly progress to respiratory distress in an individual who otherwise exhibited no evidence of respiratory difficulty, and this may result in death (Gessner *et al*., 1997).

9.3.1.3 Treatment

Symptomatic individuals should seek medical attention immediately and preferably in an urgent care setting with ongoing monitoring for the potential loss of airway patency (Grattan *et al*., 2013).

9.3.2 Amnesic Shellfish Poisoning (ASP)

9.3.2.1 Exposure

Domoic acid (DA) is a naturally occurring toxin produced by marine diatoms of the genus *Pseudo-nitzschia*. Shellfish and other marine organisms feed on toxic *Pseudo-nitzschia* blooms and concentrate the toxin within them. Subsequently, people who eat shellfish with high levels of domoic acid are at risk of serious illness and, in some cases, death. The first documented outbreak of ASP was associated with the consumption of affected blue mussels from the Prince Edward Island region of Canada (Perl *et al*., 1990a, 1990b). Measurable levels of DA have also been found in the viscera of Dungeness crabs, Pacific razor clams (*Siliqua patula)*, and other organisms of the Pacific Northwest (PNw) coast of the United States.

Historically, the greatest risk for human exposure and ASP in the United States has been through consumption of the Pacific razor clam (*Siliqua patula*) in the PNw. This is attributed to the relatively slow depuration process in the PNw razor clam, allowing them to retain the toxin for up to one year in the natural environment, or several years after being processed, canned, or frozen (Wekell *et al*., 1994). In contrast, the risk of human illness associated with eating PNw Dungeness crabs is only increased under conditions of more sustained periods of elevated DA in the marine environment. Based upon studies by Grattan *et al*. (2016a) and Tracy *et al*. (2016) described later in this section, caution should be considered for high consumers of razor clams (15 or more clams/month) with any detectable DA. Particularly vulnerable groups include pregnant women, nursing, mothers, children, and elderly adults. There does not appear to be any risk for DA neurotoxicity with repeated consumption of fewer clams for healthy adults.

In the United States, the presence of *Pseudo-nitszchia* has been reported in regions outside of the west coast. Until 2016, however, it has not been found to produce DA at levels that could potentially harm human health (i.e., greater than the currently established safety level of 20 ppm). In late September and early October 2016, DA levels of up to 100 ppm were found in shellfish harvested from the coast of Maine. As a result, approximately five tons of mussels, mahogany quahogs, and clams were recalled, and unprecedented shellfish harvesting closures were implemented. These closures extended into Massachusetts and most of the Narragansett Bay in Rhode Island while aggressive shellfish and phytoplankton monitoring procedures were implemented.

9.3.2.2 Clinical Syndrome

Most of what is known about the human health impacts of exposure to high levels of DA and ASP

traces back to the initial Prince Edward Island outbreak (Perl *et al*., 1990a, 1990b; Teitelbaum, 1990; Teitelbaum *et al*., 1990; see Lefebvre and Robertson, 2010, for review). People who ate affected blue mussels served in Montreal restaurants suffered from a variety of symptoms, ranging from mild to severe, within several hours of exposure. This included vomiting, abdominal cramps, diarrhea, headache, seizures, respiratory excretions, confusion, and coma. The health outcomes were highly variable. While some people completely recovered, others were left with a permanent and profound memory disorder, ASP, and a few died (Cendes *et al*., 1995; Grattan *et al*., 2016a, 2016b).

DA is a water-soluble amino acid that activates the AMPA/kainite subtype of glutamate receptors (Hampson and Manalo, 1998). Considered a chemical analog to kainic acid, DA binds at the same receptor sites in the central nervous system. Thus, a pattern of cerebral damage is produced analogous to kainic acid neurotoxicity. Human autopsy and cross-species research suggest that DA neuropathology in adults occurs predominantly in the hippocampus, a cerebral region with a high density of kainite receptors and critical to memory functions (Grattan *et al*., 2013, 2016a). This explains the seizures and profound memory disturbances associated with high-level exposures to DA. Less well-known, however, are the potential health impacts of lower level, chronic exposures to DA.

To address the question, a prospective epidemiological cohort study of more than 500 Native American men and women over five years was conducted (Grattan *et al*., 2016a; Tracy *et al*., 2016). Measures of memory and general cognition as well as exposure (razor clam consumption and DA levels from harvested beaches) were annually obtained. Findings indicated isolated decrements on some measures of learning and memory for people who consumed 15 or more razor clams per month with low levels of DA (range: 3–6 ppm). Meanwhile, other cognitive functions remained unaffected. The memory decrement was statistically significant but relatively small, and remained within normal limits for the participants' age and level of education. From a scientific standpoint, this raises the possibility that repeated low-level exposures may cause neurotoxicity characterized by a mild disruption in memory functions. The clinical significance of these findings, however, remains to be determined. Ongoing studies of actual everyday memory functioning are currently underway to better establish the ecological validity of the laboratory-based memory tasks.

9.3.2.3 Treatment

Similar to other marine toxin exposures, there is no antidote for DA neurotoxicity or ASP. Thus, treatment is typically supportive and focused on symptom management with close observation for illness progression. After high levels of DA exposure, hospitalization is usually required, and patients should be followed for at least one year post ingestion as there is the potential for the delayed onset of temporal lobe epilepsy (Cendes *et al*., 1995). Similarly, following the patient with neurocognitive examinations is indicated to document the nature and extent of memory deficits and develop a plan for cognitive rehabilitation therapies.

9.3.3 Neurotoxic Shellfish Poisoning (NSP)

9.3.3.1 Exposure

NSP is typically caused by ingesting clams, oysters, and mussels contaminated with brevetoxins. The brevetoxins are a group of more than ten natural neurotoxins produced by the marine dinoflagellate *Karenia brevis*, formerly *Gymnodinium breve* (Watkins *et al*., 2008). Similar to ciguatoxins (CTX), brevetoxins are lipophilic polyethers and are regarded as depolarizing substances, which open voltage-gated sodium ion channels in cell walls, leading to uncontrolled Na+ influx into the cell (Baden, 1983; Daranas *et al*., 2001; Wang, 2008). Sensory symptoms may result from the transformation of fast sodium channels into slower ones, resulting in persistent activation and repetitive firing (Watters, 1995).

The risk for NSP toxins in shellfish is typically associated with HAB or "red tides" along the Gulf of Mexico. The greatest numbers of cases appear to originate from the west coast of Florida, although this may be due to more active surveillance rather than actual differences in occurrence. Similar to other HAB-related illnesses, there is an ongoing threat of new NSP cases as HAB may be transported to new regions. Thus, outbreaks have also been reported in New Zealand and Mexico. Interestingly, the largest number of reported U.S. cases came from an unexpected bloom and single outbreak of 48 persons in North Carolina as a result of the transport of brevetoxin-producing

organisms up the eastern seaboard from the Gulf of Mexico (Morris *et al*., 1991; Tester *et al*., 1991). The clinical presentation of these cases is discussed in the next section. In Florida and other Gulf Coast regions, public health agencies have routinely monitored coastal waters for the presence of brevetoxins for the past 35 years. The majority of episodes of illness have been associated with recreational shellfish harvesters and visitors who don't follow harvesting restrictions (Reich *et al*., 2015).

9.3.3.2 Clinical Illness

The diagnosis of NSP is based upon clinical presentation and history of bivalve shellfish consumption from an endemic area. Symptom onset may begin from a few minutes to 18 hours after consuming contaminated shellfish; however, in most cases time to illness is about three to four hours (Morris *et al*., 1991; Poli *et al*., 2000). The symptoms of NSP include both gastrointestinal and neurological problems. The most frequently reported symptoms are nausea, vomiting, abdominal pain, and diarrhea; however, these are often not the primary presenting complaint. Of greater concern to most individuals are the neurological symptoms, which may include paresthesias of the mouth, lips, and tongue; peripheral tingling; partial limb paralysis; slurred speech; dizziness; ataxia; and a general loss of coordination. Reversal of hot/cold sensation, similar to ciguatera poisoning, has also been reported (Arnold, 2011). Hospitalization is sometimes necessary; however, no fatalities have been reported as a result of NSP (Arnold, 2011). Treatment for NSP typically involves supportive care, which may include fluid replacement, monitoring of respiratory functions, the administration of sedatives, and pain management.

The most common symptoms from the 48 people involved in an unanticipated outbreak in North Carolina were paresthesias (81%), vertigo (60%), malaise (50%), abdominal pain (48%), nausea (44%), diarrhea (33%), weakness (31%), ataxia (27%), chills (21%), headache (15%), myalgia (13%), and vomiting (10%) (Morris *et al*., 1991). Albeit rare, a few cases have reported respiratory discomfort and distress, and some required ventilator support (Watkins *et al*., 2008). Cases reported by Reich *et al*. (2015) as well as others confirm that hospitalization is sometimes necessary; however, no fatalities have been reported as a result of NSP, and patients typically recover within two to three days without long-term effects (Morris *et al*., 1991; Watkins *et al*., 2008; Arnold, 2011).

9.3.3.3 Treatment

Treatment for NSP typically involves supportive care, which may include fluid replacement, monitoring of respiratory functions, the administration of sedatives, and pain management.

9.3.4 Brevetoxin Inhalation Syndrome (BIS)

9.3.4.1 Exposure

A BIS is caused by exposure to the marine aerosols of the same toxin responsible for NSP. Exposure is thought to be due to wave action and aerosolized sprays along Florida beaches during "red tide" events. Exposure also occur through recreational or occupational activity in the impacted waters.

9.3.4.2 Clinical Illness

The aerosolization of brevetoxins produces an inhalation syndrome characterized by respiratory problems and eye irritation (Fleming *et al*., 2005). Adverse respiratory effects include upper airway irritation and discomfort, decreases in pulmonary function, and exacerbation of asthma symptoms. A significant decrease in pulmonary function has been reported one hour after a beach exposure to a Florida red tide (Fleming *et al*., 2009). Longer term studies of people with asthma during a similar event found that those with elevated symptoms after a one hour exposure demonstrated a further decrease in pulmonary function over the next 24 hours, and did not return to baseline for at least four days (Kirkpatrick *et al*., 2011).

9.3.4.3 Treatment

Symptoms of the inhalation syndrome appear to be transient and reversible over one to four days. Meanwhile, supportive treatment may be provided as indicated.

9.3.5 Diarrhetic Shellfish Poisoning (DSP)

9.3.5.1 Exposure

DSP is a gastrointestinal illness of relatively rapid onset caused by consuming shellfish contaminated with okadaic acid and related toxins. The most common vectors for DSP toxins are mussels, clams, scallops, and oysters. These toxins are produced by a community of dinoflagellates, particularl *Dinophysis* and *Prorocentrum* (Yasumoto *et al*., 1985; Lee *et al*., 1989; Quilliam *et al*., 1996). Outbreaks of DSP have been reported in Japan, France (see Hossen

et al., 2011), other parts of Europe, Canada, New Zealand, the United Kingdom (see Hinder *et al.*, 2011; Taylor *et al.*, 2013), and South America. With the exception of a handful of cases reported by the Washington State Department of Health in June 2011 (Taylor *et al.*, 2013), relatively few episodes of DSP have been identified in the United States. The threat for exposure in the United States has been reported in Texas Gulf coastal waters, where the responsible organisms (*Dinophysis*) and the related shellfish toxin (okadaic acid) have been identified in oysters (Barbier and Diaz, 2003; Deeds *et al.*, 2010). DSP-related toxins have also been reported in New York (Freudenthal and Jijina, 1988), although no episodes of illness were documented. Okadaic acid is a lipophyphilic polyether that inhibits eukaryotic protein phosphatases, which plays an important role in many regulatory processes in cells (Bialojan and Takai, 1988). It is thought to trigger acute diarrhea by stimulating phosphorylation of proteins that control sodium secretion in intestinal cells (Cohen *et al.*, 1990; Sobel *et al.*, 2005). There is also reason to believe that DSP toxins are tumor promoters as they have been found to increase the risk for colorectal or other digestive cancer sites (esophagus, stomach, and liver for men and stomach and pancreatic for women; Cordier *et al.*, 2000; Manerio *et al.*, 2008). Further research is needed to determine the extent to which DSP poisoning increases risk for digestive cancers.

9.3.5.2 Clinical Syndrome

Symptom onset for DSP typically occurs within 30 minutes to four hours after ingesting contaminated shellfish. The main symptom is incapacitating diarrhea, followed by nausea, vomiting, and abdominal cramps (James *et al.*, 2010). The symptoms may be severe, lead to dehydration, and persist for about three days (Barbier and Diaz, 2003). No fatalities have been reported for DSP.

9.3.5.3 Treatment

Treatment is largely supportive for DSP, with specific efforts made to prevent dehydration. The illness tends to be self-limiting, with affected individuals recovering within three days with or without medical intervention. Prevention is the best way to manage the illness.

9.3.6 Ciguatera Fish Poisoning (CFP)

9.3.6.1 Exposure

Ciguatera is the most frequently reported seafood-related disease in the United States and the most common foodborne illness related to finfish consumption in the world (Isbister and Kiernan, 2005; Villareal *et al.*, 2006). It is endemic in areas where consumption of reef fish is common, including the Caribbean, southern Florida, Hawaii, the South Pacific, and Australia; however, recent reports suggest expansion of the biogeographical range of ciguatoxic fish (Villareal *et al.*, 2007; Dickey and Plakas, 2010). Accordingly, CFP has been reported from fish originating in South Carolina and the northwestern Gulf of Mexico as well as the Canary and Madeira Islands in Europe (Perez-Arellano, 2005; Nunez *et al.*, 2012). Exposure risk goes beyond local fish consumers to include tourists and other travelers to endemic regions (Epelboin *et al.*, 2014). In addition, the increasing consumption of imported fish and seafood expands the geographical range of potential CFP.

The target source for CFP is the consumption of reef fish that have accumulated potent neurotoxins (CTX) in their flesh and viscera. The toxins are produced by the marine dinoflagellate *Gambierdiscus*, which lives on various, sometimes harmful, microalgae in coral reef ecosystems. These dinoflagellates are then consumed by herbivorous fish, and, through the process of bioaccumulation and magnification, the toxin advances through the food web via carnivorous species. There is reason to believe that more than 400 fish species have the potential for ciguatera toxicity (Halstead, 1978), and this includes farm-raised fish (DiNubile and Hokama, 1995). The risk for CFP is greatest for carnivorous, predatory fish, such as barracuda (of which >70% may be toxic), snapper, grouper, and amberjack (Langley *et al.*, 2009).

9.3.6.2 Clinical Illness

The diagnosis of CFP is based upon clinical symptoms within the context of a history of recent predatory reef fish consumption. The clinical syndrome typically arises within 12 hours of eating toxic reef fish with initial symptoms including severe gastrointestinal problems (nausea, vomiting, diarrhea, and abdominal pain). Cardiovascular problems (generally a combination of bradycardia with hypotension) or neurologic symptoms may also be present during the acute episode. The early gastrointestinal symptoms typically abate within 24 hours (Hokama, 1988). In the Caribbean and South Florida, cardiovascular disorders typically reverse within 48 to 72 hours (Hokama, 1988; Butera *et al.*, 2000). From a few hours to two weeks after exposure, a wide range of subjective neurological complaints emerge in about 70% of

cases. These may include pain and weakness in the lower extremities; painful tingling around the mouth, teeth, nose, and throat; peripheral paresthesias; headache; metallic taste; hyporeflexia; and/or dysphagia. The hallmark of CFP neurological symptoms is an unusual paradoxical disturbance of thermal sensation (i.e., cold objects feeling hot and sometimes hot feeling cold) (Pearn, 2001; Achaibar *et al.*, 2007). The possibility has been raised that subjectively reported temperature reversals reflect a misinterpretation of paresthesias, a more common neurologic symptom. Recent quantitative sensory testing of Caribbean patients by Grattan and colleagues has documented, however, abnormal performance on objective measures of temperature threshold sensation in some patients (Grattan *et al.*, 2013).

While there has been considerable agreement about the neurologic symptoms of CFP through detailed case reports, the full symptom complex remains to be fully characterized or understood. What can be stated with reasonable certainty is that recovery from acute neurologic symptoms is longer and less predictable than gastrointestinal or cardiac symptoms, ranging from 1 week to 6 months or more (Lange *et al.*, 1992; Butera *et al.*, 2000; Achaibar *et al.*, 2007). Some patients report persistent symptoms for many years. Where a long-term or chronic ciguatera syndrome occurs, it is often characterized by intractable fatigue, weakness, and/or paresthesias, and typically accompanied by depression. It is also generally accepted that CFP symptoms may reappear after a period of presumed recovery. This recurrence may be triggered by alcohol use or repeated consumption of fish with relatively low levels of CTX. Thus, persons who have had one episode of ciguatera are at increased risk for repeated illness (Morris *et al.*, 1982).

In some cases, particularly with exposures in Pacific regions, there have been reports of rapid progression from initial gastrointestinal and neurologic complaints to respiratory distress, coma, and death (Defusco *et al.*, 1993; Habermehl *et al.*, 1994). These deaths have been attributed to consuming large amounts of the fish viscera and head (CTX-intense fish parts), exposure to highly ciguatoxic fish species (e.g., yellow-edged morays) or fish caught after a storm, as well as individual susceptibility and lack of access to emergency care (Chan, 2016).

Despite the best efforts of numerous investigators, at this time there are no reliable rapid bioassays for toxin detection in fish and there is no human biomarker. Due to its high level of potency, any amount of CTX in fish is thought to pose a health risk. Preliminary laboratory studies suggest industry and consumer advisory levels of 0.10 ppb C-CTX-1 equivalent toxicity in fish from tropical Atlantic, Gulf of Mexico, and Caribbean regions and 0.01 ppb P-CTX-1 equivalent toxicity in fish from Pacific regions (Dickey and Plakas, 2010).

9.3.6.3 Treatment

Treatment for CFP involves nonspecific, supportive symptom management. In most cases, the illness is self-limiting. To prevent relapses, patients are typically instructed to avoid risky fish, nuts, or alcohol for 6 months after the poisoning episode. In severe cases (most often a result of Pacific ciguatoxins), administration of intravenous D-mannitol within 48 hours of symptom onset is generally accepted as the most effective way to manage the neurological symptoms (Pearn *et al.*, 1989). While the efficacy of this intervention was not supported in one double-blind trial where D-mannitol was compared to normal saline (Schnorf *et al.*, 2002), infusion of D-mannitol remains the treatment of choice for severe ciguatera intoxications (Kumar-Roiné *et al.*, 2010). Preliminary reports suggest that brevenal and pregabalin may also be effective in managing severe cases of CFP (Nguyen-Huu *et al.*, 2010; Brett and Murnion, 2015).

9.3.7 Azaspiracid Shellfish Poisoning (AZP)

9.3.7.1 Exposure

The potential risk of AZP to human health was first identified in the Netherlands in 1995 following an outbreak of severe gastrointestinal and neurological symptoms triggered by ingesting contaminated mussels. The mussels had been imported from Killary Harbour, Ireland (Ofuji *et al.*, 1999; James *et al.*, 2003). A second outbreak in Arranmore Island, Ireland, was also associated with consuming mussels containing azaspiracid as well as a complex of several new toxins (Lehane *et al.*, 2002). Since that time, AZA or its analogs have been identified in shellfish of coastal regions of England, Spain, France, Italy, northwest Africa, and eastern Canada. Furthermore, the potential vectors for human illness have expanded to include scallops and crabs. The potential producer of AZA is thought to be the dinoflagellate *Protoperidinium crasspie*, and shellfish are exposed through their normal grazing activity (Twinner *et al.*, 2008; Wang, 2008).

9.3.7.2 Clinical Syndrome

The complete characterization of AZP is not well elucidated due to limited data from the outbreaks to date. What is known is that the poisoning syndrome appears somewhat similar to DSP and includes nausea, vomiting, abdominal cramps, and severe diarrhea (Alfonso *et al*., 2005). Available data also suggest that most people do not seek medical treatment for their symptoms, which persist for about two to three days. Full recovery can usually be expected.

9.3.7.3 Treatment

Medical treatment (if requested) is focused upon symptom management with a good prognosis for complete recovery. Timely reporting of initial cases and ongoing biotoxin monitoring programs in endemic areas are helpful toward the goal of illness prevention.

9.3.8 Toxic Cyanobacteria

9.3.8.1 Exposure

Cyanobacteria blooms are common in freshwater and many coastal regions throughout the United States and the world. The reader is referred to Chapter 16 in this compendium for further detail regarding the frequency of occurrence, environmental factors associated with proliferation, and types of cyanobacteria-producing toxins. In summary, cyanobacteria blooms may produce a wide range of potent toxins (Codd *et al*., 2005; Meneely and Elliott, 2013). Hepatotoxins are the most commonly found and well-studied metabolites produced by cyanobacteria (Carmichael, 1992); and, in fresh water, microsystins seem to have the most profound impact on the food chain (Meneely and Elliott, 2013). It is important to note that not all species of cyanobacteria produce toxins, but when they do, a diverse range of toxins and human health problems may emerge (Funari and Testai, 2008; Backer *et al*., 2015). Although color and odor often accompany cyanobacteria-related HAB, such markers are neither stable nor reliable in their correlation with toxin presence. Four routes of human exposure have been documented, including oral, pulmonary, dermal, and hemodialysis treatments (Carmichael *et al*., 2001; Codd *et al*., 2005; Funari and Testai, 2008), and the health impacts may vary accordingly. Illness prevention involves avoiding exposures to contaminated water or food products (including vegetation irrigated with bloom or scum-infested nutritional supplements and water).

9.3.8.2 Clinical Syndromes

Health problems related to cyanobacteria may range from mild, transient symptoms to death depending upon the specific toxin, route, and duration of exposure. Oral exposures may occur from drinking untreated water, accidentally swallowing contaminated water during recreational activity, eating fish or shellfish with toxin that accumulated during production, consuming vegetables irrigated with infested water, or taking blue-green algae dietary supplements (Funari and Testai, 2008; Poste *et al*., 2011). Human health effects are diverse and may include gastroenteritis, nausea, vomiting, abdominal pains, fevers and flu-like symptoms, myalgia, and visual disturbances. Based upon human studies as well as animal models, liver and/or kidney problems (including primary liver cancer) have resulted from microcystin and nodularin exposures, and neuromuscular symptoms are thought to be associated with anatoxin-a. An ecological association relationship between cyanobacterial blooms and clusters of death attributable to non-alcoholic liver disease was reported by Zhang and colleagues (2015). While still controversial and not confirmed, some researchers suggest that dietary consumption of B-N-methulamino-L-alanine (BMAA) produced by cyanobacteria has been associated with amyotrophic lateral sclerosis and a Parkinsonism–dementia complex (ALS/PDC) as well as Alzheimer's disease (Cox *et al*., 2003; Brand, 2009).

With respect to non-dietary forms of exposure, inhalation of aerosol sprays and dermal contact have been associated with recreational and work activity in waters with toxic cyanobacteria. These exposures may trigger clinical symptoms such as a sore throat, sore mouth, ear and eye irritation, as well as rashes. Finally, when waters tainted with cyanobacteria hepatoxins (microcystins) were used in hemodialysis treatment in Brazil, 56 out of 130 patients in hemodialysis treatment died (Jochimsen *et al*., 1998; Azevedo *et al*., 2002).

9.3.8.3 Treatment

Medical treatment and management regimens for known or suspected cyanotoxin exposure vary based upon the presenting health symptoms, complaints, and coexisting medical diagnoses. Given the limited number of studies available to date, human diagnostic as well as treatment data are scarce and inconclusive. Prospective

epidemiological cohort studies of acute and chronic health effects after long-term exposure are needed.

9.4 The HAB Manager's Role in Preventing HAB-Related Illnesses

Illness prevention represents a critical component of HAB-manager activity (for a comprehensive review of mitigation, the reader is referred to Chapter 12). Widespread, proactive monitoring of coastal algal blooms and shellfish before they reach consumers through recreational or commercial harvesting sources along with rapid outreach communication methods represent effective ways to prevent most HAB-related illnesses (NSP, DSP, ASP, PSP, and AZP). Aggressive monitoring, shellfish testing, and well-enforced harvesting closures by state health fisheries departments and agencies have been successful in preventing cases of ASP, PSP, and NSP in commercial shellfish harvested from U.S. coastal regions. Unfortunately, most episodes of illness from these shellfish exposures have been associated with recreational harvesters and visitors who appear to be unaware of, or disregard, harvesting restrictions.

The best way to prevent CFP is to encourage people to avoid consumption of large predatory fish from endemic areas. This is particularly important if the individual has had prior episodes of ciguatera. People with asthma or other respiratory difficulties should avoid exposure to aerosolized brevetoxins during "red tide" events to reduce their risk of BIS especially with significant bloom concentration and on shore winds. While the environmental impacts and toxin diversity of cyanobacteria make it difficult to establish guidelines to fully protect human health, the monitoring of blooms and encouraging people to avoid all forms of contact (oral, dermal, and inhalation) are integral parts of the process. For reference, the flowchart presented in Figure 9.2, provided by the Florida Department of Health, illustrates the essential steps for monitoring cyanobacteria/blue-green algae. The HAB manager also needs to maintain awareness of cases of cyanobacterial-related animal illnesses and deaths. These are potentially useful as sentinel events for the presence of cyanobacterial toxins that may later impact human health (Hilborn and Beasley, 2015). Finally, early diagnosis and rapid reporting of sentinel patients are critical for all of the HAB clinical syndromes as this has the potential to reduce the number of secondary cases in cluster outbreaks.

9.4.1 HAB Management Exemplars

HAB managers are faced with a complex set of responsibilities to protect the marine environment as well as human health. This task is particularly challenging with the emergence of new or unfamiliar HAB-related toxins, the geographic expansion of known toxins into new locations, and the need to be sensitive to the cultural and community impacts of HAB management practices. Building upon the extensive experience of Joe Schumacker, representing the Quinault Nation in the State of Washington, and Andrew Reich from the Florida Department of Health, the following exemplars are provided. HAB managers are encouraged to refer to these exemplars as potential models or sources of guidance for the successful management of HAB-related problems in other settings.

9.4.2 The Native American Perspective from Washington State, USA: Domoic Acid and Paralytic Shellfish Toxins

9.4.2.1 Background

The fall of 1998 began a new chapter in the lifeways of the coastal tribes of Washington State. For the first time in their history, tribal members were not allowed to harvest shellfish for over an entire year. An unknown and unseen toxin had been detected in clam tissue. Concurrent water samples indicated it was the result of a HAB event not previously noted on that coast. Domoic acid and *Pseudo-nitzschia* diatoms suddenly became common terms to people that had never known them before.

For millennia, Native Americans had harvested fish and shellfish from the shores of the Salish Sea (Puget Sound and Straits of Georgia) and the Pacific coast of what is now Washington State. Generations of experience developed Traditional Ecological Knowledge (TEK) that determined when and where shellfish, in particular, were safe for humans to harvest and consume. For peoples of the outer coast including the Quinault Indian Nation and the Hoh, Quileute, and Makah Tribes, TEK had guided the harvest of Dungeness crab (*Metacarcinus magister*), butter clams (*Saxidomus gigantea*), native littleneck clams (*Leukoma staminea*), horse clams (*Tresus* spp.), and the highly prized Pacific razor clam (*Siliqua patula*).

Shellfish were historically not harvested by tribal peoples in the late summer on the outer coast, what would now be the months of August and part

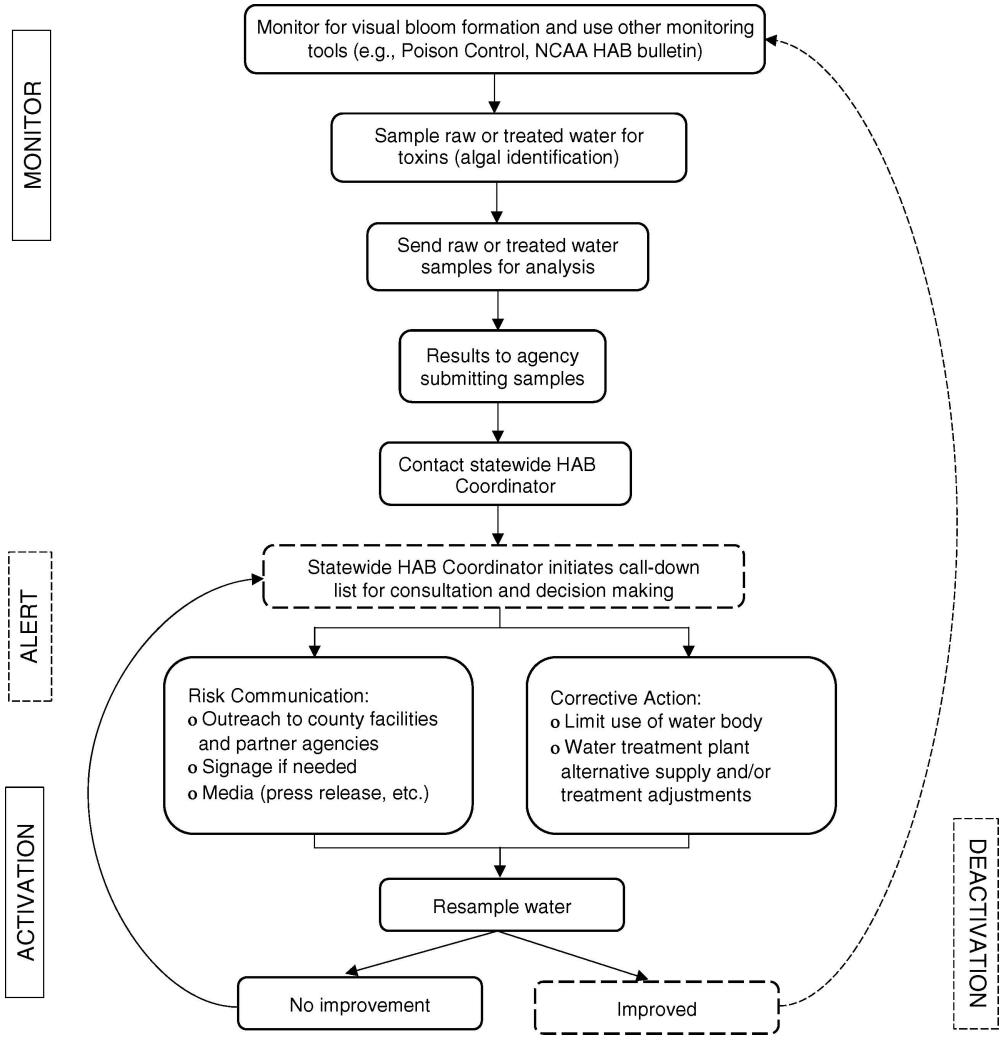

Figure 9.2 Flow chart for blue-green algae and cyanobacteria bloom response for statewide HAB Coordinator – recreational and other bodies.

of September (personal communication, Justine James Jr., Quinault Indian Nation). During that times, many would travel inland to harvest salmon, elk, berries, and collect weaving materials. When they returned to their coastal villages, generally in late September and October, shellfish would be considered harvestable once again.

In retrospect, viewed through the lens of current "western science," the pattern of non-harvest in late summer months may have had its basis in protecting human health. Data accumulated by Tribes, state agencies, and researchers on the Washington coast have documented the likelihood of HAB toxins including domoic acid and saxitoxin being present in shellfish at that time of the year versus other seasons. Tribes may have been reacting to observed health issues, but avoidance may also have been to allow clams to reproduce effectively. The TEK that protected peoples' health may have been intermingled with natural resource management that worked to assure sustained populations of healthy shellfish.

Coastal peoples of what is now Washington State have lived off the land and waters for thousands of years. Europeans established settlements in the area in the early nineteenth century, and following years of their populations being decimated by disease, loss of historic harvest areas, and repatriation, tribes shared a general mistrust of information from non-Indians. Following the 1974 landmark federal court case *United States* v. *Washington* (The "Boldt Decision") that reestablished tribal treaty rights to

co-manage and harvest fishery resources, tribes began to build capacity to manage resources using best science. To meet their co-manager responsibilities, tribes throughout western Washington State established fisheries management programs to protect their resources. It was through this building of tribes' capacity to manage, using both TEK and western science, that the foundations of trust in western science emerged.

9.4.2.2 Tribal Capacity and Inclusion

It requires clear communication, transparency, and trust to build confidence in public health warnings regarding consumption of natural foods that have not caused a death or illness in recorded memory. In 1998, few, if any, on the Washington coast, tribal or non-tribal, had heard of domoic acid or amnesiac shellfish poisoning. There were no illnesses reported other than potential gastric distress determined through Washington Department of Health telephone surveys of clam diggers. Tribes had no indication of health issues and were asked to believe scientists and managers that the foods they had eaten forever could now make them seriously ill or even kill them. It was in that atmosphere that a group of concerned tribal managers, state managers, and academics worked to form a partnership to monitor HAB, share data, and build public trust in the science of HAB on the Washington coast.

The Olympic Region Harmful Algal Bloom partnership (ORHAB) was formed in 2000, supported by federal funds, to build tribal and state capacity to effectively monitor for HAB and test for toxins. Initial funds for this project allowed the hiring of tribal samplers to collect water, identify and enumerate HAB species, test for toxins including domoic acid and saxitoxin, compile results, and share findings with the public. The inclusion of tribal members in the project was a requirement for building trust in the science. Now, members themselves could see the phytoplankton responsible, test for toxins in the water and shellfish, work closely with researchers conducting modeling and offshore sampling, and share that information with their respective tribes.

The ORHAB partnership has evolved over time, generating both tribal and state funding support to carry on its mission to increase capacity and protect public health on the Washington coast. It was the inclusion of tribal members in the science of HAB that was critical to building the trust that led to acceptance of and adherence to harvest closures from HAB events. Through their inclusion and the network of managers and researchers generated through the partnership, tribal members are now teaching the next generations about scientific methods, coastal ecosystems, climate change, and public health.

9.4.2.3 Lessons Learned

The treaty tribes of Washington State have developed scientific management capacity to meet the requirements for co-management of fisheries resources within their respective treaty areas. Throughout the United States and its possessions, many indigenous peoples depend upon fish and shellfish from coastal waters but may lack the capacity that Washington State tribes have developed. Inclusion of these communities in HAB monitoring, science, and data distribution are imperative in developing knowledge of, and trust in, potential HAB impacts on the resources they depend upon. Tribes and most indigenous peoples of the United States are "place-based," their identities tied to ancestral lands and waters. They are the best stewards for caring for these often-remote areas and should be included in any comprehensive HAB plans that require an engaged public.

9.4.3 The Florida Department of Health Perspective

The development of a public health response protocol for HAB is an important task that all states should consider. It can for provide guidance and increased capacity for public health and environmental managers to facilitate appropriate response to algal bloom events. HAB occur throughout the United States, with some states, such as Florida, having regionally specific bloom types.

Florida has an inviting subtropical climate along with 1200 miles of coastline; 11,000 miles of rivers, streams, and waterways; 7700 lakes; and more large springs than any other state. The Department of Health works to ensure that these waters are safe for recreation and that Florida seafood is safe to eat. In the Division of Disease Control and Health Protection, we protect the health of Floridians by educating people about HAB and their toxins; watching for illnesses in our communities; and working with local, state, and federal agencies, nongovernmental organizations, and our private sector partners to reduce exposures to aquatic toxins.

With over 80% of residents residing in coastal counties, Floridians are sometimes affected by algal blooms. Recreational activities such as

boating, fishing, swimming, and beach-walking are popular with both residents and tourists. In Florida, tourism depends on an abundance of healthy beaches, outdoor recreational activities, and seafood. HAB can have unrecognized economic impacts on healthcare, fisheries, and tourism industries. During a bloom, people may seek medical care for their symptoms, which results in additional healthcare expenditures. Local county and city governments may participate in beach clean-up efforts. Sometimes when *K. brevis* red tide toxins accumulate in shellfish, nearby harvesting beds are closed, resulting in revenue losses for the aquaculture industry. Local businesses including hotels and restaurants often report a decline in tourism during HAB events.

9.4.3.1 Harmful Algal Blooms

In Florida, there is *K. brevis* red tide; blue-green algae/cyanobacteria; saxitoxin-producing organisms; ciguatoxin-producing organisms; and emerging threats, including other seafood poisonings. Due to the toxicological complexity of the various HAB, and the many individual and community impacts, response planning must include a broad spectrum of stakeholders from both the public and private sectors. In Florida, county-specific response plans that address local needs, capacity, and capabilities have been developed; however, they all have in their core a statewide structure that ensure consistency in their assessment and response. This is encouraged by the centralized structure of the Florida Department of Health with all 67 local county health entities integrated as components of the Department.

The Department has developed a public health preparedness system that uses the development of standard operating guidelines (SOG) to integrate a wide variety of response actions in its action plans. The HAB SOG provides the Department's county environmental managers and environmental protection authorities with guidance to develop appropriate county-specific response plans. The Public Health Toxicology Section within the Bureau of Environmental Health in the Division of Disease Control and Health Protection works to ensure that Florida waters are safe for recreation and Florida seafood is safe to eat. The development of the SOG and its overall implementation responsibility resides in this Bureau, which also contains the Environmental Preparedness Program and the Water Program (with Healthy Beaches, Drinking Water, and Recreational Water responsibilities). This SOG provides a general approach to HAB response efforts statewide, with sections that focus on the four most important HAB occurring in Florida. The broad objectives outline general responsibilities of federal, state, and local stakeholders on how to monitor, notify, coordinate, allocate resources, mitigate, and implement control measures for HAB events.

For instance, Section 381.0031 of the Florida Statute identifies "ciguatera fish poisoning" as a reportable condition in the state. In addition to mandatory reporting, every effort should be made to obtain the implicated fish and to trace it back to the source. Consequently, patients are asked to keep any frozen samples of the suspect fish, and to document its species and size, where it was captured/purchased, and how it was stored and prepared. Samples are then collected by the Department and shipped to the U.S. Food and Drug Administration (FDA) Center for Food Safety in Dauphin Island to test for ciguatoxins using materials provided by the FDA (unless shipping materials and protocols are in place). The Department protocols for ciguatera response follow established procedures for foodborne illness investigations. This is one of the many activities that engage an effective HAB manager in Florida. Table 9.2 provides more general guidance for the role of the HAB manager.

9.5 HAB-Related Stressors and Human Resilience

Coastal communities and their residents are vulnerable to a wide range of distress as a result of HAB events. Residents and families, often dependent upon healthy environmental resources for employment in fishing, tourism, and service industries; adequate housing; maintaining cultural traditions; and quality of life may suffer losses as a result of a toxic bloom. These losses are often a source of profound distress. When these events are clearly defined with a rapid onset and a distinct endpoint, are short-lived, or are predictable, the hazard will likely be viewed as a nuisance; however, more often than not, HAB events are unpredictable and diffuse in time and space. Under these circumstances, the stresses upon individuals and their communities may wear down their psychological and problem-solving resources for coping and adaptation over time. When personal or community resources for coping are overwhelmed or depleted, various forms of behavioral reactivity

may ensue and disrupt effective educational, outreach, and communication efforts toward preventing further harm (Grattan *et al*., 2016b). Subsequently, understanding some of the basic mechanisms of individual and community resilience in the face of coastal environment distress may be useful to the HAB manager.

Resilience generally refers to the capacity to withstand or "bounce back" from stressful life events. While it is beyond the scope of this chapter to provide a thorough review of this complex topic, there are several basic premises underlying how resilience operates within individuals and communities. Understanding these mechanisms may be useful in working with coastal communities and people in distress.

1) Most individuals are inherently resilient and can and will cope with the challenges that face them.
2) Financial losses, environmental worry, and ambiguity related to environmental and health risks will increase distress, and psychological or behavioral reactivity.
3) Active coping using problem-solving strategies and turning to religion or cultural beliefs are among the most effective coping strategies in response to environmental hazards. In contrast, behavioral disengagement (avoidance, withdrawal, and drug and alcohol abuse) tend to be the least effective coping strategies in these circumstances.
4) The presence of accurate, timely information from reliable sources; social support; the ability to manage strong emotions (such as anger); optimism; and the belief that one can be successful in overcoming the distress (based upon past experience) are associated with more resilient individual outcomes.
5) Most communities are capable of self-organization and can build the capacity for learning, adaptation, and resilience in the face of environmental hazards.
6) If the coastal community has knowledgeable, prepared, and responsive institutions and has learned from past experiences, they are more likely to better manage an environmental hazard.
7) The lack of community financial resources or trusted leadership, and the presence of community conflict, will contribute to a more difficult resilience process.
8) If the community has institutions that distribute valuable resources in a fair and equitable manner, the capacity for resilience will be optimized.

9.6 Conclusion

Tackling the complex operations of HAB management is a difficult but important activity to protect the health of our citizens. Fish and seafood consumption in the United States has significantly increased over the past 20 years, and there is no reason to believe that this dietary pattern will abate. This has been related to a combination of factors, including the increased appreciation for the health benefits of fish and seafood consumption; an increase in ethnic diversity with groups that have been associated with greater consumption of fish products (e.g., Hispanics); rising incomes resulting in a greater proportion of money spent on food, restaurant dining, and a variety of fish and seafood products; and increased "globalization" of consumer food choices. With this in mind, public health specialists in collaboration with HAB managers will need to continue to advance the knowledge base of HAB-related illnesses.

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10

Marine Biotoxin and Harmful Algae Monitoring and Management

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10.1 Introduction

Routine monitoring for marine biotoxins in bivalve shellfish and other seafood is a necessary and proven approach for protecting public health. In the United States (U.S.) and internationally, it is required to test commercially harvested shellfish for all marine biotoxins of concern. Regulations within the respective countries establish the approved analytical methods, as well as the action levels, for each toxin of concern. The majority of countries have standardized on the same action levels that would prompt a harvest closure: 80 µg saxitoxin (STX) equivalents per 100 g of shellfish meat (80 µg STX eq./100 g = 0.8 ppm) for paralytic shellfish poisoning (PSP) monitoring, 20 µg/g (20 ppm) of domoic acid for amnesic shellfish poisoning (ASP), 160 µg okadaic acid (OA) eq./kg (0.16 ppm) for diarrheic shellfish poisoning (DSP), 20 mouse units/100 g (0.8 mg brevetoxin-2 eq./kg = 0.8 ppm) for neurotoxic shellfish poisoning (NSP), and 160 µg azaspiracid-1 (AZA1) eq./kg (0.16 ppm) for azaspiracid shellfish poisoning (AZP) (Tables 10.1 and 10.2). Where exceptions exist, they err on the side of safety (see examples in this chapter). A more recent concern involves the potential for freshwater toxins, produced by several species of cyanobacteria, to be transported to coastal waters where shellfish resources could be impacted. Regulatory action levels have not been adopted for these toxins in shellfish; however, there is mounting evidence of the potential for coastal seafood resources to be impacted. Miller *et al.* (2010) associated the deaths of sea otters to microcystin intoxication, and Mulvenna *et al.* (2012) developed consumption guidelines based on a risk assessment for several cyanotoxins of concern in Australian seafood (Table 10.3).

In the U.S., the monitoring requirements for commercial shellfishing exist within the National Shellfish Sanitation Program (NSSP) Model Ordinance, part of the Guide for the Control of Molluscan Shellfish (NSSP, 2015a). The NSSP is the federal/state cooperative program recognized by the U.S. Food and Drug Administration (FDA) and the Interstate Shellfish Sanitation Conference (ISSC) for the sanitary control of shellfish produced and sold for human consumption. Participants in the ISSC/NSSP include agencies from shellfish producing and nonproducing states, tribal representatives, the FDA, the U.S. Environmental Protection Agency (EPA), the National Oceanic and Atmospheric Administration (NOAA), and the shellfish industry. The state agency responsible for ensuring the state shellfish program meets NSSP guidelines is referred to as the *shellfish control authority*. The purpose of the NSSP is to promote and improve the sanitation of shellfish (oysters, clams, mussels and scallops) moving in interstate commerce through federal/state cooperation and uniformity of state shellfish programs. The ISSC is recognized by the FDA as the primary voluntary national organization of state shellfish regulatory officials that provide guidance and counsel on matters for the sanitary control of shellfish. Regulatory guidelines and procedures developed and approved by ISSC members, following FDA concurrence, are published in

Harmful Algal Blooms: A Compendium Desk Reference, First Edition. Edited by Sandra E. Shumway, JoAnn M. Burkholder, and Steve L. Morton.

Table 10.1 Action levels for algal toxins set by the U.S. Food and Drug Administration.

Toxin syndrome	Action level
ASP	20 ppm (20 μg g^{-1}) domoic acid, except in the viscera of Dungeness crab, where the action level is 30 ppm
AZP	0.16 ppm (0.16 mg kg^{-1}) azaspiracid equivalents
CFP	0.01 ppb (0.01 μg kg^{-1}) P-CTX1 equivalents for Pacific ciguatoxin and 0.1 ppb (0.1 μg kg^{-1}) for C-CTX1 for Caribbean ciguatoxin
DSP	0.16 ppm (0.16 mg kg^{-1}) total okadaic acid (i.e., combined free okadaic acid, dinophysistoxins, acyl esters of okadaic acid, and dinophysistoxins)
NSP	0.8 ppm (20 mouse units g^{-1}) brevetoxin-2 equivalents or 5000 *K. brevis* cells L^{-1}
PSP	0.8 ppm (80 μg 100 g^{-1}) saxitoxin equivalents

Source: Data from Health and Human Services, U.S. Food and Drug Administration (2011) and National Shellfish Sanitation Program (2015).

Table 10.2 FAO internationally accepted toxin levels for bivalve molluscs.

Toxin	Tissue Levels (mg kg^{-1} or ppm)
AZA	0.16
DA	20
OA	0.16
STX	0.8
YTX	1.0*

* EU limit, in Shirone *et al.* (2013).
Source: Adapted from P. Hess, IFREMER, Nantes, France, personal communication; and Lawrence *et al.* (2011).

revisions of the Model Ordinance (http://www.fda.gov/Food/GuidanceRegulation/FederalStateFoodPrograms/ucm2006754.htm). The NSSP guidelines are not codified in federal regulations, but are voluntarily agreed to by participating members and adopted specifically or by reference in state regulations.

Table 10.3 Tolerable daily intake (TDI) levels for various cyanotoxins.

Toxin	TDI μg $(kg\ d)^{-1}$
Anatoxin-a	0.1
Microcystin	40, 0.05*
Nodularin	40
Cylindrospermopsin	0.15, 0.03**
Saxitoxins	0.05*

* Farrer *et al.* (2015);
** Humpage and Falconer (2003).
Source: Data from World Health Organization (1998) and Mulvenna *et al.* (2012).

The Model Ordinance requires each shellfish-producing state to prepare and maintain a marine biotoxin contingency plan. This plan defines the administrative procedures and resources necessary to

> (a) initiate an emergency shellfish sampling and assay program; (b) close growing areas and embargo shellfish; (c) prevent harvesting of contaminated species; (d) provide for product recall; (e) disseminate information on the occurrences of toxic algal blooms and/or toxicity in shellfish meats to adjacent states, the shellfish industry, and local health agencies; and (f) coordinate control actions taken by authorities and federal agencies. (Model Ordinance; NSSP, 2015a, sec. II, chap. IV, @04.A)

Criteria must also be developed for determining when it is safe to reopen a commercial harvesting area. The Model Ordinance further describes a number of objectives for the biotoxin contingency plan, among them the need for an early warning system that includes phytoplankton and/or shellfish monitoring. The monitoring program should incorporate the concepts of *key stations* and *key species*. Briefly, this means the establishment of monitoring stations where shellfish toxins or toxin-producing phytoplankton are first known to occur and to focus initial sampling on the specie(s) known to assimilate and concentrate the toxin most readily. The frequency of monitoring should take into account the unpredictability of these toxicity events and the variability in the phytoplankton population in terms of species composition and abundance.

Somewhat similarly, the European Union (EU) has established controls on bivalve shellfish from commercial production areas, which include the need for a sampling plan for the monitoring of phytoplankton and toxin levels in shellfish. These requirements are codified as EU regulations (European Union, 2004) and adopted and codified by the member countries. The EU regulations also provide guidance on a uniform procedure for enumeration of phytoplankton (European Union, 2006). This regulation does not address sampling methodology, but focuses on laboratory procedures for microscopic algal analyses and statistical procedures for the design, optimization, and validation of methods and protocols. EU member countries develop their region-specific management plans for marine toxins that contain details on sampling sites, sampling frequency, sampling methodology, and all of the other region-specific information necessary to manage effectively the occurrence of marine toxins in shellfish to protect public health.

The NSSP Model Ordinance also prescribes the method or methods that may be used by the regulatory laboratories for analysis of the various toxins of concern. The biotoxin methods are subdivided into categories of "Approved for Use" and "Approved for Limited Use." By definition, methods that are Approved for Use are the primary methods of the NSSP. Such methods have been documented in peer-reviewed scientific literature, have been in use historically by the NSSP and other authorities, and have been determined fit for purpose through that historic use or through at least a single-laboratory validation (SLV) study and preferably a multilaboratory collaborative study. Methods that have been Approved for Limited Use are alternative, permanent methods that have been through at least an SLV study but have had limited application or have some limitation associated with their application. The latter would include methods approved for use for the specific shellfish matrices used in the SLV and/or collaborative studies. In this case, the method may be used in place of the primary method, but only for those accepted matrices. Other limited-use methods would include qualitative screening tests such as the Scotia Rapid Testing Ltd. PSP test kit, which is approved for use with the AOAC (Association of Analytical Communities) extraction used in the mouse bioassay (MBA). The ISSC/NSSP approved this test kit for screening and eliminating negative samples from the need to test with the MBA. Positive samples must be tested immediately by the MBA, and the associated harvest area placed in the closed status until the quantitative result is reported and the official harvest status determined. Until recently, the MBA has been the only method approved by the ISSC for PSP testing. An alternative method for the PSP toxins using high-performance liquid chromatography (HPLC) with post-column oxidation (PCOX), referred to as the HPLC-PCOX method (Rourke *et al.*, 2008), was approved for limited use in 2009. Another alternative method, a receptor binding assay (RBA; Van Dolah *et al.*, 2012), was assigned a split status in 2013 by the ISSC/NSSP, being approved as an alternative to the MBA for mussels and approved for limited use for PSP testing of scallops and clams. Each of these two methods lacked representation of all the shellfish matrices of regulatory concern in the respective validation studies, hence the limited-use designation. Matrix extension studies are underway, however, and if these successfully pass the ISSC approval process, the methods will be designated as Approved for Use. The State of Maine has replaced the MBA with the HPLC-PCOX method, and several states are evaluating this method and/or the RBA for replacement of the live-animal assay. Completion and approval of the current shellfish matrix extension studies will make it easier for more states to replace the MBA. It should be noted that the EU approved a pre-column oxidation HPLC method, referred to as the Lawrence HPLC method (AOAC 2005.06), as an alternative to the MBA for PSP testing in 2005. The MBA remains the reference method at this time. The analytical choices for domoic acid analysis are much simpler, as only the HPLC method coupled with UV detection (Quilliam *et al.*, 1995) has been approved for use by the NSSP. This HPLC method is the EU reference method, with an alternative method being the Biosense enzyme-linked immunosorbent assay (ELISA). The NSSP does not currently have an approved method of analysis for the toxins associated with DSP, although a mouse bioassay has traditionally been used and a liquid chromatography–tandem mass spectrometry (LC-MS/MS) method is in the early stages of validation for eventual ISSC consideration. An LC-MS/MS method for the DSP toxins (McNabb *et al.*, 2005) has been approved by the EU as the reference method, and a protein phosphatase inhibition assay (PPIA) by Zeulab is a supplementary method for the OA group of lipophilic toxins.

Although the NSSP requirements discussed above apply only to commercial harvesting and not to recreational and subsistence harvest activities, the monitoring of the latter resources is

usually conducted by the same organization(s) responsible for oversight of commercial shellfish harvesting. It is most beneficial to have a coordinated monitoring program for recreational and commercial shellfish resources, generating comparable data that provide a broader scale picture of harmful algal species distribution and toxin occurrence. Therefore, the same methodology for sampling, analysis, and harvest closures and reopenings will apply. There is likely a greater variety of shellfish species being harvested recreationally than commercially, increasing the complexity and demands for monitoring and public health protection.

The actual design and capacity of a marine biotoxin monitoring program will differ for each circumstance, depending on such things as the history of harmful algal events in the region and the toxins involved; the diversity of species and habitat, abundance, and geographic extent of shellfish and other seafood resources potentially impacted; how those resources are utilized (e.g., recreational versus commercial harvesting); and the laboratory and administrative resources available to the responsible agency to coordinate and conduct the necessary field sampling, to process and analyze samples, and to carry out the associated administrative tasks. The analytical component is significant in terms of initial cost to equip a laboratory and validate one or more of the approved methods described previously. Each of these methods varies in the amount of time needed to extract and prepare samples for analysis, the actual analysis time per sample, and the ability to automate at least a portion of the analytical process. All of these factors affect the throughput of the laboratory, an important consideration when planning a sampling program and developing response and control measures.

The majority of long-standing monitoring programs were developed in response to a history, as well as an increasing awareness, of recurring poisoning events in the region. Such was the case in California when a large PSP outbreak in 1927 spawned the initiation of routine monitoring along a portion of the coast associated with the poisoning of recreational mussel harvesters. In reporting on this particular episode, Myer *et al.* (1928) commented on the large amount of anecdotal evidence of past poisonings along the mostly rural coastline of California and noted that the Pomo Tribe was aware of mussel poisoning well before the influx of settlers. Washington State initiated monitoring of commercial shellfish several years after the 1927 California outbreak, expanding to include recreational shellfish in the 1990s (Trainer *et al.*, 2015). On the east coast of the U.S., monitoring for PSP began in Maine in 1957 following a Canadian outbreak. John Hurst, of the Maine Department of Marine Resources, developed and expanded this plan to a more comprehensive monitoring program for the entire coast in 1975 (Shumway *et al.*, 1988; Bean *et al.*, 2005). More recently initiated monitoring programs have been developed due to the sudden occurrence of a toxin not previously known to exist in the region, often associated with significant illnesses and deaths from consumption of contaminated seafood (Rodrigue *et al.*, 1990; Llewellyn *et al.*, 2002; García *et al.*, 2004). In addition, some existing programs have been augmented, typically involving the addition of phytoplankton monitoring, to improve early warning capabilities or to address the occurrence of new toxins or toxin-producing species (Langlois, 2001; Martin *et al.*, 2009; McIntyre *et al.*, 2013). Following the identification of domoic acid in shellfish and other seafood along the west coast of the U.S. in the fall of 1991, California developed a volunteer-based phytoplankton monitoring program with guidance and support from the FDA. Maine and other states likewise saw the need to augment their shellfish monitoring programs and developed phytoplankton monitoring programs during the 1990s. In 1999, NOAA created its phytoplankton monitoring network (www.pmn.noaa.gov) to help states that lacked resources to develop local volunteer networks, providing the expertise, training, equipment, and infrastructure for data management and reporting. In the 2000s, additional collaborative programs were created in numerous states, including Florida (Florida Fish and Wildlife Research Institute), the Olympic coastal region of Washington State (ORHAB; www.orhab.org), and states on the Gulf of Mexico (www.gulfofmexicoalliance.org), establishing routine phytoplankton monitoring and conducting research on HAB in partnership with NOAA and local universities. A number of community-based phytoplankton monitoring programs have been developed in several states, often as part of a larger environmental monitoring effort. Each of these phytoplankton monitoring programs has evolved to meet the unique needs of their region; however, the one common element is the reliance on citizen volunteers for sample collection and, in some cases, conducting the microscopic examinations for

identification of harmful species (see Section 10.6.1, "Diversifying Program Participation: Volunteer Monitors").

The EU requires monitoring for the presence of toxin-producing plankton in commercial shellfish production waters (European Union, 2004), while the NSSP recommends, but does not require, phytoplankton monitoring to be conducted as part of an early warning system. Because phytoplankton monitoring is not a regulatory tool under the NSSP, there are not specific approved methodologies for sampling and observation or enumeration of toxin-producing species. With one exception, the NSSP does not contain triggers for regulatory action based on cell densities. For NSP monitoring in the U.S., a cell density for *Karenia brevis* greater than 5000 cells/L requires harvest closure. States may establish their own thresholds based on quantitative or qualitative criteria. The Washington State ORHAB program is responsible for phytoplankton monitoring, with an action level of 50,000 cells/L of large-celled *Pseudo-nitzschia*, or the presence of *Alexandrium* at any density, triggering the collection of shellfish samples (Trainer *et al.*, 2015), or the increased frequency of sampling if monitoring was already in effect, by the Washington Department of Health. In California, phytoplankton and shellfish monitoring are integrated within the biotoxin monitoring program of the state public health agency, facilitating the immediate comparison of trends in the relative abundance of *Pseudo-nitzschia* with domoic acid levels in shellfish samples; any observation of *Alexandrium* or positive PSP sample triggers a response for increased sampling in and around the affected area. Maine has established a level of 15,000 cells/L for *Pseudo-nitzschia*, which triggers the use of a field test kit for domoic acid in the plankton sample. A positive result triggers a resample five days later that, if positive, triggers shellfish sampling in the affected area. Triggers based on cell densities are the norm in EU and other countries (e.g., Australia, New Zealand). Typical action levels for *Pseudo-nitzschia* range from 50,000 cells/L (Scotland; New South Wales and Western Australia) to 150,000 cells/L (Victoria, Australia; Denmark). The Center for Environment Fisheries and Aquaculture Science (CEFAS) in the United Kingdom has established a trigger level of 40 cells/L for *Alexandrium* spp., with other countries establishing thresholds of > 0 cells/L (Wales, England, Northern Ireland), 200 cells/L (Australia), and 1000 cells/L (Denmark). Action levels for *Dinophysis* spp. range from 100 cells/L (Denmark, Scotland, Wales, England) to 1000 cells/L (Western Australia).

Organizational structure of marine biotoxin monitoring programs can vary considerably. In the U.S., these programs most often reside within the state public health agency, but may exist within an agricultural or fisheries resource agency. Local and regional health agencies may also be involved, with the federal FDA becoming involved if contaminated shellfish has entered interstate commerce. Other organizational models exist, with multiple agencies and research organizations sharing the duties of shellfish and phytoplankton monitoring, implementation and enforcement of shellfish harvesting closures, and the issuance of health alerts via press outlets and educational messaging. The agency, or agencies, acting as the shellfish control authority under the NSSP would be responsible for developing and maintaining the biotoxin contingency plan. EU member countries develop and maintain marine biotoxin management plans and associated food safety regulations based on EU regulations. In many cases, there are individual regional plans within a country to address specific concerns and varying risks. In Australia and New Zealand, for example, a national program similar to the NSSP exists, with each state responsible for developing a marine biotoxin management plan. A unique aspect of this program is that significant responsibility is placed on the industry for managing the risk of marine biotoxins in shellfish, with oversight and regulation by the government (Victorian Fisheries Authority, 2015: http://agriculture.vic.gov.au/fisheries/aquaculture/publications/shellfish-quality-asurance/marine-biotoxin-management-plan). It is likely that any marine biotoxin monitoring program will have responsibilities and expertise shared among multiple organizations; therefore, it is essential to establish a system of effective communication with clear lines of responsibility among all the stakeholders to ensure a rapid response to a biotoxin event for public health protection.

It is expected that a newly created marine toxin monitoring program may initially focus on the most essential areas for public health protection, for example major areas of shellfish harvest and aquaculture, and that public health surveillance activities will focus on the most populated areas. Growth of the program should be planned, eventually reaching out to less populated areas or regions of prospective fishery and aquaculture activity. Foresight in development of a rigorous monitoring program can encourage and support

the development of sustainable fisheries, benefiting local economies that directly or indirectly rely on these resources. Regardless of their origins, the regulatory monitoring programs in existence for public health protection focus on product testing, which involves the routine sampling and analysis of high-risk seafood species before they are distributed for public consumption.

Bivalve shellfish are the most common vector associated with human illness from phytoplankton-produced marine toxins. Consequently, bivalve molluscan shellfish have been the focus of most regulatory monitoring programs for public health protection. While the following discussion focuses on bivalve shellfish, it is recognized that other seafood species can present a significant risk of poisoning from marine biotoxins (Shumway, 1995) and should be considered for routine monitoring. Some of these non-bivalve species represent commercial or recreational fisheries that are primarily offshore and therefore represent unique challenges for existing monitoring programs, a topic that will be addressed in Section 10.5, "Monitoring Other Fisheries."

As newly occurring toxins have been identified in different parts of the world, the demands on monitoring program resources have increased dramatically. Faced with testing for multiple toxins, and lacking the resources to test every high-risk seafood species for every toxin, public health monitoring programs have looked to more efficient tools to focus their sampling efforts. An obvious place to start is at the base of the marine food chain, expanding coastal monitoring efforts to include the examination of phytoplankton species composition and abundance. This leads to questions about the causative factors associated with their presence and population growth (see Chapter 1). Monitoring programs may find it useful to include data acquisition from stationary oceanographic buoys (e.g., sea surface temperature, wind speed and direction), satellite imagery (sea surface temperature, ocean color associated with chlorophyll-*a* levels), or highly sophisticated moored buoys capable of conducting onboard assays for the detection and enumeration of toxigenic species (Greenfield *et al.*, 2008) or testing seawater or cells for particulate (Doucette *et al.*, 2009) or dissolved toxin. The use of toxin-capturing resins can provide useful information on the presence of dissolved toxins, particularly in areas where shellfish are not naturally occurring, and may aid in tracking the source of toxins in the case of freshwater inputs (MacKenzie *et al.*, 2004; Lane *et al.*, 2010).

A monitoring program for harmful algae and marine toxins can vary from a simple, grassroots approach to a technologically sophisticated effort. It may consist of a few key sampling stations to dozens or hundreds of sites. A well-designed monitoring program will be successful at protecting the public from marine toxin-contaminated seafood and protecting the businesses dependent on the marketing of seafood. This, in turn, will have the added benefit of increasing consumer confidence in the safety of local seafood resources. To develop and maintain this trust in the safety of local seafood, the monitoring program must have an effective system for early detection of harmful algal events, increasing sampling and laboratory capacity with little notice, data management and quality assurance, control of potentially contaminated product, and alerting the public in a timely manner that a potential health risk exists.

While the major effort of marine toxin monitoring programs has been the protection of the public from consuming shellfish and other seafood contaminated with a known toxin, there are other applications and benefits. Phytoplankton are increasingly being used for monitoring and assessing environmental impacts and change, in both freshwater and coastal marine environments. A visible phytoplankton bloom is often a cause for concern and alarm in coastal communities. These "red tides" may or may not be associated with a toxin-producing species, but the public often assumes the latter. A nontoxic bloom can have a significant negative economic impact as the wary public avoids the shoreline and local businesses. Seafood consumption in general may be impacted, even if the product originates well outside the bloom area. Knowledge of the species involved through a routine monitoring program can help minimize public concern and the impact to local economies. Engaging the public in phytoplankton and shellfish monitoring is an opportunity for education and outreach, as is discussed in Section 10.6, "Novel Approaches and Advanced Tools to Enhance Monitoring Programs." Routine phytoplankton monitoring can also be protective of public drinking water sources when focused around the site of a desalination plant (Villacorte *et al.*, 2015). Operators can be alerted when a bloom of a particular harmful species is initiating so that preventative measures can be taken to protect the plant and the drinking water supply.

10.2 Identifying Sampling Program Needs

A variety of background information is necessary for guiding the design of a reliable monitoring program. It is assumed that some knowledge exists for the distribution of toxins, and perhaps the toxin-producing species of phytoplankton, that are present in the region, as well as the seasonality of each. Regardless, an assessment of risk should be conducted with respect to species and locations to guide the design of the sampling program. The less that is known, the more exploratory sampling may be required to develop the necessary experience and time series of data for refining the program design. Some information on the parameters above may be available from local universities, existing environmental monitoring programs, or ongoing special studies, and from a review of the scientific literature.

Decisions will need to be made regarding the type(s) of monitoring data needed (shellfish toxins, phytoplankton, physical and chemical parameters, large-scale oceanographic data), where and when to collect it, and at what frequency. It may not be feasible, or necessary, to sample in some months for areas with extreme winter weather; it is likely that the public will not have access to the resource, and historical data may support the absence of toxicity or poisonings during these periods. In these circumstances, the monitoring program may be seasonal, focusing on the months when shellfish resources represent a potential risk for marine biotoxin poisoning. In more temperate areas, there will be a need for monitoring throughout the year, with sampling intensity being dictated by the relative risk at different times of the year.

An assessment should be made of the existing programmatic resources available to support the sampling program. This will include the personnel time and costs associated with the collection of samples, transporting samples to the laboratory, the processing and analysis of samples for one or more toxins of concern, phytoplankton observations, data processing, quality control measures for each step of this process, preparation of reports and public health alerts and press releases, and managing the closure and reopening of harvest areas. An accurate assessment of the resources available to the program will guide the initial scale of the monitoring effort. It is better to start small with a limited number of stations, expanding with experience as funding allows, than to create an overly ambitious program that is unsustainable.

A survey should initially be conducted to establish the geographic location, habitat, abundance, and accessibility of commercial and recreational bivalve shellfish resources of concern. The location of bivalve populations will help determine the type and frequency of monitoring that will be feasible and most effective. Potential phytoplankton sampling locations in proximity to shellfish resources should also be identified in the survey. The distance of resources and prospective sampling locations should be noted with respect to the time and means of transport to the laboratory. One goal for an effective monitoring program is to achieve a rapid turn-around time between sample collection, delivery to the laboratory, sample processing and testing, and the availability of verified results. Shellfish resources and associated sampling stations within a short distance of the laboratory can potentially be sampled and delivered in time for an analytical result to be available by the end of the day. If the resources are a significant distance away from the laboratory, special handling and transportation procedures will be required, resulting in turn-around times of two or more days. The NSSP Model Ordinance Laboratory Evaluation Checklist for PSP testing requires that the time from collection to initiation of the sample extraction should not exceed 24 hours, but provides for those situations where distance and transportation time prevent the sampler from meeting this time frame. These contingencies are detailed in the discussion on shellfish sampling. Although there is no regulatory holding time for phytoplankton samples, the goal is to conduct an examination of the sample as quickly as possible to allow early detection of the appearance or increase of toxin producers. Initial observations of the live sample can be made in the field with portable microscopes developed for this purpose. It will, however, usually be necessary to add a preservative to the sample prior to transport to the laboratory. Observations of live versus preserved samples can differ significantly, as long-chain-forming diatoms and motile dinoflagellates can remain in suspension and thus be undersampled in the former. It is therefore advisable to reexamine field-identified samples in the laboratory after preservation and settling. Conversely, some species, like the unarmored dinoflagellates *Cochlodinium* and *Akashiwo*, may lyse upon preservation and will be

underrepresented or completely missed, so an initial field observation of the live sample can help to document their presence.

The habitat occupied by the shellfish species of interest will dictate the amount of effort needed for collecting samples and associated data. Intertidal mussels can easily be pried from rocks and pier pilings on low tides, with sample collection taking less than 30 minutes. Intertidal benthic clams, particularly deep burrowers such as butter and horseneck clams, can take significantly longer, often requiring the entire low tide to obtain an adequate sample. Subtidal populations will require significantly more programmatic resources, possibly including certified divers, boats, and specialized sampling gear such as dredges for benthic clams or the specialized hydraulic systems used to liquefy the sediment for harvesting deep-burrowing subtidal and intertidal geoduck clams (*Panopea generosa*).

If comprehensive regional coverage is not feasible, focusing sampling effort on areas of greatest shellfish abundance will at least represent the majority of resources being harvested recreationally and will be the most efficient for sample collection. The relevant resource agency can provide additional guidance on the areas with the highest population densities that are popular with harvesters. Commercial harvesting and aquaculture operations are typically licensed to operate by one or more governmental agencies; therefore, information on their location, the list of species they are approved to harvest, and harvest quantities are readily available. All of this information can be useful for establishing sampling stations that are representative of the harvest area.

Accessibility to the shellfish resource, particularly those used by recreational harvesters, is a function of the habitat (intertidal versus subtidal), as well as tidal and climatic conditions. Organisms found in the lower intertidal zone may only be reachable on exceptional low tides, limiting sampling to a period of several days per one or two low tide sequences each month, at best. There may be no reasonable access for several months, due to either inadequate low tides or inclement weather. It is presumed that these limitations also prevent the recreational harvester from accessing the resource during times when monitoring is not feasible. Unfortunately, the interruption of the time series of data interferes with early detection and/or tracking of an event.

10.3 Developing a Sampling Program for Shellfish Monitoring

10.3.1 Shellfish Sampling Stations

Sampling stations should be established at representative locations to protect the recreational/subsistence and commercial shellfish resources identified in the initial survey. For monitoring marine biotoxins over a broad area, a common approach is to establish primary and secondary sampling stations. Primary stations, or *key stations* in NSSP terminology, are sampled at some routine frequency and are intended to provide the earliest warning of a potential toxic bloom. Over time, these regularly sampled sites will also provide the time series of data that is necessary for understanding existing and changing trends in toxin distribution. When harmful algal blooms (HAB) are known to originate offshore or alongshore, as opposed to originating inside protected bodies of water, these primary stations will be located along the open coast. Secondary stations may be established inside bays and estuaries where an existing coastal harmful algal event may be transported (Shumway *et al.*, 1988). This approach won't always be applicable, as large interior bodies of water such as Puget Sound in Washington State can experience harmful algal increases and significant shellfish toxin levels that can be disconnected from coastal processes. Cox *et al.* (2008) reported that some PSP events in Puget Sound may be related to local cyst beds, and Anderson *et al.* (2008) noted the possible link of anthropogenic sources of nutrients and HAB that initiate within the Sound. Secondary stations may also be established to provide greater spatial resolution in conjunction with primary stations, or to represent additional shellfish species in the area. These secondary sites would be activated when samples from the associated primary station show the first signs of toxin occurrence or the presence of toxin-producing phytoplankton. Environmental heterogeneity may influence the number of sampling stations needed. Target species for routine monitoring will differ with habitat; therefore, sampling stations should be selected to represent these different areas. For example, the Pacific coast of the U.S. consists of a mix of rocky intertidal habitat with an abundance of mussels (*Mytilus californianus*) and sandy beaches where razor clams and other bivalves can be abundant. Stations may be

needed to reflect the various species in different habitats, even if they are in close proximity. Ideally one primary species, known to concentrate the toxin(s) of concern rapidly, is available to be sampled throughout the region of interest. The patchiness of phytoplankton distribution and abundance will also affect the number of sampling stations needed in a region. A long stretch of homogeneous habitat and shellfish, for example miles of rocky intertidal mussels, may not experience the same impact from a harmful algal event. Unique local features such as freshwater inputs, wastewater treatment plant discharges, and topographical differences such as points of land that serve as upwelling centers or, conversely, protected areas that serve as "upwelling shadows" that retain a water mass for a long period of time (Ryan *et al.*, 2009) can all result in patchiness in the species composition of the local phytoplankton community.

Routine sampling should also be conducted in the bays and estuaries used for digging clams or harvesting mussels and oysters. One or more sampling stations should be established, based on the distribution of the various shellfish species and the history of toxin distribution and magnitude. If there is unequivocal evidence that toxin-producing algal blooms do not originate in these protected waters, but are transported from the open coast, it can be advantageous to have one or more primary sampling stations near the entrance to a semi-enclosed body of water to provide an advance warning of a toxin increase. A positive result at one or more outer stations can prompt the sampling of secondary stations within the bay. In larger semi-enclosed bodies of water, it may be possible to have a tiered approach to activating secondary sampling sites, beginning in the outer reaches and, if toxin levels increase or transport of the toxin producer is observed, extending further inside the bay.

An adjunct to the traditional sampling of naturally occurring populations of shellfish is the use of sentinel stations. In general, a sentinel station consists of a cage or mesh bag containing the sentinel species to be used for early detection of toxicity. The ideal sentinel organism for routine toxin monitoring will model the relative risk associated with a harmful algal event, with toxicity increasing in the sentinel species as cell numbers increase, followed by a near-synchronous decrease in toxin concentration as toxin-producing cell numbers decline and return to baseline levels. This sentinel species should also be sensitive to the presence of low cell densities of toxin-producing species that, despite sub-bloom cell numbers, can produce dangerous levels of toxin. Mussels are the most common species used for this purpose. Collection of individuals for stocking the sentinel station should be conducted in the same general area that the sentinel bag will be deployed to prevent the movement and introduction of other species or disease organisms. The mesh size of the sentinel bag or cage is not critical but should be small enough to exclude common predators like starfish and crab while allowing water to flow freely through the container. Smaller predators such as the "oyster drill" snail (*Urosalpinx cinerea*) are difficult to exclude and must be periodically removed. Maintenance of the sentinel bags or cages is also necessary to remove fouling organisms and plants that can prevent adequate water circulation needed to ensure the mussels can feed and thus accurately represent toxin levels in the area.

There are different configurations for the sentinel station. One or more sentinel containers can be staged with a large number of mussels in each that can be subsampled as needed. Alternatively, a larger number of containers, each with enough individuals for one sample, can be deployed, allowing for the quick retrieval of an individual sample. In the latter case, it is advisable to include extra individuals to compensate for mortality or occasional predation. The container can be suspended from a pier, boat dock, or buoy, or affixed to a stake or anchor in shallower waters. This arrangement permits ready access for sample collection regardless of tides or weather, allowing the program to increase sampling frequency in high-risk areas. Site selection is critical for a sentinel station and should represent the resources in the surrounding area. In a bay or estuary with a predominance of benthic clams (butter clams, horseneck clams, littleneck clams), it can be much easier to sample a sentinel mussel station representing the area. If toxin is detected in sentinel mussels, then the more laborious and time-consuming collection of clam samples can be initiated. Sentinel stations are most successful where there is no, or limited, public access, reducing the loss of materials due to theft or vandalism. A side benefit to sentinel bags of mussels is their potential to act as nurseries and refuges for small fish and invertebrates. This tiny community can be an entertaining teaching tool when educating school and environmental groups about marine biotoxins and monitoring efforts in their local coastal area.

Areas of commercial shellfish harvest or aquaculture are required to be sampled routinely. Fortunately, these samples are usually the easiest to obtain, as it is a common regulatory requirement for the shellfish industry to provide representative samples of harvest areas and species on a regular basis and more frequently as needed. The easiest point of sampling is the newly landed harvest lots of shellfish. If harvest locations vary, this sampling strategy will potentially result in a poor time series of data if the different harvest areas represent higher or lower risks for toxin occurrence. This will make it difficult to reliably track the presence, increase, and transport of toxins in the region. To improve data quality for developing reliable time series and tracking data, it is best whenever possible to establish fixed primary sampling locations that represent a broader harvest area. Sampling at these stations may focus on the shellfish species being harvested (e.g., oysters) or may include representative samples of all high-risk species present. The use of strategically located sentinel mussel stations can add a layer of protection to the monitoring program, providing an early warning of the initial presence of toxin before commercial oyster or clam beds are affected. The number of stations needed will be dictated by the number of companies and the diversity of the area relative to toxin occurrence. The number of high-risk species in the region will also have to be considered when establishing sampling stations. In some situations, the shellfish industry may have the primary responsibility for sampling and ensuring that samples arrive at the laboratory in a timely manner to allow for analysis and possible response actions. Their agreed-upon responsibilities will be detailed in the biotoxin contingency plan.

10.3.2 Monitoring Shellfish Toxicity

It is unlikely that all shellfish species in a region, whether commercial or recreational, can be routinely monitored because of the effort and cost involved. If it is known that different shellfish species in an area accumulate marine biotoxins at different rates, the species can be ranked in order of risk to the consumer. This risk information can be used to focus the sampling program and also can guide the potential for species-specific harvest closures. It should be noted that this ranking may be different for the different toxins of concern, potentially complicating the sampling program design. Ideally, the species representing the highest risk will also be ubiquitous throughout the region to be monitored, or can be deployed as a sentinel station, so that it can serve as the primary sample type, or indicator species. Mussels are used by many different monitoring programs for just that reason. They are widely distributed, are very efficient at concentrating many different marine biotoxins, and are also capable of depurating the toxin quickly once the toxin-producing cells have returned to low or undetectable densities. Mussels are also very resilient and have a high survival rate when harvested and transplanted in sentinel stations.

The NSSP does not prescribe specific sampling frequencies for shellfish toxin testing in commercial growing areas, leaving it to the state authorities to determine the appropriate interval between samples. The EU regulations specify at least weekly sampling of harvest areas for biotoxin testing, with a provision for less frequent sampling in certain areas, or of certain species, if they are determined to be of low risk based on a risk assessment that is periodically reviewed and updated. In practice, weekly sampling of primary stations representing harvestable areas is common. In addition, sampling should be conducted as early in the week as possible to allow for sample transport, analysis, and initiation of follow-up sampling and possible control actions in the event of toxin presence. It is advantageous to monitor routinely all primary stations to maintain a rigorous time series of toxin data regardless of whether there is active harvesting occurring. In response to the detection of toxins or an increase in the toxin-producing algal species, sampling frequency should be increased to multiple samples per week, and may include additional species and/or secondary stations. Various site-specific control measures that have been established as part of a contingency plan may be implemented, such as a tiered approach in managing toxin levels in individual species as discussed in Section 10.7, “Management Considerations.”

The frequency of sampling for primary recreational stations will often be lower than achieved in commercial shellfish areas due to more limited access to the resource or lack of programmatic resources to travel long distances to sample. For the latter, the use of local trained volunteers for sample collection and shipment to the laboratory can significantly strengthen the sampling program. Details on the use of citizen scientists is discussed below in Section 10.6.1, “Diversifying Program Participation: Volunteer Monitors” (and see Chapter 11). The desired sampling frequency will also be dictated in part by the survey information gathered on the historical distribution and

magnitude of toxins and toxin-producing phytoplankton, and the ability of the shellfish species in a given area to concentrate the toxin(s) of concern. Realistically, routine sampling of representative sites for protection of recreational and subsistence harvesting areas may only be feasible once or twice per month, as tidal conditions and resources allow. As mentioned for commercial area management, a tiered approach can be taken, with secondary stations and species being sampled only when toxins or toxin-producing phytoplankton are detected at primary stations. Even this approach can be problematic for secondary sampling stations that have limited access. If a sample result approaching a toxin action level is reported and follow-up samples cannot be obtained for several days or more, a preemptive health alert or recreational harvest closure may be necessary to alert the public. When follow-up samples are finally obtained and analyzed, the alert can be adjusted as warranted.

The minimum number of individuals that constitute a sample is determined by regulatory requirements and the requirements of the analytical method(s) to be employed. For the sake of minimizing sampling error, it is preferable to obtain as many individuals, and as much tissue, as is practical for the laboratory to process. The NSSP requires at least 12 individuals, or more if necessary, to provide a minimum of 200 g of tissue. Smaller species like littleneck clams (*Tapes semidescussata*) may require 50 or more individuals. Specific biotoxin management protocols of local control authorities may specify a minimum number per species required for a representative sample. The Scottish biotoxin protocol, for example, calls for 100 individuals for rope-cultured mussels (1.0 kg) and 1.5 kg for shore mussels (Food Standards Agency Scotland, 2009). The NSSP-required minimum sample mass of 200 g will provide enough material for multiple toxin analyses (e.g., PSP, domoic acid, DSP), as well as for re-analysis should there be a laboratory accident or a questionable result requiring retesting. The individual size of shellfish being collected should be representative of the typical harvest size for that species. Selecting individuals that are significantly smaller or larger than harvest size can bias the results and be unrepresentative of the shellfish being consumed by the public. Individual shellfish should also be healthy (e.g., not gaping). The shellfish collected should be rinsed to remove any mud or debris on the shells and placed in a clean, waterproof container that can be sealed and labeled with the collector's name, type of shellstock, harvest area, sampling station, date, and time. The sample should immediately be placed in dry storage, such as an ice chest, with enough ice packs or wet ice to maintain the temperature between 0 and 10 °C throughout transport to the laboratory. The time from sample collection to extraction should not exceed 24 hours. In many cases, this holding time may not be feasible, for example at remote sampling stations. These exceptions will be known in advance and can be addressed by the development of a laboratory contingency plan agreed upon by the laboratory and the sampling program. For samples shipped live, the provisions of the contingency plan must ensure that samples remain within the holding temperature range and animals are alive upon receipt. As an alternative, the contingency plan can address field and/or laboratory-processing procedures that ensure the integrity of the sample, or sample extract, until initiation of the assay. For example, samples are washed, shucked, drained, and (a) refrigerated or frozen until extracted; or (b) homogenized and frozen until extracted; or (c) extracted, with the supernatant decanted and refrigerated or frozen until assayed. Individual shellfish damaged during shucking should be discarded.

Another exception to the basic sampling requirements above concerns the number of individuals per sample. In the case of larger bivalves, for example geoduck and butter clams, it is impractical to collect, homogenize, and extract 12 individuals. The contingency plan can document these exceptions and provide details on alternative sample sizes and processing procedures. For example, it is impractical to apply the standard composite sample size for the larger bivalve species such as the butter clams, horseneck or gaper clams, geoducks, and surf clams. The Washington Department of Health requires providing three to six geoduck clams to the laboratory, where the stomachs (referred to as *gut balls*) are combined and homogenized as one sample, then tested for toxins of concern; the PSP toxins do not accumulate in the edible meat. In some circumstances, such as when little is known about toxin uptake and sequestration in a larger species, it may be more advantageous to analyze multiple specimens individually when they are large enough to meet the minimum sample mass needed for extraction. This approach can provide valuable data on the variability of toxin concentration among individuals, information that is lost when pooling samples. There can be a significant difference in toxin concentration among individuals

collected from the same site (White *et al.*, 1993), so this variability should be assessed for each species if the intent is to analyze individuals. A high degree of variability in toxin load among individuals would argue for a large number of individuals per sample to assess more accurately the risk to human health. Additional information can be gathered by separating tissues into edible (siphon, foot) and inedible (viscera, gills) subsamples for the larger species and analyzing individually; pooling similar tissue types is another option for smaller individuals or if analytical resources are limited. Knowing how toxins are sequestered may provide options for the safe marketing of the nontoxic tissues of a species (e.g., adductor muscles from certain scallop species; Shumway and Cembella, 1993). While it is strongly advised that the requirements for testing of commercial shellfish be applied to recreational species, there may be more latitude for the latter with respect to sample numbers and sample volume. At times, it may not be possible to obtain the minimum number of individuals at a particular site due to accessibility or safety concerns; however, the information provided by a smaller sample may still be helpful in determining the presence of toxin and thus be of value for the goal of public health protection.

10.4 Developing a Sampling Program for Phytoplankton Monitoring

10.4.1 Phytoplankton Sampling Stations

Phytoplankton monitoring is intended to provide an early warning for the initial presence of a toxin-producing species and to assess an increase in population numbers signifying the potential start of a bloom reliably. This early warning can trigger the initiation, or an increased frequency, of shellfish sampling for toxin testing. With some genera, such as the dinoflagellate *Alexandrium* spp. that is responsible for production of PSP toxins, the presence of even low numbers of cells can result in significant toxin levels in shellfish. Observations from routine phytoplankton sampling also provide guidance on the toxin(s) that may be of immediate concern. As with the design of the shellfish sampling program, it is advantageous to have fixed primary stations representing recreational and commercial shellfish resources along the coast and inside bays and estuaries that were identified in the initial survey. Routine samples from set locations will allow the program to develop a time series of data on specific HAB events over many years for evaluating longer time scale patterns. Secondary stations can be established as needed for the purpose of increasing resolution of observations on the distribution and abundance of a toxin producer or another harmful algal species. Secondary stations may also be desirable for representing areas of reduced risk, for example far inside a bay or estuary beyond the normal transport of toxin-producing species. In this circumstance, these secondary stations may be activated when observations at primary stations in the outer reaches indicate a harmful algal species is being transported inside the body of water. Although the NSSP Model Ordinance does not require phytoplankton monitoring in commercial shellfish growing and harvesting areas, it is a mutually beneficial activity to engage the industry in this activity. Samples can be quickly and easily collected by work crews and, in some cases, a company staff person can be trained to conduct the field identification of the sample. In addition to providing an extra layer of protection from a harmful algal increase, the information gathered on other species present may be of use to the industry in understanding certain aspects of their shellfish farms, such as variability in the rates of growth, mortality, and seed survival.

A key factor in determining phytoplankton sampling station locations is safe access. Piers and boat docks provide stable platforms for sampling regardless of tides and weather. Sampling sandy beaches is feasible under calm ocean conditions and in areas where beach slope is gentle, avoiding the potential for deadly rip currents. Boat-based sampling provides increased flexibility in the selection of primary sampling locations and in collecting opportunistic samples during a harmful algal event. This approach, however, can represent a significant expense and is often not feasible given the budget constraints of most programs. Nonetheless, offshore stations can significantly improve the program capability for early detection of harmful algae; therefore, an effort should be made to identify other sampling resources capable of collecting these samples. Random offshore locations can also be incorporated in the sampling program by way of engaging seagoing program participants, such as fishermen, dive clubs, kayak clubs, and so on. Specific approaches to sampling these different areas and enlisting volunteer samplers are discussed later in Sections 10.4.2, “Monitoring

Phytoplankton," and 10.6.1, "Diversifying Program Participation: Volunteer Monitors."

Co-locating phytoplankton and shellfish sampling stations is desirable for establishing relationships between cell abundance and toxin concentrations in the shellfish. Developing this understanding can help the program anticipate the magnitude of impact to shellfish resources based on observed species abundance. Likewise, based on the maximum toxin levels reached and the rate of decline in the toxin producer, the program may be able to estimate the time needed for shellfish toxin concentration to return to safe levels. This information, in turn, can guide the determination of sampling frequency needed for this assessment, potentially saving program resources. For example, weekly or biweekly samples of razor clams contaminated with high levels of domoic acid may not be warranted when it is anticipated that it will take months for this species to return to safe toxin levels. The degree of correlation in data from paired phytoplankton and shellfish samples can also provide the needed feedback for adjusting sampling frequency of both. A poor correlation would indicate the need for more frequent phytoplankton samples, or an adjustment to the sampling procedures for the less abundant toxin producers, to improve the predictive capabilities.

10.4.2 Monitoring Phytoplankton

The program design for phytoplankton monitoring will be based on the risk assessment for species of concern. The important elements in this design will include the geographic and seasonal trends for distribution and blooms of the species of concern, the frequency of occurrence and rates of increase and decrease, and a determination of the type and quality of data required. Data quality objectives include (1) a determination of sampling frequency; (2) whether quantitative or qualitative data are needed for species abundance; (3) whether the objective is to identify only the toxin-producing species, all suspect HAB species, or all species present; and (4) a decision about the taxonomic level at which cells should be identified: genus or species (or a combination of the two).

As with shellfish sampling for toxin analyses, the more frequently phytoplankton is monitored, the more likely an increase in a harmful algal species with be detected, triggering increased monitoring of shellfish toxin levels in the affected area. Dynamic areas where coastal water masses have a very low residence time may experience a rapid turnover in phytoplankton composition. Multiple samples per week may be necessary to capture each shift in species accurately. In addition, the sampling frequency may vary by region based on the relative risk. Practically speaking, there are limits to what program resources can manage. In practice, weekly sampling of the primary stations is desirable in areas where toxin-producing phytoplankton have been observed or marine toxins have been detected. Biweekly or monthly sampling may be adequate in areas with no history of toxicity or in low-risk areas. Links to several example sampling protocols are provided at the end of this chapter.

Either quantitative or qualitative sampling and analysis methods, or a combination of each, can be used for collecting data on phytoplankton distribution and abundance. Each approach has its merits and limitations, and it is up to the program to decide which approach is the best fit based on data needs, as well as on sampling and laboratory resources that are available. Likewise, it may be determined that only the toxin-producing species will be monitored for expediency, or that all species observed will be recorded to provide a broader ecological picture of the phytoplankton community. The latter approach allows tracking of shifts in major assemblages (e.g., diatoms, dinoflagellates), which can provide insight into the likelihood of an increase in a given harmful algal species. A strong shift from a dinoflagellate-dominated community to diatoms may signal the potential for a bloom of *Pseudo-nitzschia*, allowing the program to focus sampling in regions where *Pseudo-nitzschia* and domoic acid have been most prevalent. Given the time-consuming nature of quantitative phytoplankton sampling and analysis, a combination of approaches may be warranted if large numbers of samples must be collected and processed. The program may use quantitative methods for toxin-producing species and qualitative methods for tracking the relative abundance of the rest of the phytoplankton assemblage.

Quantitative sampling methods involve the collection of rather small volumes of unconcentrated water and will produce an estimate of cell densities for each species identified in the sample, usually expressed as cells per liter (cells/L). Rare species can potentially be missed with this approach. A known volume of unconcentrated water, referred to as a *whole water sample*, must be collected at each sampling site. Because phytoplankton can be stratified relative to depth, the sampling method chosen should provide an accurate representation

of the entire water column, at least over the maximum depth of concern. There are several different sampling methods discussed below that can be used for this purpose. The best approach will be determined by the conditions at each sampling station, for example depth, currents, wind, and the distance of the sampler from the water surface. Multiple methods may be needed if a variety of different conditions exist among the sampling stations. Several programs collect a concentrated net tow for identification of toxigenic species in low abundance and a whole water sample for quantitation of known or suspect toxin producers. A standard operating procedure (SOP) should be created for each specific sampling method to ensure uniformity among those collecting samples.

For shallow-water stations, a simple grab sample can suffice. The CEFAS protocol uses grab samples in depths less than 2 m (approximately 6.5 ft.). A pole and water bottle sampler is recommended for collecting three grab samples at different depths from a beach. Discrete water samplers such as a Van Dorn or Niskin sampler can also be used when sampling from a pier or dock. In very shallow water, where it is not possible to collect subsamples at various depths, a bucket may be used. For stations in deeper water, the surface grab sample is not appropriate because it will not represent the variety or density of species that may be stratified subsurface. Conversely, this approach can overestimate cell densities for the water column if a given species is stratified at the surface. For deeper water, a common approach is to use a tube sampler to take a core of the water column. The tube can be of rigid PVC or a more flexible plastic material (e.g., Tygon© tubing) and must have a mechanism to stopper both ends of the tube before retrieving. In the case of the flexible tubing, it is also possible to have a line attached to the bottom of the tube, which can be used to lift the lower end to the surface, capturing the core of water. To collect a sample of the water column, the tube is gently lowered into the water, keeping it as vertical as possible and avoiding hitting or disturbing the bottom sediments. The flexible tubing will require a sizeable weight attached to the bottom to help reach the bottom and remain vertical. The top opening is closed by stopper or valve, and the bottom opening is either closed or retrieved to the surface and kept at the same height as the top of the tube prior to decanting. Alternatively, a Van Dorn or Niskin sampler can be used to collect discrete samples at a variety of depths. These samples can be analyzed individually for depth profile information, or they can be combined and mixed into one integrated sample.

For each method of collecting a whole water sample, the sampler will collect a larger volume than needed in the final sample. The initial sample will be decanted into a large, clean bucket and thoroughly mixed. A screw-cap sample bottle of standardized volume, typically 500 mL or 1 L, is immersed in the bucket and filled to the neck, allowing enough room for the addition of a fixative if needed. The bottle is removed from the bucket, fixative added, and the cap attached and tightened before removing from the water. The sample bottle is labeled with a unique sample identification number or code, the sampling location name, the date and time of collection, the collector's name, the depth or range of depths sampled, and the method of collection. For obvious reasons, it is a good idea to fill in all the label information, or affix a completed label, before immersing the sample bottle into the bucket of water. This information should be kept in a field sheet or logbook; ancillary data on water temperature, salinity, turbidity, tidal stage, wind speed, and direction should also be recorded.

Qualitative sampling methods involve some means to concentrate the phytoplankton assemblage present in an unmeasured volume of water, which increases the probability of observing a toxin producer that may be present in very low densities. The most common form of qualitative sampling involves the use of a plankton net, a conical fine-meshed nylon net with a metal ring attached to the mouth to keep the net open and a collecting bucket at the cod end to contain the concentrated sample. Some collecting buckets may have openings covered in the same mesh material to assist draining and concentrating the sample, but this feature is not required and represents a potential point of failure for the sampling equipment. A small ring (bridle ring) and crossbar, or three-cable system and bridle ring, are connected to the mouth ring for attaching a tow rope. A 20-μm mesh net is adequate for sampling most toxin producers of concern. A 10-μm mesh net may also be used, but it is more easily clogged and takes significantly longer to drain compared to the 20-μm mesh net. In shallow water, the net can be towed horizontally along a boat dock or in the surf zone or in tidal channels in calm conditions. For sandy beach surf zones, it is often more practical to collect buckets of seawater and pour these through a supported net. This technique allows most of the suspended sand particles to settle to the bottom of the bucket before pouring just the seawater

through the net, resulting in a much clearer sample. In deeper water, the net will be lowered into the water, allowing it to sink to the maximum desired depth. The collecting bucket at the cod end of the net routinely traps air and floats like a cork, so it is necessary to raise and lower the net to "burp" the air out so the entire net will sink. The net should never touch the bottom, as disturbed sediments will collect in the net, clogging it and resulting in a poor-quality sample. If unsure of the depth, a small weight attached to a fishing line or thin rope can be lowered to the bottom to provide an estimate. Once lowered to depth, the net will be retrieved as vertically as possible at a very slow rate. A "hand-over-hand" retrieval of the net, with each hand touching the other as a length of rope is grasped, will help ensure a slow retrieval. The net can be pulled out of the water and lowered again, repeating this process until an adequate sample has been collected. This can usually be determined by observing the rate at which the excess water in the net drains. As cells (or sediment) concentrate, it will take longer and longer for the net to drain. Alternatively, a set sampling effort can be established to ensure consistency. An equivalent of a 50-ft. tow (e.g., five net retrievals at a 10-ft. depth) is recommended as a starting point by the California Phytoplankton Monitoring Program (California Department of Public Health, 2016a). Exceptions occur, such as when sampling a bloom where a single short tow will result in a clogged net full of cells. Observing the rate at which the net drains after each vertical haul can guide the sampler on the number of tows to conduct. Net sampling from piers can be an adventure when currents and winds increase. The nylon net material is very fragile, so it is important to avoid contact with obstacles such as pier pilings and railings. If currents or wind push the net dangerously close to an obstacle, it is best to retrieve the net quickly and start over. Positioning oneself on the downwind or downcurrent side of the pier is usually advised. In windy conditions, it is a good idea to attach a weight to the cod end of the net for stability while lowering it and to facilitate sinking once in the water. A weight can also be gently placed inside the collecting bucket, something to remember when decanting the sample. Rather than pull the net out of the water and into the wind, the net can be "fished" by giving the rope a sharp tug as the mouth approaches the surface, then providing some slack line. This will cause the net to do a U-turn as it approaches the surface, the slack line helping sink the mouth of the net ahead of the cod end. A weight can be added to the bridle ring to help sink the mouth of the net. In this way, there is no risk of air getting trapped in the net. Once the net has been retrieved, the collecting bottle at the cod end is removed, and the sample is swirled or mixed thoroughly, then decanted into the sample bottle. A preservative can then be added (or the bottle pre-filled with the preservative), the cap attached, and the collection information recorded on the label and in the logbook. In addition to the collection data mentioned above, the depth at which the net was lowered and the total tow length (depth multiplied by the number of vertical retrievals) should be recorded. The net should be rinsed in freshwater as soon as possible and definitely before collecting at another location to prevent the transport of species. The cleaned net should be hung up to dry, avoiding exposure to direct sunlight.

There are some common aspects to both quantitative and qualitative sample collection, including the procedures used for sample preservation and observation. Unless the samples are observed immediately upon collection, it is advisable to preserve them immediately to minimize the number of cells/species lost. The more fragile species may disappear from the sample in just a few hours. If samples will not be preserved until arriving in the laboratory, they should be transported in an upright position in an insulated container with an ice pack to keep the inside of the insulated container cool. The container should be handled gently to minimize shaking of the sample, which can cause chained diatoms to break apart and may cause unarmored dinoflagellates to rupture or aggregate. There are a number of options for short-term and long-term preservation, with the choice dependent upon the types of cells being preserved. For short-term storage, either a neutral formaldehyde or an acidified Lugol's iodine solution will be adequate. The former will distort or destroy some of the unarmored dinoflagellates but is adequate for diatoms and armored dinoflagellates (Throndsen, 1978). Formaldehyde will bleach the cell pigments over time, degrading the quality of samples during long-term storage. Diatoms and dinoflagellates, both armored and unarmored, will be preserved adequately with Lugol's iodine. Cells will be stained yellow to brown, sometimes obscuring cell features and also making specimens less desirable for photography or fluorescence microscopy. The cell staining can be removed by treating the sample with a solution of sodium thiosulfate. Lugol's iodine adds weight to the cells, which will facilitate settling. This preservative does degrade over time, so long-term sample storage requires periodic replacement of the iodine solution. For

either solution, the quantity required for sample preservation will vary with the density of cell mass present. For short-term preservation, a 1–2% solution of formaldehyde or a 0.5% solution of Lugol's iodine is satisfactory.

Samples collected either qualitatively or quantitatively will be observed using basic light microscopy to identify the genera and species present. The standard quantitative approach for cell identification and enumeration is called the Utermöhl method, which uses an inverted microscope. This is a time-consuming process but can provide the highest quality data on species densities. The small volumes used, however, may result in the rarer species being overlooked or underestimated (Rodríguez-Ramos *et al.*, 2014). Cermeño *et al.* (2014) reported that these small-volume subsamples used for settling underestimated species richness by as much as 50%. This can be problematic if a toxin-producing phytoplankton of concern such as *Alexandrium* spp., when present, occurs at relatively low cell densities. It is recommended that a qualitative net tow sample also be collected at each whole water sample station. This concentrated sample can be examined quickly in the field or laboratory, as described below, to make a quick assessment of species composition, diversity, and cell densities. The net tow will be more likely to pick up those rare species that the whole water sample and Utermöhl method miss. The preliminary observations of the net tow can alert the observer to the presence of a toxin producer and will also guide the procedures for quantitative analysis of the sample. A thorough protocol for the Utermöhl method can be found in Edler and Elbrächter (2010). To summarize, a subsample of the quantitative whole water sample is transferred to a settling chamber of known volume, and the cells are allowed to settle in a counting chamber prior to microscopic examination. Each step in this process must be standardized to minimize variability in sample quantitation, and these procedures are documented in a standard operating protocol. The volume of the settling chamber used will vary with the density of cells in the sample: using too large a chamber volume for a dense phytoplankton sample will result in a mat of settled cells that will be impossible to identify and count. Common chamber volumes range from 2 to 100 mL; however, care should be taken when using the larger volume settling chambers (e.g., greater than 50 mL), as some species such as chain-forming diatoms can adhere to the chamber walls and convection currents can prevent some species from settling (Hasle, 1978). The settling chamber and counting chamber should be calibrated to determine their actual volume, as this can vary from the manufacturer's specification. All equipment and samples should be brought to room temperature. A standard mixing technique should be used for resuspending cells in the sample bottle prior to decanting into the settling chamber. The EU Standards for Phytoplankton (EU, 2004) specify that the sample should be mixed by hand, using a combination of rolling motion and rotating the bottle upside down for 2 minutes. The sample bottle should not be vigorously shaken or a vortex motion used. The mixed whole water sample is poured into the settling chamber, until just overflowing, and the top of the chamber sealed. The cells are allowed to settle into the counting chamber over a 12 to 24-hour period. There are a number of recommendations in the scientific literature concerning the appropriate settling time, many of them volume-dependent. Edler and Elbrächter (2010) recommend settling times from 3 hours for a 2 mL chamber to 24 hours for a 50 mL chamber for samples preserved with Lugol's iodine. Samples preserved with formalin will require longer settling times, and a minimum of 24 hours is a common recommendation. Once the sample has settled, the settling chamber is moved and a coverslip placed over the counting chamber, which will contain a known volume of sample (usually 1 mL). The slide is placed on the stage of the inverted microscope and a systematic approach taken to view the slide, identifying and counting all cells of interest. One of two different ocular inserts can be used to facilitate this process, one containing a grid (Whipple graticule) or one containing two parallel lines. Either can facilitate the systematic movement across the entire slide or the examination of a specific proportion of the slide when there are too many cells present. The ocular grid can be calibrated with a stage micrometer at each magnification for determining sizes of cells. A standard data form containing grouped lists (e.g., diatoms, dinoflagellates, cyanobacteria) of the most common genera and species of interest should be used to record cell counts. Volume corrections can be made to calculate the number of cells per liter for each species. To facilitate sample observations by program volunteers, the Maine phytoplankton monitoring program collects 10 L of seawater at a 1 m depth, which is filtered using a 20-μm sieve, then backwashed and collected to produce a final volume of 15 mL. A 1.0 mL portion is observed with light microscopy using a Sedgewick–Rafter slide, as described below.

Observation of the qualitative phytoplankton sample can be done in the field or in the laboratory. Field observations can be conducted with a portable microscope, an example of which is based on the original Macarthur field microscope developed in the 1930s and currently commercially available (Brunel Microscopes Ltd., Unit 2 Vincients Road, Bumpers Farm Industrial Estate, Chippenham, Wiltshire SN14 NQ, UK; tel: [+44] 01249 462655; www.brunelmicroscopes.co.uk). If observations and cell counts are conducted at the sampling site with a field microscope, the use of a flat glass capillary tube in place of the counting slide and coverslip can simplify the process of observing a sample in less than ideal conditions. A practical size for the capillary tube is 100 mm long with an internal height and width of 0.3 mm and 3.0 mm, respectively, providing a sample volume of approximately 0.1 mL. The tube is placed into the sample bottle, and the sample fills the tube by capillary action. Digital microscopy is also making it easier to observe samples in the field, and attachments are becoming available for use with smartphones.

Laboratory observations can be done with basic light microscopy, using a standard slide and coverslip and following the quantitative procedures above for sample mixing, systematic observation of the microscope slide, and data recording. There are two basic approaches for estimating the relative abundance of each species of concern for samples collected qualitatively. Similar to the quantitative approach for cell identification, cell counts can be made for each species identified. Enumeration of the species observed can be accomplished by using a Palmer–Maloney slide or a Sedgewick–Rafter slide to count cells in a known volume of the sample (0.1 mL and 1.0 mL, respectively). When cell numbers are too high to count on the entire slide, a grid in the ocular or on the slide can be helpful for observing a specific percentage of the sample. Maine standardizes the counting process by enumerating the cells of interest on 200 squares of the grid. Although the volume of water originally sampled by bucket or net tow is unknown, the relative abundance of each species identified can be calculated based on the total number of cells counted. Counting cells is a time-consuming process and may not be feasible if large numbers of samples must be observed. A second, more qualitative, but faster approach to estimating the relative abundance of each species forgoes the counting of cells. Using a microscope slide and coverslip, the entire slide should be observed systematically as previously described. The cells of interest should be identified and notations on relative abundance recorded on a data form as the analyst works through the slide. This can be in the form of tick marks, with a greater number signifying the most common species. These relative abundance notations will be refined as more of the slide is observed because the species present will probably not be uniformly distributed across the slide. After the sample observation is completed, an estimate of the percentage of each species identified can be made. Although it is a qualitative sample and the notations on relative abundance are also qualitative, it is possible to rank the abundance of each species observed relative to all other species in the sample. A simple ranking system for abundance can be used to assign a rank to each species observed. For example, the following ranking system is based on estimated percent composition (California Department of Public Health, 2016b): Rare (<1%), Present (1–9%), Common (10–49%), and Abundant (≥50%). To simplify the assignment of ranks for qualitative observations, the categories of Presence, Absence, and Elevated can be used for HAB species of concern and major groupings such as diatoms and dinoflagellates (NOAA). The latter approach also includes the collection and enumeration of whole water samples when the qualitative observations indicate a potential bloom of concern. Whichever ranking system is used, with experience the analyst will be consistent in estimating percent composition and assigning a relative abundance rank. It is important to standardize a new analyst when training to ensure consistency in identifications and ranking among analysts.

The ranking or estimated percent composition data can be very useful in tracking general trends over time for the increase or decrease of the species of concern. Changes in the general assemblage can also be observed, for example a shift from dinoflagellates to diatoms. The user, however, should be aware of the limitations of these qualitative estimates. The use of the percent composition data can be misleading because it does not account for the mass of cells in the sample. A 90% composition of *Pseudo-nitzschia* in a sample containing only 100 cells is quite different from the same percentage in a sample containing tens of thousands of cells. To adjust for this, a volumetric estimate of the settled cell mass can be recorded. Using a standard-sized sample bottle (e.g., Nalgene 125 mL) and having a volumetrically calibrated and marked bottle to compare the samples against is a simple way to estimate the settled mass. The percent composition of detritus in the sample should also be recorded so that its contribution

to the total settled mass estimate is accounted for. The settled mass will often be a function of the sampling effort; therefore, the total tow length should be recorded at the time of sample collection. To normalize the percent composition of each species to account for the influence of cell mass and sampling effort, a relative abundance index (RAI) was developed (California Department of Public Health, 2016c) to normalize the data for differences in sampling effort and cell mass:

$$\text{RAI} = [(\text{settled cell mass volume}) \times (\text{species percent composition})]/\text{total tow length}$$

For a harmful algal species that does not typically bloom, such as *Alexandrium* spp. along the California coast, a RAI value of 0.5 or greater would be significant and of concern. For bloom-forming species such as *Pseudo-nitzschia* spp., RAI values greater than 1.5 and as high as 99 have been associated with blooms and the detection of domoic acid in shellfish samples (California Department of Public Health, unpublished data).

10.5 Monitoring Other Fisheries

As mentioned previously, there are other species that present a risk to consumers from marine toxin poisoning, including a variety of crustaceans, finfish, and carnivorous gastropods (Shumway, 1995; Deeds *et al.*, 2008; http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Seafood/ucm2018426.htm). The EU also includes echinoderms and tunicates as species to be monitored for marine toxins when appropriate. Unfortunately, not all states have the capacity to sample and analyze these other species routinely because their programs were designed and funded specifically to manage nearshore bivalve shellfish harvesting. Yet the responsibility will naturally fall to these programs when there is a HAB event that impacts non-bivalve fisheries. In practice, when other seafood species are potentially impacted by a HAB event, the responsible state program may have to divert resources from other programs to increase their capacity for sampling and laboratory analyses. This crisis management approach will not be sustainable if HAB events increase in frequency and magnitude and more seafood species are routinely impacted. State governments will have to reevaluate the need for expanded monitoring programs, including those offshore fisheries that are at risk of becoming contaminated. Some states have included other species in their monitoring programs, such as Maine and Washington, which test carnivorous gastropods for PSP. The west coast states of Washington, Oregon, and California test Dungeness crab (*Metacarcinus magister*) each year prior to the season opening, but these programs were severely stressed in 2015 when a massive west coast *Pseudo-nitzschia* bloom resulted in persistent, elevated concentrations of domoic acid in crab and various shellfish species in each state. The Californian commercial anchovy fishery was also closed. The Californian Dungeness crab fishery was most heavily impacted from this event, with commercial losses estimated to be greater than $48 million (https://nrm.dfg.ca.gov/FileHandler.ashx?DocumentID=116284). The commercial and recreational rock crab fishery, which is open throughout the year in California, was also heavily contaminated with domoic acid and closed for an extended period of time. The experiences with offshore HAB events on the east and west coast of the U.S. have shown that the existing nearshore and estuarine monitoring programs for phytoplankton and biotoxins in bivalve shellfish cannot be relied upon as an early warning for potential impacts to offshore fisheries.

Another issue for some state shellfish monitoring programs is the potential for offshore aquaculture development, which is being promoted by the NOAA Office of Aquaculture (http://www.nmfs.noaa.gov/aquaculture/). Because these prospective aquaculture operations will be in federal waters, the state food safety regulatory programs do not have jurisdiction to manage these areas to ensure they meet NSSP requirements for sanitation and marine biotoxin monitoring. The FDA is responsible for the safety of seafood harvested in federal waters, but the legal authority to close an impacted fishery lies with the National Marine Fisheries Service within the Department of Commerce (Etheridge, 2010). Regardless of jurisdictions, it is clear that bivalve shellfish and other at-risk species grown and harvested offshore must meet NSSP requirements if they are to be landed legally in a state port and enter intra- or interstate commerce. It is also clear that alternative, innovative approaches will need to be considered in order to ensure the continued operation and expansion of important offshore fisheries and aquaculture while ensuring public health protection. One possibility is for a consortium of federal agencies to assume the responsibility of the shellfish control authority, ensuring that aquaculture in federal waters meets the same requirements that the state shellfish

industry must meet. An example for this approach exists in the Georges Bank shellfishery in the Gulf of Maine, which first experienced elevated PSP levels in 1988, resulting in an extended harvest closure for this valuable fishery. It was not feasible for the states involved to establish a routine monitoring program for this offshore area, so the fishery remained closed for years. A collaborative effort involving federal agencies (FDA, National Marine Fisheries Service, EPA), North and Mid-Atlantic state shellfish control authorities, and the Atlantic Fishery Management Council developed a protocol for rigorous testing of shellstock harvested from Georges Bank. As part of a larger NOAA-funded modeling project in the Gulf of Maine (https://coastalscience.noaa.gov/projects/detail?key=150), a pilot program was developed in 2008 to evaluate this "Onboard Screening and Dockside Testing Protocol" for shellfish harvested from closed federal waters, with the states of New Jersey, Delaware, Rhode Island, and Massachusetts participating. Based on the success of this effort, in 2011 the ISSC approved a modified version of the dockside testing protocol as an acceptable marine biotoxin control strategy. The FDA concurred, and this control strategy was added to the NSSP Guide for the Control of Molluscan Shellfish (http://www.issc.org/client_resources/2011%20summary%20of%20actions/with%20fda%20concurrence/proposal%2011-116.pdf). The approved proposal recognized the increased demand on state resources that this approach might have and identified an option for industry to pay for the costs associated with sample collection, screening tests, transportation, analysis, inspection, enforcement, and other related expenses.

For regions with widespread or multiple offshore fisheries, it is advisable to implement some level of offshore environmental monitoring for phytoplankton and toxin levels in seafood of concern. Any such effort will be expensive, so determining the most cost-effective approach will be essential for sustaining this effort and developing a long time series of data to improve the management of offshore HAB events. Using a tiered approach to sampling and analysis is one option, with some environmental condition or model forecast triggering the collection of phytoplankton samples in the area of concern. The phytoplankton sample observations would guide the need to collect samples of seafood species in the area. This is clearly a simplified example, and there are many obstacles to this approach. Identifying resources for reliable offshore sampling will be one of many challenges. Engaging the at-risk fishing industries is an obvious place to start; it may be difficult, however, to convince fishermen to go out of their way to collect samples if the projected bloom location does not happen to be where the fishery is operating. The commercial fishing industry's effort could be supplemented with participation from recreational charter boats focused on at-risk fisheries (e.g., crab) and other "ships of opportunity" (e.g., other boats commonly in the area for research, marine sanctuary activities, diving, or ecotourism). The existence of offshore aquaculture operations would be a valuable component to routine offshore monitoring. With a tiered approach, there is some cost-savings by initiating seafood sampling only when toxin-producing phytoplankton are observed at levels of concern. This approach does introduce an additional delay for determining the current status of toxin levels in seafood species of concern, but this can be minimized by sampling landed product. A simpler approach is to coordinate sampling efforts of fishermen for phytoplankton and seafood wherever the fleet is fishing. This minimizes the fisherman's efforts, the transport time and cost, and the turn-around time for analytical results. The results of the phytoplankton sample observations could still serve to determine the need to analyze the seafood samples, but would probably not provide the data needed for field validation and refinement of the forecast system.

Even if a program for routine phytoplankton and seafood sample collection is developed, there are inherent delays in delivering these to the laboratory. Most, if not all, states operate a centralized public health laboratory for analyzing all biotoxin samples, which can be far removed from the landing ports. An option for minimizing the turn-around time for sample collection and analysis is to decentralize laboratory services by establishing accredited regional laboratories to serve the local fisheries. Local government public health labs could potentially provide this service if given the necessary staff, equipment, and training. The use of local commercial laboratories is another option, assuming that there would be enough routine samples to make it economically attractive. Too few samples would likely result in a high cost per sample and long turn-around times for results. The development of a network of accredited local labs also has the advantage of transferring the cost of analysis to the fisheries themselves, easing the burden on state or federal public health laboratories. This, in turn, would help mitigate the "boom and bust" aspect of HAB event response in a centralized laboratory, although it complicates

the data management needs of the regulatory program(s) that must make quick decisions on health alerts and fishery closures. Regional labs could alternatively serve to conduct the initial screening of samples, with all positive samples forwarded to the state regulatory lab for confirmation. The continued development and approval of simple and quick analytical methods for routine regulatory testing for seafood toxins will facilitate a decentralized lab model.

The significant cost and amount of effort involved for sample collection, analysis, coordination of sampling, management of data, and other associated activities are probably beyond the current capacity of most states. It is clear that any offshore monitoring program will have to be managed at the federal level with state participation. In the absence of a rigorous offshore environmental monitoring program, alternative approaches to supplement traditional monitoring should be considered, and some of these options are discussed in the following section. If coastwide offshore monitoring is not feasible, it may be necessary to forgo environmental monitoring and focus on dockside screening of landed product. The use of field toxin screening kits can identify negative samples that do not need to be tested further, reducing the demand on laboratory resources (see Section 10.6.2, "Field Testing for Toxins," for more detail). As with the Georges Bank protocol, positive samples would result in lot rejection and product destruction, a potentially costly gamble.

10.6 Novel Approaches and Advanced Tools to Enhance Monitoring Programs

10.6.1 Diversifying Program Participation: Volunteer Monitors

Monitoring for marine toxins has long been the purview of state agencies charged with protecting the public's health. Sampling has traditionally been conducted by biotoxin program staff with cooperation from the shellfish industry for sampling commercial harvesting areas. Many existing programs are already operating at capacity, with increasing demand on limited resources resulting from budget and staff reductions, increasing need due to a growing industry, and the occurrence of new toxins that must be addressed. Increasingly, states have turned to the use of citizen volunteers, or citizen scientists. Once dismissed as unreliable or incapable of providing reliable and reproducible data, volunteerism has become an increasingly accepted approach to strengthen long-term monitoring programs for water quality and harmful algal monitoring. With a simple and clear scope of work, and proper training and oversight, volunteers can provide the effort and continuity needed to sustain these long-term sampling programs. California and Maine initiated statewide, volunteer-based phytoplankton monitoring programs in 1992 and 1996, respectively. Aided by the FDA's Office of Seafood and using the nets and field microscope described earlier, both programs identified and trained local citizens to collect phytoplankton samples, with some participants also trained to conduct field identifications of the species of concern. In 2005 the Maine program adopted a quantitative sampling procedure, described earlier in the "Monitoring Phytoplankton" section. Over the years, each program has developed into a reliable source of valuable information on the distribution and abundance of harmful algal species. The near-real-time data from the volunteer efforts has been incorporated into the decision-making process of the biotoxin monitoring programs, providing an early warning of potential toxin events and identifying the need for focused shellfish sampling. In subsequent years, several other states and tribal governments have developed phytoplankton monitoring programs, each having unique designs that reflect the needs of the program, the community, and the shellfish industry. In 2000 the Florida Fish and Wildlife Research Institute established a volunteer-based phytoplankton monitoring program, the Red Tide Offshore Monitoring Program, to assist research efforts on *Karenia brevis*. NOAA established the Phytoplankton Monitoring Network in 2001 to help those states without the resources to create and maintain their own program (Schaefer *et al.*, 2004). The network also assists states with active monitoring programs by providing additional observations. Volunteer monitors were an integral part of the ORHAB project. Recently, the native tribes of southeast Alaska, in response to regional PST poisoning, created the Southeast Alaska Tribal Toxins (SEATT) partnership. The SEATT program monitors subsistence shellfish harvesting area for phytoplankton concentrations using methods developed by the NOAA Phytoplankton Monitoring Network and tests shellfish for both domoic acid and PSTs. Additional information on these

citizen science programs can be found in Chapter 11.

Phytoplankton and shellfish sampling are ideal activities for volunteer involvement. Both involve relatively simple equipment, the protocols are quick and easy to complete, and they are fun activities for many. In addition to the obvious benefit of providing low-cost samples to the monitoring program, the participation of citizen volunteers provides other rewards and benefits. The samples provided to the monitoring program are typically analyzed as soon as they are received, so feedback to the collectors is quick. The fact that they are providing a service with an immediate consequence for protection of public health is a rewarding experience for many volunteers. Informed of the observation of a toxin producer in their phytoplankton sample or the detection of toxin in their mussel sample, many volunteers are quite willing to increase their sampling effort during the event. Participants in the phytoplankton monitoring effort are often in contact with the public on the piers and docks used for sampling. Natural curiosity leads to questions about what they are trying to catch with their odd-shaped nets. So, in fact, the volunteers often serve as "ambassadors" and educators to the local community, providing information about their activities, marine toxins and phytoplankton, and the public health protection efforts of the monitoring program. Some states also involve volunteers for the collection of shellfish and other seafood species for toxin detection. Some volunteers may simply live in proximity to the coast, so collecting a dozen or so mussels is not a huge investment of time and effort. People who enjoy recreationally harvesting mussels and clams are justifiably concerned about marine toxins and rely on the biotoxin monitoring program for protection and guidance. When informed of the gaps in sampling stations or sampling frequency, many will offer to assist in sample collection to help fill that need. Likewise, sport divers collecting scallops or crustaceans such as lobster are often quite willing to provide at least the internal organs of their catch for toxin testing. While not necessarily representative of the toxin load in the edible tissue, data from these other non-bivalve species can provide insight into toxin occurrence and help assess the need for more intensive monitoring of the fishery.

There are many different ways in which to initiate a volunteer-based monitoring program and many different forms such an effort can take. Focus can be placed solely on commercial harvesting areas, the most common recreational harvest areas, or a combination of both. Some volunteer efforts are part of a more comprehensive environmental monitoring effort, as is the case with the Kachemak Bay National Estuarine Research Reserve phytoplankton monitoring program in south-central Alaska. Monitoring program managers will need to define the areas and types of sampling where help is needed, based on the surveys of monitoring needs and program resources discussed earlier. Safety is always a prime concern, so locations with safe and easy access are obviously preferable for volunteers. There are many ways to recruit volunteers, requiring varying levels of staff resources. Passive efforts include posting information on the program web page about the need for volunteers, explaining what a volunteer does, illustrating this with photos and examples, and providing a contact number or email address for more information. Many states maintain a toll-free phone line with a recorded message reporting current health advisories or quarantines. This is an ideal opportunity to reach out to people interested in shellfish safety by mentioning the need for volunteers and offering to have a staff scientist return their call. More active efforts involve recruiting volunteers by focusing on local communities, contacting school science teachers, environmental groups, and government agencies that can direct you to other community groups. There is a tremendous variety of people who get involved in a volunteer effort, ranging in age (from schoolchildren to retirees) and in experience, from those with a science background to non-science people interested in learning. This diversity in participation needs to be taken into account when designing the sampling protocols and training materials you expect them to follow, particularly for phytoplankton sampling and observation. Developing materials at several levels of increasing detail and complexity is ideal so that volunteers can choose the level they are comfortable with. For example, a phytoplankton field identification form based on pictures of the most common genera may work best for non-science volunteers, while a more detailed species list is more suited to those with a strong science background.

Providing adequate training to new volunteers is essential for ensuring that they have the confidence and demonstrated ability to meet the quality control requirements for sampling and data collection. Meeting and working with volunteers in person also establish a personal working relationship and demonstrate the program's commitment and

appreciation of their efforts. Shellfish sampling is a relatively straightforward and simple process. Those that volunteer typically are already harvesting shellfish recreationally and are therefore probably as efficient as the program staff, perhaps more so when it comes to specialized activities like digging razor or butter clams. Ideally, program staff will meet the new volunteer at the sampling location to assess access and discuss safety. There should also be a discussion of any legal requirements pertaining to the species being collected, such as size restrictions and a limit on the number of individuals taken under a sport fishing license. If the volunteer is collecting under a special scientific collecting permit, then there may be notification requirements for the intent to collect on a specified date and time, as well as reporting requirements for species and numbers collected. It's advisable that the program take responsibility for as much of the license or permit requirements as possible; burdening volunteers with excessive administrative paperwork is a sure way to lose their involvement. If allowed by the regulatory laboratory SOP, it can be a tremendous help to the lab if the shellfish sample is shucked into a sample bottle by the volunteer following an approved protocol. The sample can be frozen if necessary before shipping to the laboratory in an insulated container with ice packs. Not all volunteers may be keen to do the dirty work, so some flexibility on sample processing may be required.

For phytoplankton volunteers, a centrally located pier is an ideal location for group or individual trainings. The collection of phytoplankton samples is an easy task, so there is ample time for each person to become familiar with the equipment, ask questions, and discuss the program. Discussion of personal safety while sampling is essential, as is mentioning the fragility of the net material. The procedures described in the "Monitoring Phytoplankton" section above should be followed, with each new volunteer collecting their own sample so program staff can work with them on proper sampling procedures. It is also a good time to discuss assignment of sampling locations and the frequency at which they can commit to sample if this has not already been agreed upon. If portable microscopes are available, the training session can also include the viewing of toxin-producing species. Once volunteers discover the beauty and variety of phytoplankton in their "backyard," they will often want to pursue learning to identify them.

Alternatively, it may be more desirable to conduct a separate training in phytoplankton identification at the program offices/laboratory, or remotely via a web teleconference as is done by the NOAA Phytoplankton Monitoring Network. The latter is a great approach when participants are long distances from the program facilities. The same basic approach for identification training will be taken regardless of location. The program should have a number of documents prepared for distribution to new volunteers, including the SOPs for sampling and microscopic observation of samples, identification guides, and equipment loan forms. An injury waiver form may also be required. Participants in the training will be oriented to the various parts of the microscope and taught how to prepare a sample slide for observation. The instructor(s) can set up demonstration slides for the species of concern, with reference specimens made available for the class to work through individually. The obvious focus will be on the harmful algal species, but it is also useful to have samples containing a variety of common species. If the participants had previously been trained in sample collection, it is good to encourage them to collect a sample from their station to bring to the training. Live material always has surprises, and it is rewarding for the collector to see what they actually captured in their sample. Phytoplankton identification guides and books should be available for the participants to use. Useful references within the U.S. for phytoplankton identification include Tomas (1997) and Horner (2002), with Smith and Johnson (1996) providing a very good introductory guide for phytoplankton and zooplankton. The existing phytoplankton monitoring programs have developed their own identifications materials, often with photos of local species, and are quite willing to share with other programs. When everyone has had time to view all of the reference specimens and live samples, time should be spent discussing the method used to work systematically through the slide and the procedure for recording observations. The latter can involve a form designed for the level of the volunteer, being picture-based or a detailed species list of common dinoflagellates and diatoms. Time should be spent explaining each data form and the proper way to use it. The students can then work through a few additional reference samples using these methods. This reinforcement is essential before ending the training session, as it could be a week or more before the new program volunteers put their new skills to use. In practice, it is important for the volunteers conducting microscopic observations of their samples to report this information as soon as possible.

Sample identification forms can be emailed or faxed to the biotoxin program, or an online data entry form may be created for the volunteers to use.

Ensuring the quality of the volunteer's sample collection is usually straightforward. Samples with heavy sediment loads usually indicate that the net has been allowed to disturb the bottom, so a quick reminder to the collector is often all that is required. The opposite problem of few or no cells in the sample may take a few repeated samplings to sort out. These sparse samples may accurately reflect the lack of productivity at the sample site, which may be cause to reevaluate the location. Some sites may be "boom or bust," with very low cell numbers being quite common, interrupted infrequently by a bloom. If the collector is suspected to be at fault, it is good practice to conduct a site visit and collect samples side by side. The volunteer's sampling technique can be observed and the samples compared to help determine if a correction is needed.

The quality control (QC) of volunteers' field identifications will require more time and oversight. One approach is to require the collector to send in a portion of the preserved sample for program staff to observe, comparing their observations with the volunteers. Alternatively, the volunteer can provide digital photos via email or a web portal for any suspect toxin producer identified and other common species or unknowns of interest. Over time, program staff will be able to evaluate the volunteers' ability to identify the species of concern and correct any systematic misidentifications. Decisions can then be made about which volunteers are capable, perhaps requiring sample QC only when a suspect toxin producer is reported, and which volunteers will require more oversight and training. While this QC effort can catch false positives (i.e., a nontoxic species mistakenly reported as a toxin producer), it can only catch false negatives (i.e., a toxic species that is present but not identified) if every volunteer sample is screened by the trained program staff. This of course defeats the purpose of decentralizing the sample identification process with the use of volunteer observers.

Periodic field visits are recommended to observe sampling and recordkeeping practices and to maintain contact, even for the most reliable volunteers. Handling and maintenance of the net and any other equipment can also be observed. In addition to the laboratory and field QC efforts, it is important to maintain contact with program participants to remind them of how valuable their efforts are to the program. This can take the form of personalized emails to individuals or organized gatherings. If the program happens to be centrally located to the coastal sampling sites where volunteers are located, it may be possible to have large group trainings at least once per year. These are ideal opportunities to bring in experts as guest speakers to discuss the program and highlight past data collected by the volunteer program. Other programs that have participants spread along hundreds of miles of coastline may find it more practical to provide web-based seminars or to organize local gatherings for the small number of volunteers in that area.

In addition to the laboratory and field QC efforts, it is important to maintain contact with program participants to make sure they are comfortable with the procedures and to track their sampling activity. It is to be expected that some volunteers will become busy and forget to sample, sometimes for weeks or months. Some volunteers will lose interest or decide to pursue another interest. Others may move out of the area without notification. It is important for the program to catch these trends early and contact the volunteer to remind them of their importance to the program. Allowing too much time to elapse can make it difficult or impossible to recover loaned equipment and supplies, no matter how stern the language in the equipment loan form. It is important to keep in mind that the people who volunteer their time and effort have many other demands on their time besides assisting the monitoring program. It is the program's responsibility to ensure they are productive and enjoying the opportunity to head to the seashore and collect shellfish or phytoplankton samples as part of an important real-time public health program.

10.6.2 Field Testing for Toxins: PSP and ASP

A valuable addition to routine shellfish monitoring is the use of commercially available field screening kits for certain toxins. There are a number of important parameters for validation of a qualitative (e.g., presence/absence) test kit that will be defined by the accrediting organization such as the ISSC/NSSP. To be used for decision making in the field, there can be no false negatives (the failure to detect toxin when it is present at levels of concern) and minimal false positives (the apparent detection of toxin when not present). The latter can also occur when the test kit is more sensitive than the

regulatory method; however, this is not a true false positive and actually a potential benefit in terms of the early warning aspect of the lower detection limit. To be practical and useful in the field, a test kit would ideally be fast and consist of a minimum of steps, be simple and rugged in design to withstand outdoors conditions, use common and easily obtained reagents, not require sophisticated laboratory equipment, and be inexpensive. The protocols for the existing field test kits are intended to be simple and reproducible and can be conducted by trained program staff, shellfish harvesters, and citizen scientists. As with any analytical procedure, there should be appropriate QC measures implemented as part of a documented SOP for field testing. This could include periodic field audits and retraining to confirm and maintain acceptable performance with the test kit. The QC program should also include the testing of a mix of negative and positive samples, with their identity unknown to the analyst (i.e., blinded). These relatively simple tests can provide an early warning of toxicity in shellfish. Some test kits can also be used with phytoplankton samples. Coupled with routine phytoplankton monitoring field observations, the use of the test kit for a phytoplankton and/or shellfish sample can be triggered by some threshold of toxin-producing cells in a phytoplankton sample, as is done by the Maine phytoplankton monitoring program. The qualitative test kits currently available from several manufacturers for PSP and ASP share the basic principle of lateral flow immunochromotography (also referred to as a lateral flow immunoassay), while differing with respect to extraction procedures, reagents, and assay protocol. One such kit (Scotia Rapid Testing Ltd.) has been approved by the ISSC/NSSP for use in regulatory screening of shellfish samples for PSP. Its use, however, is tied to the standard AOAC acid extraction used in the mouse bioassay, an impractical procedure to carry out in the field. This is somewhat ironic, given the origins of the mouse bioassay and acid extraction as a method to be used for field testing in the early PSP research by Sommer and Myer (1937; Wekell *et al.*, 2004). The other extraction and assay procedures used by the different field kits vary in reagents and, with one exception, have not been approved for use by the ISSC/NSSP. The Scotia Rapid Testing Ltd. rapid extraction method for PSP has been approved for use in combination with the Biosense PSP ELISA for the Georges Bank shipboard testing, as discussed earlier. Some of the alternative extractions are compatible with the approved laboratory-based quantitative method, which could save time when a confirmatory analysis is needed for the field result. Shellfish sample homogenization can be accomplished in the field in a number of ways. These include the use of a battery-operated food processor, or a rechargeable drill fitted with a paint mixer blade or egg beater from a hand mixer. The latter will have a longer battery life and does a more thorough job of homogenization, key to an efficient extraction. A standard corded home blender can also be used with a 12-volt converter and a car battery, an option when on a boat or when your vehicle is near the sampling site. A small portion of the homogenate will be extracted following the manufacturer's protocol, a portion of the extract added to a proprietary buffer, and an aliquot of this mixture introduced to the test strip. A Control line should always develop; its absence or irregular appearance denotes an invalid test. The more toxin present, the less developed will be a second Test line. A decided difference among kits is the time needed for the test strip to develop and be read. The Neogen Reveal 2.0 ASP kit has an incubation period of 10 minutes and must be read within one minute of that endpoint, as the color development of the indicator lines is not stable (http://foodsafety.neogen.com/en/reveal-2-asp). In contrast, the Scotia Rapid Testing Ltd. kits require 35 to 60 minutes to develop, and the indicator lines are stable and can be read within a reasonable time of development (http://www.jellett.ca/psp_test.php?page_id=13 http://www.jellett.ca/). The Mercury Science field test for domoic acid is formatted differently, with a triangular test pattern providing two colorimetric endpoints, one below and one above the action level (http://www.mercuryscience.com/products.html). Qualitative lateral flow assays all involve some ambiguity in interpreting the test result when the toxin concentration is close to the method detection limit. The detection limit for PSP tests can vary among regions with different subsets of STX analogs, as the cross-reactivity will vary for each analog and not in proportion to its specific toxicity (Laycock *et al.*, 2010). Test kits may also have been designed to have different detection limits to serve a particular purpose, from early detection to exceeding the action level for that toxin. To address this issue, some companies have developed portable instruments to read the test strip and provide a more consistent interpretation of the result. This of course increases the capital expense for employing the test strip, making it infeasible as a tool to put in the hands of numerous staff and/or volunteers. To be used properly, the various test kits should be compared against an

approved method for local shellfish samples to understand the test's performance characteristics at different toxin concentrations. It will also be necessary to validate the kits for each of the shellfish matrices to be tested to ensure there are no interferences that affect the result.

10.6.3 Screening Tests for Toxins: DSP and PSP

There are no true field test kits currently available for the DSP toxins. Screening assays are available that use either lateral flow immunochromotography, ELISA, or protein phosphatase 2A inhibition (PP2A) technology. Each requires instrumentation, including a heating block for alkaline hydrolysis of the ester forms of the toxins (OA, DSP1, and DSP2) and a microwell plate absorbance reader, and is therefore not an option for field testing. None of the commercially available kits have been approved by the ISSC/NSSP at present, although single and interlaboratory validation studies have been conducted on a PP2A inhibition assay (Smienk *et al.*, 2012, 2013). Comparison studies on each technology have revealed some issues with each (Sholz *et al.*, 2013; Eberhart *et al.*, 2013), although the PP2A assay appears the most promising at this time.

10.6.4 SPATT

Another potential tool for early detection of marine toxins in the environment is the use of synthetic resins to adsorb marine toxins. This solid-phase adsorption toxin tracking (SPATT) method is based on the assumption that extracellular, as well as intracellular, toxins associated with a harmful algal increase will be available for specific resins to adsorb and retain (MacKenzie *et al.*, 2004). The resins can be packaged in a fine mesh bag and deployed similarly to sentinel mussels, as well as on a vessel in combination with an autosampler as part of a "ferrybox" system for autonomous sampling. Extraction of the resin and analysis by HPLC-MS/MS allows the detection of very low levels of toxins, without the concerns of matrix effects possible with various shellfish species. At least some bivalve shellfish species are known to biotransform certain toxins (Bricelj and Shumway, 1998). Shellfish analyses may therefore be detecting a different set of toxin analogs than originally present in the phytoplankton. This is appropriate for public health concerns unless biotransformation continues during product distribution such that the health risk is increased. SPATT resins capture the native toxins produced by the associated phytoplankton species, a valuable trait for ecological studies. The majority of research has focused on the ability of these resins to adsorb various lipophilic toxins (e.g., OA, DTX1, DTX2, azaspiracids, pectenotoxins), although some effort has recently been placed on using this approach for domoic acid and STX and its analogs (Lane *et al.*, 2010). Some studies have demonstrated that SPATT can provide an early detection of toxin presence, prior to the detection by traditional phytoplankton and shellfish toxin analyses, at least for *in situ* blooms (MacKenzie, 2010). One study reported that, for a bloom that developed elsewhere and was transported to the area of concern, the detection of toxin was simultaneous in SPATT and shellfish (Fux *et al.*, 2009). That same study noted a correlation in the rate of toxin uptake by SPATT and in mussels when the causative phytoplankton species was present, but high toxin concentrations in the water in the absence of the toxin-producing species did not result in toxicity in the mussels. In another study, domoic acid was detected in SPATT several weeks before it was detected in sentinel mussels from the same location (Lane *et al.*, 2010). The ability of SPATT to model shellfish toxin uptake has varied, at times providing a good correlation and at other times not (Scholz *et al.*, 2013). Similarly, this method has been documented to provide an early warning for toxin occurrence in some circumstances (MacKenzie *et al.*, 2004; MacKenzie, 2010; Lane *et al.*, 2010), while failing to do so in others (Rundberget *et al.*, 2009). As a potential replacement or adjunct to shellfish monitoring, SPATT suffers from the same constraint of weekly sampling and the potential to be behind the curve of toxin detection (e.g., if a bloom initiates the day after a weekly sample is collected). A significant improvement to this sampling limitation for shellfish or SPATT would be a continuous monitoring device for toxins and toxin-producing phytoplankton. It is clear that putting a new tool such as SPATT to work in a routine monitoring program is not a straightforward process and will involve experimentation to determine if it provides value to the program. As with any new monitoring or analytical tool, there is a need for validation studies to be conducted in the regions of potential use to understand its performance, benefits, and limitations, in order to determine the best application.

The question for any new potential tool is how to best incorporate it into long-term public health

monitoring programs for marine biotoxins so it can provide an extra layer of protection. The resin bags are small, making SPATT attractive for deploying in high densities to characterize toxin distribution and magnitude in a new area. They can also be stationed in areas where natural populations of shellfish do not exist or sentinel shellfish cannot be maintained, including aboard vessels. For programs with limited resources, the options for use of this technology will be restricted to areas of easy access such as the piers and boat docks used for sentinel mussels. As a result, there will be far fewer possible SPATT sampling locations than existing wild shellfish and phytoplankton access points. While many of the successful research projects have suggested SPATT as a surrogate for traditional biotoxin monitoring, SPATT is unlikely to supplant existing phytoplankton and shellfish monitoring programs for a number of reasons. It can potentially serve as a valuable addition, however, improving the ability for early detection of harmful algal events in some areas. One major obstacle to replacing the use of shellfish for public health monitoring programs is that, at present, there is not an established relationship in toxin uptake between SPATT and shellfish. It therefore cannot serve as a proxy to determine when a toxin action level has been exceeded in shellfish. There are also regulatory requirements that must be met for shellfish monitoring in commercial harvest areas. There is the attractive possibility of using the greater sensitivity and easy deployment of SPATT at offshore stations for early warning to trigger increased sampling for phytoplankton and shellfish in nearby coastal areas. This tool may also be of value for assessing risk to offshore pelagic fisheries; assessing the risk to offshore benthic populations is complicated by the potential for long-term exposure to toxins captured in the food web and the ability of some species (e.g., Dungeness crab, surf clams) to sequester toxins. While phytoplankton observations of toxin producers may lag behind SPATT toxin detection in some circumstances, there is still tremendous value in the routine observations of species composition and relative abundance. This additional environmental information on phytoplankton community composition can be of immediate importance for managing a harmful algal event, and the long-term data sets compiled over time will be invaluable for assessing seasonal and multiyear patterns. Routine phytoplankton observations can provide a quick picture of the relative abundance of the species of concern, and trends in the assemblage can be tracked; for example, a shift from diatoms to dinoflagellates, providing insight into the risk of occurrence for a given toxin producer. The growth state of the toxigenic phytoplankton species population may be closely paralleled by toxin concentrations in shellfish, providing insight into the rate of increase and decrease that in turn can provide guidance for anticipating the possible duration of the event and the need for public health warnings and harvest closures. SPATT can be complementary to this aspect of shellfish monitoring because of its ability to retain toxin in the absence of the toxin producer, although the availability of that dissolved toxin to the shellfish is in question and may differ for the various toxins potentially present (Fux *et al.*, 2009). On the other hand, this conservative property of SPATT may provide an alert that a toxin-producing species was present sometime between sampling days. With the possibility that the phytoplankton species was present in low numbers and missed during sampling, there may be an increased probability of its reoccurrence. Either way, it is a signal to the monitoring program to investigate the area more intensively.

Rather than view new tools like SPATT as replacements for existing methods of monitoring marine toxins for public health protection, it may be more productive to investigate how they can be incorporated to provide an additional layer of safety, serving as a valuable addition to the program by improving the ability for early detection of harmful algal events in some areas. Practically speaking, many existing monitoring programs are operating at capacity and cannot add another responsibility for program and laboratory staff. Laboratories will always be required to conduct shellfish toxin analyses for public health protection, so the adoption of a new method will not supplant that work but add to it. For other programs that have the resources to investigate new tools, however, the use of SPATT is a practical and potentially useful tool to explore.

10.6.5 Oceanographic Data

The desire to predict when and where a toxic bloom will occur has been a consistent theme since HAB became a topic of increased focus for both researchers and government monitoring programs in the early 1990s (see Chapters 1 and 3). In efforts to aid in the understanding of these events, there have been attempts to draw on existing data sources and to develop new tools to fill the gaps that were identified. Along the way there have been

various approaches on how to present that information so it can serve a wide range of end users, from government and university scientists to biotoxin program managers. One challenge is to identify, or create, data products that can be easily obtained and understood and that provide immediate value. Another challenge, as discussed earlier with monitoring programs in general, is that one size does not fit all; each region has its differences and will present unique challenges for developers of tools intended to improve these programs. There are an unprecedented number of resources for oceanographic data now available on the web but, without guidance and training, it is unlikely that biotoxin program managers have the time and expertise to determine what products are of greatest value and how best to use them. Many managers have independently experienced similar struggles in trying to link their regional knowledge of marine biotoxin occurrence in shellfish with the variety of data sources and the myriad of options within each. The following is a general overview of some of the information sources currently available and how to access them, and is not intended to be an all-inclusive review, nor a detailed explanation of the technology.

Data for sea surface temperature (SST) are an obvious place to start, as each phytoplankton species or group (i.e., diatoms and dinoflagellates) has an optimum temperature range for growth. Observing trends in temperature change may provide insight into the potential for increases in a particular toxin producer, or at least in diatoms versus dinoflagellates. SST data are intuitive to understand and are available in different formats from a variety of online sources. The NOAA National Buoy Data Center (NBDC; www.ndbc.noaa.gov) provides access to near-real-time data from a network of buoys and land-based stations operated by a variety of organizations. Current and historical data are available for SST, wind speed and direction, and other oceanographic and atmospheric measurements at many of these locations. The frequency of data collection can vary, with at least hourly readings available on most, if not all, of the NBDC buoys. Data for each buoy can be easily downloaded as an ASCII file for various time intervals, from the most recent 24 hours to historical archives of annual data going back 20 years or more. This system represents the most extensive network of *in situ* monitoring that is readily available and easy to access, download, and plot. It is also the most reliable source of SST data, not suffering from the same shortcomings as the satellite-derived temperature data mentioned below. Data from a particular buoy can be easily processed, for example as average daily or weekly SST, and plotted as a time series in conjunction with shellfish biotoxin concentrations or phytoplankton relative abundance from the nearest sampling stations. Comparison of the SST data with current and historical data for periods of major harmful algal events can help determine if there is an obvious relationship between a major shift in water temperature, perhaps representative of an upwelling or relaxation/downwelling event, and toxin increase. For example, major PSP events along the west coast of the U.S. are thought to be associated with the relaxation of upwelling and perhaps a subsequent downwelling event (Lewitus *et al.*, 2012). To look for possible trends over broad areas, it is necessary to track multiple buoys, as each represents a single point of information representing the local area. While a number of the coastal buoys are a considerable distance offshore and therefore of questionable use for nearshore biotoxin monitoring, there are increasing numbers of nearshore buoys providing data that may be valuable to biotoxin program managers. The offshore buoys may be of greater value in relation to assessing risk to offshore fisheries. They would also possibly provide the existing infrastructure for integration of newer HAB-specific technologies.

While potentially quite valuable for assessing changing conditions in SST, it is a daunting task for individual managers to keep pace with the volume of data available for areas with extensive coastline and/or a large number of monitoring buoys. The prospect of having a large area "picture," or synoptic image, of color-coded water temperature along the coast becomes quite attractive in comparison to capturing and processing the *in situ* buoy data. The NOAA CoastWatch Program, created in part in response to a significant HAB event, provides a variety of satellite data products over the web (http://coastwatch.noaa.gov/cwn/index.html#). Using the Data Access option (http://coastwatch.noaa.gov/cgi-bin/cwn_most_recent.cgi?region=ALL&product=sst&sensor=AVHRR&daysback=1) for near-real-time imagery, the user can specify the region of interest, and one or more imagery products available for that region will be listed (e.g., SST, chlorophyll-*a*). Each product, in turn, will have one or more sensors associated with it. The West Coast SST option, for example, has five different sensors listed (Imager, AVHRR, Multi, SEVRI, VIRS), and a brief description of each can be found elsewhere (http://coastwatch.noaa.gov/cwn/cw_products_sst.html). One should be aware that data may

not be available for each sensor for the day specified. When data are available, a series of thumbnail images will be provided with the option to download a raster image (PNG), a georeferenced TIFF file, or a Hierarchical Data Format (HDF) file. The latter can be opened with the CoastWatch Data Analysis Tool (CDAT) software available for free download elsewhere on the CoastWatch web site (http://coastwatch.noaa.gov/cwn/cw_software.html). CDAT represents the temperature data as colored pixels, the color varying with the temperature value. A number of different color "palettes" are available, each of which assigns a range of colors to the data set. The CDAT Help information notes that, for temperature data, the Blue-Red palette (representing cold to warm) is most commonly used. Using the CDAT Color Enhancement tab, the default temperature range can be modified to the appropriate range for the user's area of interest, using the histogram of data values for guidance. Care must be taken when setting the minimum and maximum temperatures for a given palette, as a critical temperature range within the extremes, for example encompassing the optimal growth temperature of a particular dinoflagellate, may be obscured by a wide band of uniform color. The sliders for the minimum and maximum temperatures can be used to alter the image interactively, revealing or obscuring temperature information. The Enhancement Function option can also help reveal important aspects of the image by providing different transitions from one color-coded value to the next. A linear transition is the default, with Stepwise (including the ability to set the number of steps in each transition) and Logarithmic transitions also available. There are a number of other image-processing tools within CDAT, but the ones discussed here will allow for fairly quick viewing of CoastWatch SST imagery. The relatively easy access to this CoastWatch imagery allows the user to view the location of major water masses quickly on the basis of SST.

The CoastWatch data access described above provides imagery for numerous regions, although not all regions have each possible set of data (e.g., SST, chlorophyll-*a*). It provides a simple interface for viewing multiple thumbnail images, making it easy to identify usable imagery. There is, however, increasing effort required to customize the selected products. Image files must be downloaded and displayed with compatible software capable of modifying the display properties, producing an optimized image for the area of interest. While simple enough, this daily process may be beyond the capacity of small and/or understaffed biotoxin programs. Some of the sources of the SST data used to compose the imagery, such as infrared sensors, are affected by cloud and fog cover, often obscuring large portions of the coast. The absence of usable imagery can last for extended periods of time, often during the peak months for phytoplankton productivity, compromising the ability of program managers to rely on this information. The NOAA CoastWatch Pacific Fisheries Environmental Group provides access to a composite SST image produced by the National Aeronautics and Space Administration (NASA) Jet Propulsion Laboratory, accessible through the West Coast Node on the CoastWatch home page. The Multi-scale Ultra-high Resolution (MUR) SST image is a blend of a variety of images from different sensors and other data sources, producing a more reliable daily image of SST (http://coastwatch.pfeg.noaa.gov/erddap/griddap/jplMURSST41.graph). The MUR-SST image is global in scale, but the user can zoom in to specific regions. The ERDDAP/grddap data server's "make a graph" interface gives the user several tools for customizing the online image.

With increasingly reliable sources of SST imagery being made available to the public, the question remains as to what exactly to do with it in the context of a marine biotoxin monitoring program. If the program has a routine phytoplankton monitoring effort, then the comparison of species composition data with SST imagery can provide some insight into the optimal conditions for a dominance of diatoms or dinoflagellates in the region of interest. If the program has the resources, then the data sets can be combined in a geographical information system (GIS) to produce map layers for a visual comparison. In the absence of a robust time series of phytoplankton data, the SST imagery may be of limited value unless the relationship between temperature and species composition has been well studied and is understood for the region of concern.

Although water temperature is an easy parameter to understand and obtain data for, it is not the only driver for phytoplankton growth and is by no means a simple predictive tool. Another data set that is readily obtained through many of the same sources provides a synoptic view of ocean productivity by measuring chlorophyll-*a* (e.g., http://coastwatch.pfel.noaa.gov/data.html#). While SST imagery may provide guidance on what types of phytoplankton (e.g., diatoms or dinoflagellates) might dominate the assemblage in a given water

mass, or might become dominant when there is a significant increase or decrease in temperature, there is no evidence that they are actually there. The chlorophyll-*a* imagery provides that missing information relative to regions of varying primary productivity, but does not provide insight into which species or groups may be present. Plankton blooms are typically common in the spring through summer months, so evidence of high chlorophyll concentrations does not provide much insight into the possible presence of a toxin producer in most cases. The exception to this may be the Gulf of Mexico, where blooms of the brevetoxin-producing *Karenia brevis* is a routine occurrence. The observation of phytoplankton in nearshore sampling stations helps in that regard, although there is no guarantee that the same assemblage will exist within the same water mass farther offshore. As with the SST imagery, the chlorophyll-*a* data are negatively impacted by cloud and fog cover. This can be partially resolved by using imagery that averages several days to a month of data, providing a more complete picture of productivity over a broad region (http://coastwatch.pfeg.noaa.gov/erddap/griddap/erdVH3chla8day.graph). There is also the problem that some harmful species, in particular *Alexandrium* spp., do not have to be present at bloom levels to pose a serious public health threat. In those situations, the chlorophyll-*a* signal will be dominated by the most abundant species and will not provide useful guidance for the toxic species present in lower concentrations. Additionally, as both SST and chlorophyll-*a* are specific to the surface of the ocean, there is potential significant error associated with the presence of subsurface blooms that will go undetected. There is also very little ground-truthing, or verification, of the satellite-derived data. Biotoxin program managers are not trained image analysts, and few may have the time to pursue even the cursory tracking of this type of data.

One possible solution to the shortcomings of imagery alone as a tool for early warning of a potential HAB is the development of predictive models (see Chapter 3), a need recognized in the 2014 National Science and Technology Council's "Harmful Algal Blooms and Hypoxia Comprehensive Research Plan and Action Strategy: An Interagency Report" (https://www.whitehouse.gov/sites/default/files/microsites/ostp/NSTC/habs_hypoxia_research_plan_and_action_-_final.pdf) and the 2008 West Coast Governors' Alliance Action Plan (http://www.westcoastoceans.org/media/WCGA_ActionPlan_lowest-resolution.pdf). The potential advantage to predictive models is that a variety of data sources are utilized, helping to overcome their individual shortcomings. Similar to the requirements discussed for field-based toxin-testing kits, the usefulness of models for biotoxin monitoring programs is partly dependent on the lack of false-negative predictions (i.e., a HAB event occurring in the absence of its prediction) and an acceptably low frequency of false-positive forecasts. The latter can result in a significant waste of program resources if each prediction results in increased sampling and analysis, even more so if offshore sampling is involved. Additionally, reliable transport models linked to the HAB forecast are essential for predicting landfall so that a general area and time of impact can be identified. Some minimum level of spatial resolution is also needed for the area encompassed by the model so that it is useful for managing shellfish and other seafood resources. Regional forecast models have been, or are in the process of being, developed in a number of areas, including: the Gulf of Mexico (*Karenia brevis*), with a localized effort in Texas also in progress; the Gulf of Maine (GOM; *Alexandrium*); and the western U.S. along the California coast (*Pseudo-nitzschia*) as well as in Puget Sound, Washington (*Alexandrium*). At present, NOAA is providing operational forecasts of red tides associated with the brevetoxin-producing dinoflagellate *K. brevis* in the Gulf of Mexico. This Harmful Algal Bloom Operational Forecast System (HAB-OFS) uses a variety of data, including satellite imagery, cell density data, wind data, and field observations of health impact reports and phytoplankton monitoring, to produce a forecast and validate the acquired data. A Condition Report is generated that includes a forecast of the potential for respiratory irritation associated with these blooms (https://tidesandcurrents.noaa.gov/hab/). The other models mentioned are being developed as research efforts in collaboration with NOAA scientists and, in the case of the PCMHAB-funded GOM model, a formal stakeholder group. Each region is unique in the set of variables used for their model and in how they represent the model output. When fully operational, the other models may be incorporated into the HAB-OFS program. More information on each of these forecasting efforts is available at the links provided at the end of this chapter.

The Gulf of Mexico forecast system has been operational for a number of years, so this effort can provide insight into what can be expected from this approach by way of the documented successes and limitations. Brevetoxins from the *K. brevis*

blooms that occur routinely in the summer and fall can cause shellfish poisoning as well as respiratory irritation, the latter having a significant impact on beach tourism. This forecast system produces at least weekly Condition Reports that provide guidance on the relative predicted respiratory health risk along the coast. These reports are delivered to program managers and are also available on the HAB-OFS web site. This is an important difference in strategy compared to updating a web page, as the proactive delivery of the forecast is likely to achieve a higher success rate for being viewed and acted upon by program managers. It seems a small distinction, but the more passive approach of updating a web page and expecting end users to seek out that information is likely to result in less use because of the other demands on managers' time. Having a product that is routinely sent to multiple subscribers in a program can ensure that someone is always aware of the current status. The forecast system includes validation feedback from a variety of data sources, including twice-daily beach-specific reports from lifeguards on incidents of respiratory irritation experienced by beachgoers. Over time, it was demonstrated that the relatively high frequency of false-positive forecasts of respiratory events (80%) for a specific beach could be reduced significantly (to 22%) by broadening the reporting area to encompass multiple beaches (Stumpf *et al.*, 2009). This report also noted that it was important to match the resolution of the model, which was affected by limited satellite image resolution and patchy quality due to cloud cover, with that of the validation data (e.g., frequency of beach reporting on respiratory effects, distance between phytoplankton monitoring stations, and sampling frequency at each). The satellite technology will undoubtedly improve over time, but increasing the resolution of sensors without a corresponding increase in resolution of the validation tools will be ineffective. This model assessment noted a lack of validation for the chlorophyll-*a* satellite imagery due to the absence of offshore phytoplankton sampling and low sampling resolution along the coast. As a result, the HAB-OFS forecasts are made at the county or half-county level (30–60 km; Stumpf *et al.*, 2009).

It is clearly beyond state monitoring programs to assume ownership and management of operational models, so the responsibility for maintaining these products will fall to federal agencies (e.g., NOAA, NASA). This makes sense from the perspective of establishing a larger scale regional monitoring effort, something that will be needed for forecasted blooms that cross state lines and that may impact other fisheries in federal waters. The challenge for all involved parties is to determine how best to use the modeling predictions to benefit the public health monitoring programs. The marine biotoxin threat in the Gulf of Mexico is unique in having the potential for both shellfish poisoning and human respiratory impacts associated with the brevetoxins. While the latter lends itself to a tiered approach to forecasting potential health risk, analogous to weather forecasts for storm severity, the question remains as to how the forecast of a toxic bloom will improve existing biotoxin monitoring programs. States with monitoring programs for phytoplankton and shellfish toxins already have baseline monitoring activities along their coasts and inside estuaries and bays with shellfish resources. A prediction of a HAB will therefore not necessarily result in a significant increase in sampling effort, and states cannot proactively close shellfish harvest areas before toxin alert levels have been reached. Sampling personnel and volunteers are not re-deployed around a state like firefighting teams; it falls to the same people in a given area to collect additional samples when needed. The stated benefit, therefore, of forecasts allowing state monitoring programs to focus sampling efforts is not manifested as a dramatic shift in resources, but more likely an intensified effort by the same available personnel. Accurate forecasts could, however, raise awareness within the monitoring program(s). A HAB prediction, or perhaps more importantly the landfall forecast of a known offshore bloom, can be a motivational tool for sampling networks, lending an increased importance and urgency to requests for samples from the target region. This is where the matter of false-positive forecasts can be damaging, particularly to the relationship with sampling program participants. The worst case is a scenario analogous to Aesop's fable of "The Boy Who Cried Wolf," with frequent false alarms eventually leading to a lack of response from the sampling network when there finally is a harmful algal event. Given the resolution issues in the various data sources identified by Stumpf *et al.* (2009), forecasts are unlikely to be specific to a given shellfish harvest area inside small embayments, but on a broader scale may be applicable to coastal and offshore resources. This could certainly change as instrument resolutions improve and/or more localized and continuous *in situ* monitoring is employed to provide data for more sophisticated forecast and transport models.

As mentioned briefly above, there is the potential for HAB forecasting models to benefit offshore

fisheries and aquaculture operations. The majority of shellfish biotoxin monitoring programs lack a robust offshore environmental monitoring component for phytoplankton and the various seafood species that can accumulate toxins. Obstacles associated with the logistics and cost of developing an offshore monitoring program may be difficult to overcome and would require the involvement of federal agencies, research institutions, offshore aquaculture ventures, and commercial fisheries to assist the state programs in managing biotoxin risks in non-shellfish species. Some regions are exploring technological solutions for automated offshore *in situ* monitoring, such as the environmental sample processor (ESP) developed by the Monterey Bay Aquarium Research Institute and the Imaging Flow Cytobot developed by Texas A&M University. The data generated from these offshore sensors should be of use for providing part of the validation data needed for associated models. The GOM forecasting project has deployed several ESP units, which are capable of onboard testing to identify toxigenic algal species and the presence of toxins, have the ability to archive samples for later laboratory analysis, and can transmit the data. A network of continuous *in situ* monitoring devices could solve the logistical problems identified earlier for implementing offshore monitoring programs for phytoplankton and toxins. Frolov *et al.* (2012) estimated that seven ESP units strategically located offshore (20 km) could provide HAB detection for the entire California coast, although noting that observations more than 2–4 km offshore would not be informative for nearshore bloom activity. The advanced technological tools can also be used for nearshore *in situ* monitoring to provide an alert of a harmful algal species approaching shellfish beds. This highly localized approach would need to be evaluated from a cost–benefit perspective to determine its practicality. As noted above with respect to operational models, state monitoring programs will not be able to afford the cost associated with deployment and maintenance of expensive monitoring instrumentation. This is another financial burden that will have to be absorbed by federal agencies if this approach to HAB monitoring and prediction is to be sustained for the long term.

There will always be the need for rigorous testing programs for marine toxins in shellfish and other seafood that the public harvests or purchases commercially. The various regional HAB forecast models are for a single species, while many states are at risk from multiple HAB species. Whatever benefits may be gained from a single-species predictive model will thus be incremental, as monitoring and toxin testing for the other toxins of concern will remain unchanged. The structure of existing marine biotoxin monitoring programs currently provides the highest resolution of nearshore data on phytoplankton composition and shellfish toxin levels and remains the most effective means of public health protection. The technologically sophisticated tools being developed and used operationally, from satellite imagery to models and high-tech *in situ* environmental sensors, may be the best approach for offshore HAB monitoring and prediction. What remains unclear is the appropriate response to a prediction of an offshore HAB in the absence of routine environmental monitoring. Increased product testing at the point of landing is an option, but how long is that effort sustained in the absence of toxin detection when a model continues to predict an event? The monitoring programs are potentially in a compromised position, for to discontinue product testing when a HAB has been forecasted will likely be perceived as not serving the needs of the public. Conversely, the public concern generated by advertised false-positive forecasts will certainly have a negative impact on all fisheries in the region. The most cost-effective approach may be some level of routine end-product toxin testing for offshore fisheries, with that effort increased for ports receiving product from a forecasted bloom area. It can be argued that any commercial seafood species capable of accumulating dangerous levels of marine toxins should be monitored routinely, as is the case for bivalve shellfish. The option of offshore sentinel monitoring (e.g., mussels, SPATT) could be more cost-effective for meeting this need and should be explored. To answer some of the questions on implementation, there will certainly be the need for a coordinated approach to evaluating new tools and technologies, involving a variety of stakeholders. An example of such an approach is the NOAA Prevention, Control and Mitigation of Harmful Algal Blooms program (PCMHAB). This program includes the requirement for any funded project to identify and involve end users (e.g., local, state, and federal resource and public health managers; nonprofit organizations; and businesses) in the three program phases (Development, Demonstration, and Technology/Information Transfer). The Technology Transfer phase requires that there will be a commitment by end users to continue the project operations or the use of the developed tool or technology beyond the lifetime of the funded project. For any project, whether funded by PCMHAB or other sources,

it is essential to engage stakeholders throughout the process rather than after the work has been completed. If this stakeholder approach is taken seriously by all involved, it will hopefully help identify what tools and data are of the most potential value and reduce the time needed to develop and objectively evaluate new approaches to minimize the impacts of harmful algal events and improve public health protection.

10.7 Management Considerations

10.7.1 Commercial Shellfish

The primary goals for managing marine biotoxin impacts to shellfish resources are protecting public health, while minimizing unnecessary impacts to commercial and recreational shellfishing activities. It is a difficult balancing act given the uncertainties associated with harmful algae events. There are a number of criteria and tools that can be employed at different stages of the event, depending on the resources of the shellfish control authority. Management options may be more limited for recreational harvest activities compared to the more tightly controlled and monitored commercial shellfish harvesting. As part of the survey of organizational resources discussed earlier, program managers must evaluate the level of complexity and flexibility it can afford in managing shellfish resources. Similarly, existing programs will need to reevaluate themselves periodically in this regard if demands on the program increase with industry growth, detection of new toxins, or a loss of program resources.

The basic requirement for each state shellfish program under the NSSP is to monitor toxin levels in all commercial harvest areas and close those with concentrations at or above the relevant action level, ensuring that contaminated product does not enter commerce. Beyond that, it is up to each state to determine if additional layers of protection can be added, such as phytoplankton monitoring, forecast models, field testing of shellfish for toxin presence, or other novel approaches mentioned earlier. The control authority can decide to implement more conservative action levels based on the need to provide a greater level of protection to the consumer. For example, the Republic of the Philippines established a regulatory limit of 40 µg STX eq./100 g in 1983, revising this limit to 60 µg STX eq./100 g in 2000 based on analysis of the intervening years of toxicity and epidemiological data (Arcamo *et al.*, 2014). Whatever the monitoring and management strategy, a key component will always be the timely communication of observations and results to the shellfish industry in the area. Without frequent toxin status updates, these businesses cannot make an informed decision about how to operate safely, particularly during the initial stages of an event.

Routine phytoplankton monitoring can be used as the first level of protection, with an increase in cell densities triggering more frequent sampling of phytoplankton and shellfish tissue in and around the affected area. Ancillary data such as reported increases in marine mammal strandings or seabird mortalities can provide an advanced warning of a HAB event. De la Riva *et al.* (2009), for example, found a positive correlation between marine mammal strandings in southern California and the relative abundance of *Pseudo-nitzschia*. Having a reliable time series of phytoplankton data at primary stations provides information on whether or not the toxin producer is increasing and expanding geographically and, if so, at what rate. This detail can help the program anticipate how quickly the bloom may reach critical levels, which can inform the program's sampling strategy (e.g., determine the frequency of sampling needed to detect when toxin concentration exceeds the action level; decide when lower risk species should be sampled) and provide a time frame for preparing closure notices and public advisories. Programs that conduct quantitative estimates of phytoplankton density may initiate this increased monitoring effort when a toxin-producing species exceeds an established threshold (e.g., 50,000 cells per liter for *Pseudo-nitzschia*). In addition to this primary threshold for increased sampling effort, some countries employ a secondary, higher threshold that can trigger the voluntary or mandatory closure of a harvest area until additional shellfish samples can be collected and tested for toxin levels and/or a public health advisory issued. For monitoring programs conducting qualitative phytoplankton monitoring, the trigger for expanded monitoring or other control actions can be based on the mere occurrence of a toxin producer such as *Alexandrium*, the presence of unusually high numbers of a particular toxin producer, or a pattern of increase over several samples in cell mass and relative abundance of a species like *Pseudo-nitzschia*. An increase in a toxin producer above a certain threshold, whether quantitative or qualitative, can trigger the use of a field kit for testing phytoplankton or shellfish for the presence of

toxin. A positive field test can allow the sampler to collect additional samples opportunistically over a broader area, perhaps including secondary stations and shellfish species that are not routinely sampled under low-risk conditions. The use of field test kits by the shellfish companies can also serve as a primary indicator for an impending harmful algal event. Given the probability of missing an initial occurrence of a toxigenic species when sampling once every seven days, the additional use of a field test kit on harvest lots can increase the potential for early detection of toxicity.

As toxin levels become detectable and begin increasing toward the regulatory threshold, there are a number of actions that can take place. Increased phytoplankton and shellfish sample collection will undoubtedly be underway to determine the geographic extent of the event and to track the rate of cell and toxin increase in one or more species of shellfish. A company may decide to cease harvesting voluntarily based on the reported information to avoid the risk of distributing shellstock containing a dangerous level of toxin. Companies with wet storage or cold storage capacity can increase harvest activity before toxin levels approach the action level, helping to maintain business operations if a harvest closure is implemented. Aquaculture operations may have the opportunity to move product to another certified location in a lower risk area of the same body of water. Moving product between water bodies, however, is problematic due to concerns of transporting unwanted "hitchhiker" species or disease organisms and may require the approval and oversight of fish and wildlife resource staff. Depending on the program resources and capabilities, an entire harvest area may be closed when shellfish from one sampling station exceed the action level, or increased harvest restrictions may be implemented based on location and/or species. Species-specific closures are common, prohibiting the harvesting of a high-risk species (e.g., mussels) that quickly accumulates toxin and has exceeded the regulatory threshold, while allowing the continued harvesting of lower risk species (e.g., some clam species) that do not readily concentrate the toxin and remain safe. In some bays and estuaries, it may be possible to allow harvesting to continue farther inside as outer beds are closed, assuming the toxin producer is being transported from the outer coast and a manageable delay exists for its transport to the interior of the bay. Additional controls on harvesting based on sub-threshold toxin levels may also be imposed rather than allowing uncontrolled harvesting until the regulatory threshold is exceeded. A tiered approach can be established that increasingly limits the harvest "window" (i.e., the time interval for harvesting relative to the most recent acceptable shellfish sample result). The actual number of tiers, threshold values, and time intervals may vary among areas depending upon the history of marine biotoxin activity and the determined risk. An example of this approach consists of four tiers or alert stages: (1) a positive PSP result below 50 μg STX eq./100 g would limit harvesting to within 48 hours of the most recent acceptable sample result; (2) toxin concentration between 50 and 59 μg STX eq./100 g would reduce this harvest window to 24 hours; (3) toxin levels between 60 and 79 μg STX eq./100 g would trigger a batch harvest testing protocol, with each harvest lot being held in cold or wet storage until a representative sample is analyzed to determine if that batch can be released for marketing. The batch release process is labor intensive and may not be feasible if the laboratory does not have the capacity to support the increased sample analyses. At least twice-weekly sampling is also conducted in the affected area, and monitoring is expanded to include adjacent growing areas not yet impacted by the toxin. And (4) toxin concentrations that meet or exceed the action level of 80 μg STX eq./100 g result in immediate harvest closure (California Department of Public Health, 2015). As the harmful algal event declines and shellfish toxin concentrations decrease, the harvest restrictions step back through the tiers, requiring two successive samples at the appropriate concentration to move to a lower tier. This tiered approach has proven effective in California for controlling harvest areas that experience a rapid increase in toxin concentration, with the first low positive sample immediately triggering a shortening of the harvest window, as well as for protected bodies of water that may experience persistent low levels of toxin before exceeding the regulatory threshold. The State of Maine manages high-risk areas and species more cautiously during the bloom season. High-risk species are blue mussels (*Mytilus edulis*), sea scallop (*Plactopecten magellanicus*), European oyster (*Ostrea edulis*), and Atlantic surfclam (= hen clam, *Spisula soldissima*). In 2013, regional mussel closures were implemented, beginning in May of each year and continuing through August along the Maine coast. "Exception areas" were established, allowing potential harvesting under tightly controlled conditions. A prospective harvester must enter into a memorandum of understanding (MOU) with the Maine Department of Marine

Resources. Both high-risk areas and high-risk species require testing and monitoring requirements above the routine weekly sampling conducted by the state during bloom events. Scallop aquaculture operations must also have an MOU with a private lab accredited for biotoxin testing and pay for all analytical costs. A three-tiered system is implemented for PSP toxicity and domoic acid concentrations that increases sampling frequency and implements a batch release process at the third tier. The tiers used by Maine for PSP and ASP, respectively, are: (1) < 40 μg STX eq./100 g and < 10 ppm; (2) 40–59 μg STX eq./100 g and 10–14 ppm; (3) 60–79 μg STX eq./100 g for PSP and 15–19 ppm; and (4) harvest closure at ≥ 80 μg STX eq./100 g and ≥ 20 ppm (https://www.maine.gov/dmr/shellfish-sanitation-management/forms/documents/highriskguidance2016.pdf). For any scheme employed, as the management of harvest activities increases in complexity, there is a need for more intensive oversight and frequent monitoring of phytoplankton and shellfish toxin levels, with rapid turn-around times for all analyses.

When toxin levels exceed the alert level in a given area, the affected industry is immediately notified of a harvest closure and the disposition of any recently harvested product is determined. Specific guidelines for trace-back and recall procedures exist within the NSSP; the individual state food regulations and recall procedures will not be addressed here. Guidance on the geographic extent of a closure, based on the location of samples exceeding the action level, as well as the species affected, will be documented in the program's management plan and must also be communicated. If recreational shellfish resources occur in the same area as the affected commercial shellfish, the public will be warned through multiple avenues, including press releases distributed to major media outlets, the posting of signs at common recreational shellfish access points by local authorities, and updates to web pages and recorded telephone messages. Sampling frequency will increase in the affected and surrounding area to track the distribution and status of the event accurately. If samples of other, lower risk species have not been tested recently, then it may be necessary to prohibit their harvesting, at least temporarily, until samples can be collected and analyzed. An understanding of the relationship among species for the relative rate of toxin increase and the maximum concentrations they may reach can also guide this decision. For example, littleneck clams (*Tapes semidescussata*) are much slower to accumulate PSP toxins and do not reach the magnitudes detected in mussels. When the toxin concentration is just above the action level in mussels but remains below the threshold in littleneck clams, the shellfish control authority may allow the clam fishery to remain open if it has the resources to manage the harvest status of multiple fisheries independently. The criteria for reopening a closed area to harvesting are also an element of the biotoxin contingency plan and will usually require at least two successive shellfish samples from the primary stations, collected some minimum (and maximum) number of days apart, with a concurrent decreasing trend in toxic phytoplankton abundance and toxin levels in the surrounding area. The criteria established in the biotoxin contingency plan are intended to be protective of public health while factoring in environmental and economic concerns. The time interval between reopening samples that has been established will be based at least in part on knowledge of species-specific toxin cleansing rates and the potential for toxins to increase again after a period of decline. It might be suspected that there would be significant variability among different geographic areas (e.g., countries, states, distinctly different areas within a state) that would result in different time intervals between reopening samples. This, in fact, is the case. The NSSP does not contain a specific requirement, although the guidance document for developing marine biotoxin contingency plans in the NSSP Guide (Chapter 2, Section 2.2) recommends that at least three samples be collected over a minimum of 14 days. A casual review of several contingency plans from around the world revealed a range of intervals between successive samples, from two to seven days. It is important to remember that each of these approaches has been demonstrated to be protective of public health and is therefore a reasonable approach for that area.

When commercial shellfish harvest areas are closed because of elevated toxin concentrations, there are few postharvest options currently available for destroying the toxin or accelerating the detoxification of shellfish to safe levels. The NSSP provides for heat treatment as a control option in areas where low levels of PSP toxins routinely occur. Thermal processing does not destroy all of the toxin but merely reduces the PSP toxin concentration. The processor is required to provide proof of the adequate destruction of the toxin and to establish controls to ensure that the treated product is safe for human consumption. A control procedure must be developed, defining the toxicity limits for processing, the controls for harvesting

and transporting the shellstock to processor, the special marking used for unprocessed shellstock, all scheduled processes, and end-product controls on the processed shellfish. There is abundant information in the scientific literature documenting attempts with various cooking strategies for reducing PSP toxin concentrations (frying, steaming, boiling, autoclaving, canning, retorting, smoking, and various combinations thereof). All have had limited success such that the finer considerations of palatability and elasticity were not addressed. A summary of these various attempts can be found at: http://www.fao.org/docrep/007/y5486e/y5486e0b.htm.

Depuration, the process of holding shellfish in a controlled system of clean seawater for cleansing purposes, is used successfully throughout the world for the reduction of fecal bacterial pathogens; however, it has not yet been successfully applied for the removal of marine toxins. The rate of depuration varies among species and for the different toxins within a given species. The actual processes involved in contamination and distribution of toxins within the organism, as well as detoxification, are poorly understood. A number of variables may affect the depuration rate, including the initial toxin concentration, the size and age of the individual, the location of the toxin (e.g., confined to the digestive gland or distributed intracellularly and potentially bound to other tissues), temperature, and salinity. There are a number of conflicting study results for these parameters, and Lassus *et al.* (2014) provide a detailed review of research on the intoxication and detoxification of shellfish and other species for a number of marine toxins. Bricelj and Shumway (1998) categorized a variety of shellfish species as rapid or slow detoxifiers. The former follow a single-phase model for detoxification, while the latter reportedly fit a two-phase model, exhibiting a fast initial rate of detoxification followed by a slower rate of decrease. For practical reasons, the potential use of depuration for detoxifying shellfish will likely be limited to those species known to detoxify quickly. Depuration is a costly and heavily regulated process, so economics certainly factor into its applicability to transient shellfish intoxications. It would be an expensive proposition to maintain a certified depuration facility if it was only used for a short period of time each year when blooms may or may not occur. The applicability of depuration to non-bivalve seafood species (e.g., crustaceans such as crab and lobster) has not been determined. Little is known about the natural depuration rates of marine toxins in these other species. With the apparent increase in frequency, distribution, and magnitude of HAB, however, depuration may receive increased attention and become more economically viable for some seafood species.

An option currently limited to some scallops is the removal and marketing of just the adductor mussel, a process that is only applicable to those species that do not accumulate marine biotoxins in this tissue. The use of only those edible parts that are known not to accumulate marine toxins, or that accumulate very low concentrations well below the level of concern, may have application to other non-bivalve species such as crustaceans (e.g., crab, lobster). Evisceration prior to marketing may be an option when toxin levels in the viscera (including the hepatopancreas) are above the action level but below a defined threshold. Similarly, it needs to be determined if crab legs and claws remain safe if toxin levels in the body meat have exceeded the regulatory threshold. It is not clear if either of these approaches is economically feasible, as the live product has the most value. Faced with an extended HAB event and fishery closure, however, these secondary markets may be slightly more attractive as a way to at least partially sustain the fishery.

10.7.2 Recreational Shellfishing

As with commercial areas, the management of marine biotoxin impacts in recreational harvest areas is greatly aided by a robust phytoplankton monitoring effort and strategically placed sentinel mussel bags or cages where feasible. Identification of a toxin producer in a particular region can trigger an increased sampling effort for phytoplankton and shellfish, with both primary and secondary stations being targeted in and around the affected area. If the phytoplankton observations catch a significant increase in a toxin producer before toxins are detected in shellfish samples, managers may have the time needed to initiate sampling of other species. Additional control actions (e.g., public notifications or closures) may be determined by the authority based on an established cell density threshold. If a sample result approaching a toxin action level is reported and follow-up samples cannot be obtained for at least several days, a preemptive health alert or recreational harvest closure may be necessary to alert the public. When follow-up samples are finally obtained and analyzed, the alert can be adjusted as warranted. If toxin concentrations increase

above the action level in the primary monitoring species (e.g., mussels), a public health advisory and/or fishery closure needs to be publicized, requiring decisions to be made quickly on the extent of the area to close and the species to include. In some cases, the phytoplankton and toxicity monitoring may indicate a minor event with toxin levels reaching a plateau and remaining only slightly above the action level. In that circumstance, a decision may be made to exclude the low-risk shellfish species (e.g., certain benthic clams) from the control actions, at least initially. The ability to monitor phytoplankton levels and collect frequent shellfish samples is obviously key to managing species-specific closures. Unfortunately, recreational harvest areas are usually sampled at a lower frequency than commercial areas due to limited access, typically because of unsuitable tides, or lack of staff resources. The use of local volunteers can help for the latter. Seasonal closures may be necessary in regions that experience frequent biotoxin events that are unmanageable due to their unpredictable nature or the inability to monitor at an adequate frequency and spatial resolution. California imposes an annual quarantine on the sport-harvesting of mussels along the entire coast, including all bays and estuaries, from May through October. Washington State closes its Pacific Ocean beaches (outside of the Olympic National Park) to mussel harvesting from April through October, and Maine implements a regional mussel closure from May through August of each year (as detailed in the "Commercial Shellfish" section above). A lack of frequent toxin data updates due to infrequent sampling can result in managers being behind the curve of increasing toxin levels, limiting management choices. In that circumstance, advisories and fishery closures will include all species that present a public health risk. The lower spatial resolution in sampling stations may not accurately capture the patchiness or complete distribution of toxins, making it impossible to define the area of impact accurately. As a result, recreational harvest closures often blanket a larger region than indicated by the data, providing a margin of safety because of the unknowns. Common landmarks or governmental boundaries are often used to define a closed area because they are known and understood by the public. On the other hand, if recreational harvesting is limited to a small number of discrete, well-defined areas (e.g., specific embayments), it may be possible to manage the area of impact, if not the species-specific impacts, more finely. The decision to reopen a recreational harvest area will usually rely on the same criteria applied to commercial harvesting: downward trends in the toxin producer numbers and in shellfish toxicity in the surrounding area, and at least two successive shellfish samples, collected a defined minimum number of days apart, which have acceptably low and declining toxicity. In most cases, the lack of access due to unsuitable tides will automatically impose a longer time interval between samples. This can actually be problematic if the follow-up sample cannot be collected for many weeks or months. If several initial samples were collected in the surrounding area and found to be acceptable, the program may decide to lift the advisory and reopen the recreational fishery without the follow-up samples. A separate issue is the impact to high-risk species known to retain a toxin for a long time (e.g., butter clams [PSP] and razor clams [ASP]). It may be necessary to extend the advisory/closure for these species, sampling periodically to determine when they are safe again. Extended closures can be problematic in terms of public acceptance. The longer a closure continues, the less some people will respect the risk. It is critical that the agency or agencies involved communicate sampling results frequently to the public and periodically reissue an updated advisory to make clear that a public health risk still exists.

From the discussion of managing marine biotoxin events, it is clear that there are a number of variables involved, many of which are outside of the control of program managers. Marine biotoxin events are dynamic, and no two are exactly the same, even when they occur in the same area. To manage these occurrences properly, quick and informed decisions are required based on the knowledge and experience that exist within the program. As regulatory requirements become more exacting and uniform, the ability to use professional judgment will be lessened. There is a definite need to tailor marine biotoxin management to the local environment, factoring in the region's history of harmful algal occurrence and biotoxin events, the observed variations in these events over time that may signify a shift in traditional patterns, local shellfish resource utilization, and the needs of the public and commercial enterprises that use and rely on this resource. It is hoped that the impetus to standardize biotoxin monitoring programs with a one-size-fits-all mentality does not remove the ability of individual programs to use their knowledge and judgment. To do so will inevitably result in longer recreational and commercial shellfishery closures than are warranted.

10.8 Phytoplankton Sampling Protocol Examples

Gulf of Mexico:
http://gulfofmexicoalliance.org/documents/pits/wq/goma_hab_toxin_resource_guide.pdf

Centre for Environment, Fisheries and Aquaculture Science (Cefas). 2015. Protocol for sampling and transport of water for the purpose of Official Control Monitoring of classified shellfish production areas under EU Regulation 854/2004, England and Wales Programme:
https://www.cefas.co.uk/media/52578/protocol-habs-collection-of-water-for-toxin-monitoring-ew-v7-july-15.pdf

Irish Shellfish Monitoring Programme. 2016. Ireland Code of Practice for the Irish Shellfish Monitoring Programme (Biotoxins):
http://www.sfpa.ie/Portals/0/Food%20Safety/Shellfish/CoP%20on%20Biotoxin%20Monitoring%20Ver%205%20Dec%202014%203.pdf

Sound Toxins. 2016. Manual, Puget Sound Harmful Algal Bloom Monitoring Program:
https://wsg.washington.edu/wordpress/wp-content/uploads/Sound-Toxins-Manual-2016.pdf

California Department of Public Health, Marine Biotoxin Monitoring Program sampling protocols:
By request at redtide@cdph.ca.gov

10.9 HAB Forecasting Links

Gulf of Mexico: https://tidesandcurrents.noaa.gov/hab/

Texas coast, Gulf of Mexico: https://coastalscience.noaa.gov/projects/detail?key=114

Gulf of Maine: http://www.whoi.edu/website/northeast-psp/forecasting

California: http://www.cencoos.org/data/models/habs

Puget Sound, Washington: https://catalyst.uw.edu/workspace/banasn/14943/82765

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California Department of Public Health. 2016b. Marine Biotoxin Monitoring Program Standard Operating Procedure for Phytoplankton Observations. California Department of Public Health, Sacramento.

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11

Harmful Algal Bloom Education and Outreach

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11.1 Introduction

Harmful algal blooms (HAB) are a naturally occurring phenomenon characterized by a higher than normal growth and accumulation of microscopic algae in coastal waters, both marine and freshwater. They can result in discolored waters as well as mortalities of fish, shellfish, birds, and marine mammals, and pose a threat to public health and local economies (see Chapters 4 through 9). As such, these blooms garner considerable public attention. Better public understanding of issues surrounding these blooms is an important part of resolving these complex and critical issues. While the public generally has limited understanding of the ocean, the more people know, the more they are willing to support policies to keep the ocean and the public healthy (Steel *et al.*, 2005). Understanding complex systems like the ocean is difficult, and engaging learners in experiences focused on the ocean that motivate them to become ocean literate and to act on behalf of the ocean helps them build personal connections to the coasts and their local environments. This chapter outlines existing educational and outreach materials, and highlights projects that might be used as case studies to educate the public regarding HAB.

Education/outreach is an integral aspect of communicating science to the nonscientist and works best when scientists team with outreach specialists. The ultimate goal of an effective HAB outreach program is for people to take actions to mitigate risks. Therefore, a successful HAB outreach program should fulfill three functions: (1) raise awareness of health risks to people associated with HAB, (2) educate people to recognize the potential of toxin production in a particular water body, and (3) clearly indicate what precautions people should take if HAB are present. To accomplish these functions, the outreach program should include elements that address both education and awareness of HAB, and about toxicity levels or presence of a toxic bloom.

Several states have developed protocols for notifying the public when blooms are present; however, at present there are no national guidelines for effective communication strategies to alert the public of HAB presence or for providing information on health risks associated with HAB. Outreach and education efforts at the state level include the production of posters (Figure 11.1), fact sheets (Figures 11.2 and 11.3), and web-based bulletins. Table 11.1 lists some state websites with additional outreach materials, fact sheets, and bulletins. For example, in Florida, a state with annual human exposures to freshwater and marine HAB, agencies have employed a variety of outreach strategies to enhance information sharing and convey research results to the public (Kirkpatrick *et al.*, 2004). One activity, an automated call-processing menu system allowing callers to speak directly with trained poison information specialists in Spanish or English, was the first known HAB educational outreach effort to be evaluated for use and satisfaction (Fleming *et al.*, 2007). Other effective efforts used in Florida include basic print material, an interactive website, and video and social networking. Florida investigators concluded that the best way to reach specific stakeholders was

Harmful Algal Blooms: A Compendium Desk Reference, First Edition. Edited by Sandra E. Shumway, JoAnn M. Burkholder, and Steve L. Morton.

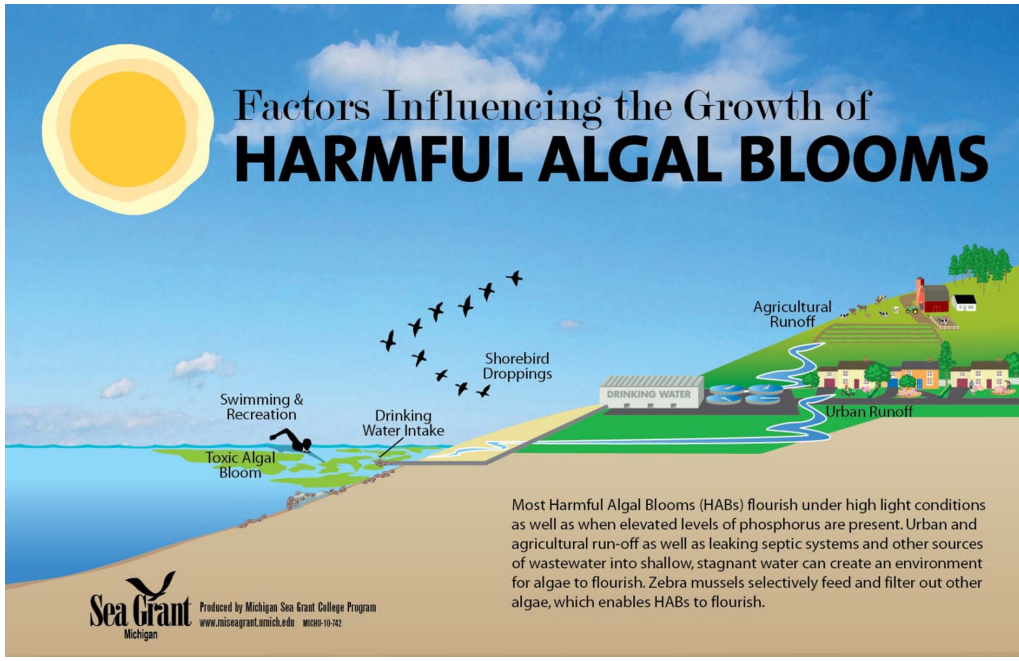

Figure 11.1 Example of an educational poster produced by Michigan Sea Grant.

to develop unique products for specific needs (Kirkpatrick *et al.*, 2004).

During the 1990s, the North Carolina *Pfiesteria* outbreak illustrated some of the many possibilities for outreach materials to be useful in getting the message out to the public. After *Pfiesteria* killed fish and sickened people in the Mid-Atlantic region, Sea Grant programs from New York to North Carolina teamed up to produce video programs, design websites, publish handbooks, and distribute other information to help everyone from seafood processors to journalists to consumers understand the risks of noxious algae and dinoflagellates (Kleindinst and Anderson, 2001). The purpose of the program was to answer questions posed by public citizens and policy makers, and it addressed the roles of science and citizens in a water quality issue. Maryland Sea Grant prepared a documentary entitled *The Pfiesteria Files* (trailer: https://www.youtube.com/watch?v=f0OJNmCoDYc&feature=youtu.be) that not only explained the scientific challenges surrounding *Pfiesteria*, but also detailed the responses of people, including the media, to this most puzzling of organisms. Maryland Sea Grant helped take the lead in preparing a resource notebook for journalists and others interested in basic information about *Pfiesteria* and other harmful algae, including facts on biology, ecology, human health, and seafood safety. North Carolina Sea Grant developed a commercially published guide for educators, *Algae: Source Book for Teaching about Harmful Algal Blooms* (Truitt *et al.*, 2000), through a federal-state-university partnership. Delaware Sea Grant produced *Understanding Mid-Atlantic Residents' Concerns, Attitudes and Perceptions about Harmful Algal Blooms*: *Pfiesteria piscicida* (Falk *et al.*, 2000), based on a survey of 3500 coastal residents from New York to the Carolinas. The results showed that *Pfiesteria* outbreaks might dramatically affect consumers' travel choices, reducing tourism in an affected area by at least 40%, with a significant impact on coastal communities (Falk *et al.*, 2000). Seafood sales might also plummet, with nearly two-thirds of respondents saying they would eat less locally harvested seafood if *Pfiesteria* had been reported in their state waters.

The Olympic Peninsula coast of Washington State has an abundance of shellfish for both recreational and subsistence fishers. The remoteness of this area makes sampling infrequent and sparse, making traditional risk management programs for marine biotoxins difficult. These limitations required that large sections of the area be closed when toxins were discovered in shellfish in order to ensure public safety. Concerned residents of the area formed a citizens' committee to see if funding could be obtained to create a research program for the improvement of monitoring of marine toxins

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OHIO SEA GRANT AND STONE LABORATORY

10 things I should know about algal blooms



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Ohio Sea Grant, based at The Ohio State University, is one of 33 state programs in the National Sea Grant College Program of the National Oceanic and Atmospheric Administration (NOAA), Department of Commerce. Ohio Sea Grant is supported by the Ohio Board of Regents, Ohio State University Extension, other universities, industries, and associations.

OHSU-FS-104
Updated April 10, 2014

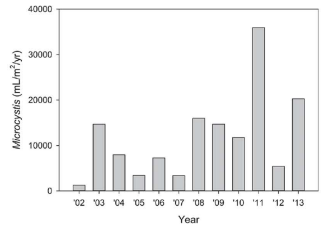
1. Algal blooms are a global problem.
Blooms are actually bacteria called cyanobacteria (also called blue-green algae) and occur in warm waters worldwide.

2. Excess fertilizers in lake water cause blue-green algal blooms.
The same fertilizers that grow plants on the land will grow algae in lakes. Sources include sewage from wastewater treatment plants and leaky septic tanks, animal manure, and commercial fertilizers for agriculture and lawn care.

3. Blue-green algae produce toxins.
Blooms produce toxins that damage the liver, nervous systems, kidneys, and the skin of humans, pets, livestock, fish, and wildlife.

4. Lake Erie blue-green algal blooms were common during the mid 1900s.
Lake Erie was labeled a "dead lake," but upgrades to sewage treatment and removing phosphate from detergents helped the lake to recover.

5. Blue-green algal blooms returned to Lake Erie in the early 2000s.
Changes in farming and more spring storms resulted in more nutrients (such as phosphorus and nitrogen) entering the lake. The record-breaking bloom of 2011 was followed by a small bloom in 2012, which indicates the lake can recover again, and quickly. However, the small bloom of 2012 was followed by the second largest bloom ever, which indicates a bloom can happen in any year.



Bridgeman et al., 2013. A novel method for tracking western Lake Erie *Microcystis* blooms, 2002-2011. J. Great Lake Res. 39, 83-89.

6. Lake Erie blue-green algal blooms form near the Maumee River.
High amounts of nutrients enter Lake Erie through the Maumee River because of lots of farming in the watershed. Larger blooms occur in years with more spring rainfall.

7. How do I keep my family safe?
- Never swim in a lake during a blue-green algal bloom.
- Keep pets out of blue-green algae.
- Never drink or cook with lake water (green or not) because pathogens and contaminants may be present and boiling will not remove them.

8. What can I do to prevent blue-green algal blooms in Lake Erie and other lakes?
- Use phosphate-free lawn care products.
- Regularly check your septic system, as damaged septic systems can contaminate nearby waters.
- Minimize the amount of water you send to the water treatment plant by installing low-flow toilets and rain barrels.
- Plant native plants along shorelines and ditches. These plants can filter out fertilizers and are essentially maintenance-free.

9. What is Stone Lab doing about the problem?
Ohio Sea Grant and Stone Laboratory scientists conduct numerous research projects each year that focus on solving the problem, and provide the results to agencies, managers and the public so they can make well-informed decisions. Ohio Sea Grant and Stone Lab are also bringing together farmers, fertilizer companies, scientists and management agencies to find ways to prevent blooms through new management practices and public outreach.

10. Where can I find more information?
- Ohio EPA, Department of Natural Resources, and Department of Health: epa.ohio.gov/dsw/hab.aspx
- NOAA Great Lakes Environmental Research Laboratory: glerl.noaa.gov/res/Centers/HABS
- Stone Lab tours: go.osu.edu/SLtours
- Aquatic Visitor Center at Put-in-Bay (free to the public): ohioseagrant.osu.edu/avc

Sea Grant | The Ohio State University

Figure 11.2 Freshwater HAB fact sheet produced by Ohio Sea Grant and The Ohio State University.

in the Olympic region. Local residents and coastal communities, in response to seemingly random closures of the shellfisheries due to outbreaks of marine biotoxins (paralytic shellfish poison and domoic acid) in razor clams, formed the Olympic Region Harmful Algal Bloom (ORHAB) Partnership in June 1999. Through this project, a number of outreach materials including restaurant place mats (Figure 11.4), newsletters, and playing cards (Figure 11.5) were developed. A HAB lesson plan was also developed for high schools, which linked to the Washington teachers' standards (https://www.nwfsc.noaa.gov/hab/outreach/education.cfm). SoundToxins, a citizen scientist project, was also initiated at this time (see the "Citizen Science" section). Because the ORHAB and SoundToxins projects provide weekly phytoplankton levels at several beach locations, the State of Washington

Harmful Algal Blooms

A Fact Sheet from the Southern California Coastal Water Research Project

December 2012

What Is a Harmful Algal Bloom?

An algal "bloom" occurs when algae grow rapidly and form dense accumulations. Harmful algal blooms (HABs) are those that negatively affect the ecosystem, humans, and/or wildlife. HABs occur in both fresh and marine waters.

Why Are HABs a Concern?

HABs have a wide range of harmful consequences, but the hazard most often associated with HABs is release of toxins. Algal toxins, if ingested via shellfish or water consumption, can be lethal to wildlife, domestic animals, and humans. The direct physical effects of excessive algal growth can also be harmful to the ecosystem.

Physical Effects

Direct physical effects of HABs include:

- Oxygen depletion (as algae decompose)
- Water discoloration and odor creation
- Light reduction to aquatic plants
- Irritation and clogging of fish gills
- Hypothermia in seabirds covered by algal foam

Causes

Algal blooms occur when water conditions (e.g., light, temperature, circulation, and nutrient levels) are conducive to algal growth. For example, natural coastal upwelling of deep, nutrient-rich waters may help to fuel an algal bloom.

The indirect triggers for algal blooms are not fully understood, but recent research suggests human influences, such as reduced water circulation or excess nutrient loads from land-based sources, can contribute to increased bloom frequency and/or the severity of harmful effects.

Red Tides vs. HABs

Though often used interchangeably, these terms are not equivalent. "Red tides" occur when pigments in algae make the water appear red or brown, a common occurrence in southern California coastal marine waters. Not all red tides are harmful, and fewer than 10% of all southern California HAB species cause red tides.

Fact Sheet 005 - 1

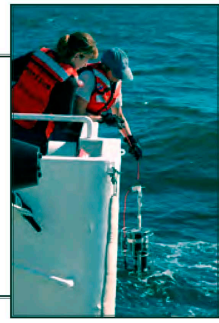
Figure 11.3 Marine HAB fact sheet produced by the Southern California Coastal Water Research Project.

reduced the number of razor clam samples to be tested prior to beach opening for harvest. This resulted in reduced cost and faster analysis. The ORHAB project also assisted managers in Washington State by enhancing communication between regulatory agencies, tribes, and HAB experts. The Washington State Legislature recognized the value of ORHAB in 2003 by establishing a permanent source of funding for it. A small fee was added to the cost of recreational shellfish licenses to maintain the monitoring effort. Each year, these fees contribute $150,000 to a special

SCCWRP actively engages in research related to HAB causal factors as well as collaborative statewide HAB monitoring and response networks.

Investigating HAB Causal Factors

SCCWRP studies how HABs form and move in relation to multiple natural and anthropogenic factors, including nutrient supplies and chemical forms of nutrients. Recent research evaluates nutrient availability on different spatial scales, including region-wide trends and specific HAB "hot spots."



Freshwater HABs

Freshwater HABs are not as well-studied as marine HABs, but can have similar ill effects. Freshwater HAB toxins are more likely to affect water supplies, domestic animals, and livestock, and can also reach marine environments via rivers and storm drains. In southern California, toxins produced by blue-green algae (cyanobacteria) have been detected in many freshwater systems. SCCWRP is conducting ongoing research to document toxin occurrence and improve understanding of triggers.

Developing Monitoring Technology

New monitoring technologies are being tested in southern California to characterize bloom events, track algal toxins, and investigate the water quality conditions associated with HABs. These include fixed environmental sensors and autonomous underwater vehicles deployed remotely to augment information from existing satellite data collection and ship-based water sampling. Passive sampling devices called SPATT (Solid Phase Adsorption Toxin Tracking) bags are another new technology being tested to detect and track toxins in the water.

Engaging in HAB Networks

To advance the application of scientific findings to HAB management efforts, SCCWRP coordinates and participates in several work groups and monitoring networks.

- In addition to establishing an ongoing statewide monitoring network, the **California Harmful Algal Bloom Monitoring and Alert Program (HABMAP)** facilitates information exchange among scientists, managers, and wildlife rescue centers. HABMAP seeks to determine how to respond to HAB events and mitigate their impacts.
- The **Blue-Green Algae Work Group**, made up of water quality managers, public health managers, and scientists, focuses on addressing HABs in California's fresh water bodies. The group is working to develop guidelines and toxicity action levels for local, state, and tribal regulators.

For more information on SCCWRP research, visit: **www.sccwrp.org**

Fact Sheet 005 - 2

Figure 11.3 *(Continued)*

account managed by the University of Washington Olympic Natural Resource Center (ONRC). ONRC uses these funds to maintain the ORHAB coastwide sampling effort, which collects data on toxins, environmental conditions, and plankton blooms. ONRC has launched a public education and outreach effort to inform local residents and visitors about HAB impacts and the benefits of ORHAB monitoring.

Communicating complex scientific principles to the public is challenging. This requires an understanding of not only the science and the scientific

Table 11.1

State	Department	Web site
Alaska	Division of Environmental Health Food Safety and Sanitation Program	http://dec.alaska.gov/eh/fss/seafood/rec_shellfish_harvest.html
California	Department of Public Health Seafood and Shellfish Safety	http://www.cdph.ca.gov/programs/Pages/FDB%20ShellfishSeafoodSafety.aspx
	California Departments of Fish and Wildlife	https://www.wildlife.ca.gov/Fishing/Ocean/Health-Advisories
	California Enviromental Protection Agency	http://www.waterboards.ca.gov/water_issues/programs/swamp/freshwater_cyanobacteria.shtml
Delaware	Department of Natural Resources and Environmental Control	http://www.dnrec.delaware.gov/Pages/RedTideINformation.aspx
Florida	Florida Fish and Wildlife Conservation Commission	http://myfwc.com/research/redtide/
	Florida Department of Health	http://www.floridahealth.gov/environmental-health/aquatic-toxins/index.html
Hawaii	Hawaii Department of Health	http://health.hawaii.gov/docd/dib/disease/ciguatera-fish-poisoning/
Maine	Department of Marine Resources Marine Biotoxin Monitoring Program	http://www.state.me.us/dmr/shellfish-sanitation-management/
Maryland	Department of Natural Resources	http://eyesonthebay.dnr.maryland.gov/hab/hab_sightings.cfm
Massachusetts	Department of Natural Resources	http://www.mass.gov/eea/agencies/dfg/dmf/programs-and-projects/psp-red-tide-monitoring.html
Michigan	Department of Environmental Quality	http://www.michigan.gov/deq/0,4561,7-135-3313_3681_3686_3728-383630--,00.html
New Hampshire	Department of Enviromental Services	http://www.des.nh.gov/organization/divisions/water/wmb/beaches/cyano_bacteria.htm
	Fish and Game Department	http://www.wildlife.state.nh.us/marine/redtide.html
New York	Department of Environmental Conservation	http://www.dec.ny.gov/outdoor/64824.html
North Carolina	North Carolina Environmental Quality	http://deq.nc.gov/about/divisions/water-resources
Ohio	Ohio Enviromental Protection Agency	http://epa.ohio.gov/habalgae.aspx
Oregon	Department of Enviromental Quality	https://www.oregon.gov/deq/wq/Pages/Harmful-Algal-Blooms.aspx
	Oregon Department of Agriculture	http://www.oregon.gov/oda/programs/foodsafety/shellfish/pages/shellfishclosures.aspx
Texas	Texas Parks and Wildlife	http://tpwd.texas.gov/landwater/water/environconcerns/hab/redtide/status.phtml
Washington	Washington State Department of Health	http://www.doh.wa.gov/CommunityandEnvironment/Shellfish/RecreationalShellfish/Illnesses/Biotoxins
Wisconsin	Wisconsin Department of Health Services	https://www.dhs.wisconsin.gov/water/bg-algae/index.htm

process, but also the ability to communicate these principals and processes effectively to nonscientists. A recent survey conducted by the California Academy of Sciences found that four out of five adults did not know basic scientific principles (2009). The health of Americans in the twenty-first century will depend upon the development of a larger number of scientifically literate citizens (Miller, 2010); however, the complex, multidisciplinary nature of the HAB phenomenon can be an excellent platform to raise scientific literacy of the general public. This chapter does not focus

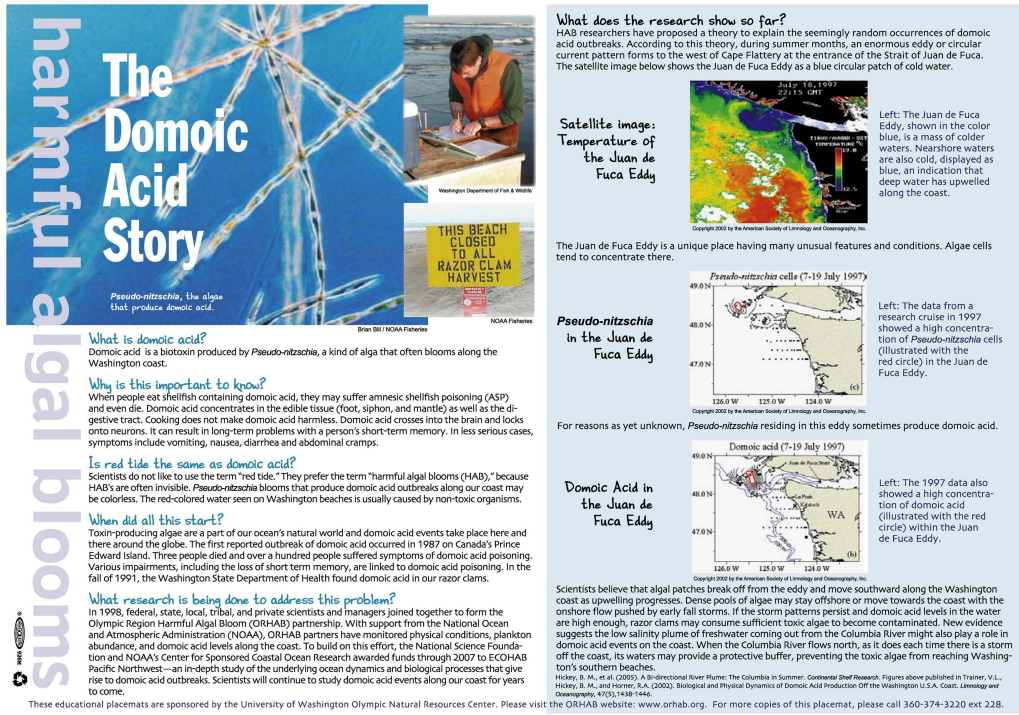


Figure 11.4 Place mats for restaurants developed by the ORHAB project. *Source*: Courtesy of Vera Trainer.

on higher education, because HAB research covers fields ranging from biology to chemistry to physics. It does present the types of outreach materials available and methods to inform the general public regarding the risks associated with HAB. The multidisciplinary nature of the HAB phenomenon makes it an excellent platform for increasing an ocean-literate populace and STEM (science, technology, engineering, and math) education.

Ocean literacy is defined as an understanding of the influence of the ocean on humans and, conversely, their influence on the ocean. The Ocean Literacy Framework comprises two consensus documents, "Ocean Literacy" (http://www.coexploration.org/oceanliteracy/documents/OceanLitChart.pdf) and the more detailed "Ocean Literacy: Introduction to the Scope and Sequence" for grades K–12 (http://oceanliteracy.wp2.coexploration.org/ocean-literacy-framework/conceptual-flows2/). The Ocean Literacy Framework has guided the educational program of the National Oceanic and Atmospheric Administration (NOAA) and the National Science Foundation (NSF). Following this framework is a requirement by both NOAA and the NSF for ocean science research funding. There are seven principles laid out in the first "Ocean Literacy" document (http://www.coexploration.org/oceanliteracy/documents/OceanLitChart.pdf, p. 5):

The Essential Principles of Ocean Sciences

1) The Earth has one big ocean with many features.
2) The ocean and life in the ocean shape the features of Earth.
3) The ocean is a major influence on weather and climate.
4) The ocean made Earth habitable.
5) The ocean supports a great diversity of life and ecosystems.
6) The ocean and humans are inextricably interconnected.
7) The ocean is largely unexplored.

"Ocean Literacy: Introduction to the Scope and Sequence" for grades K–12 describes the seven most important ideas, or Essential Principles, about the ocean that all students should

Figure 11.5 Phytoplankton flash cards developed by ORHAB. *Source*: Courtesy of Vera Trainer.

understand by the end of high school (grades 9–12). The Essential Principles are supported and explained by 45 Fundamental Concepts. The "Scope and Sequence" then provides educators with guidance as to what students need to comprehend in grades K–2, grades 3–5, grades 6–8, and grades 9–12 in order to achieve full understanding of the Essential Principles. These progressions show how student thinking about the ocean may develop in ever more complex ways across many years of thoughtful, coherent science instruction. The "Scope and Sequence," represented in a series of conceptual flow diagrams that include cross-references, also shows how concepts about the ocean are interconnected.

The Ocean Literacy Framework was developed by scientists and educators representing a wide audience from the ocean sciences education community. Their efforts built on previous work to define ocean literacy, assess what the public knows about the ocean, and redress the lack of ocean-related content in state and national science education standards, instructional materials, and assessments. National organizations such as the National Marine Educators Association (NMEA), the European Marine Science Education Association (EMSEA), and the Asian Marine Educators Association (AMEA) have led coordinated efforts to develop educational plans and build awareness about the ocean.

11.2 K–12 Education

In order to have lesson plans accepted for use in the classroom, they must meet individual state science standards. Each state has developed its own science standards, and they are not uniform or even coordinated throughout the United States. The Next Generation Science Standards is a multistate effort to create educational standards arranged in a coherent manner across disciplines and grades to provide students an international benchmark for science education. These standards

were developed by a partnership of the National Science Teachers Association, the American Association for the Advancement of Science, the National Research Council, and a number of nonprofit organizations. The Next Generation Science Standards are intended to help students develop a deeper understanding of core scientific concepts, and help educators develop curricula based on case studies with emphasis on critical thinking and primary investigations. This link demonstrates the Next Generation Science Standards alignment: http://oceanliteracy.wp2.coexploration.org/next-generation-science-standards-2/.

One of the few publicly available lesson plans was developed by NOAA in partnership with the National Science Teachers Association (http://oceanservice.noaa.gov/education/lessons/bad_algae.html). This lesson plan, called "Bad Algae!", requires approximately one to two, 45-minute class periods to complete. The plan includes background information, learning procedures, extra resources, and links to the National Science Education Standards and ocean literacy standards. *Algae: A Sourcebook for Teaching about Harmful Algal Blooms* by Anderson and Stubbs (2000) contains a series of lesson plans. After each lesson plan, the National Science Education Standards applying to the lesson are listed.

Additional lesson plans on HAB and phytoplankton can be found on The Bridge clearinghouse of ocean science lesson plans. The Bridge (http://web.vims.edu/bridge) is an online collection of marine education resources. The site is supported by the National Sea Grant Office, the National Oceanographic Partnership Program, and the National Marine Educators Association. Other examples include:

Bridge Ocean Education Teacher Resource Center
http://www2.vims.edu/Bridge/
An ocean of free teacher-approved marine education resources

Deep-Sea Discoveries in the Atlantic Onboard the NOAA Ship *Okeanos Explorer*
http://www.coexploration.org/oe2014/
An online workshop to advance transatlantic ocean science literacy, March 3–April 4, 2014.

The Bridge is composed of a network of educators who serve on the Clearinghouse Coordination Committee and assist with national information and site review, and a network of scientists who serve on the Scientific and Technical Advisory and Review to advise on scientific content. The Bridge is a resource center for vetted lesson plans, professional development opportunities, and all marine education topics. The Bridge also hosts the listserv Scuttlebutt, a forum for marine educators to talk informally and discuss marine educational ideas.

University of Georgia, Marine Extension and Georgia Sea Grant produced a curriculum guide based on the Coastal Georgia Adopt-A-Wetland Program; the link to download is: http://marex.uga.edu/uploads/documents/AAW_Curriculum.pdf.

The training guide for the Coastal Georgia Adopt-A-Wetland Program is at: http://marex.uga.edu/wetland/.

11.3 Web-Based and Distance Learning Education

Web-based education has become a very important branch of educational technology. For learners, it provides access to information and knowledge sources that are practically unlimited, enabling a number of opportunities for personalized learning, tele-learning, distance learning, and collaboration, with the clear advantages of classroom and platform independence (Brusilovsky, 1999). In addition, teachers and authors of educational material can use numerous possibilities among web-based authoring tools for developing web-based courseware, and cheap and efficient storage and distribution of course materials, hyperlinks to suggested readings, digital libraries, and other sources of references relevant for the course (Devedzic, 2003). *Web-based distance education* is often used interchangeably with *distance learning*; strictly speaking, however, distance learning is only one aspect of web-based learning, and the desired outcome of distance education. Distance education uses a wide spectrum of technologies to reach learners at a distance, not only the web: written correspondence, text, graphics, audio- and videotape, CD-ROM, audio- and videoconferencing, and interactive TV and Skype. In addition, several educational videos have been produced to be viewed on platforms such as YouTube (Table 11.2).

Several governmental and nongovernmental agencies have produced numerous interactive websites to educate the public on HAB. The "Harmful Algae" web page (https://www.whoi.edu/redtide/home) is maintained by the U.S.

Table 11.2 Examples of YouTube phytoplankton educational videos.

Title	URL
Florida Red Tide	https://www.youtube.com/watch?v=14zmRgr6OaI&list=UUqTycG9RmJ9Jv_GmsBXi6Xg
What is Red Tide?	https://www.youtube.com/watch?v=0LdLWPwdwVs
The Secret Life of Plankton	https://www.youtube.com/watch?v=xFQ_fO2D7f0&t=116s
The Power of Plankton	https://www.youtube.com/watch?v=u1Vtdz4J7J8&t=223s
Five Reasons to Thank Plankton	https://www.youtube.com/watch?v=23mrtGCkAH8
The Ocean's Green Machines	https://www.youtube.com/watch?v=H7sACT0Dx0Q
Ocean Drifters	https://www.youtube.com/watch?v=ziGtmjiUlJQ

Office of Harmful Algal Blooms with funding from the NOAA Center for Sponsored Coastal Ocean Research under the authorization of the Harmful Algal Bloom and Hypoxia Research Control Act (HABHRCA). The site contains information on HAB species and their impacts, along with national and international HAB resources. Links to various state toxin hotlines are also given. The site itself receives approximately 7000 web views per month with 33% of these hits being international (J. Kleindinst, personal communication).

Federal agencies such as NOAA (www.noaa.gov), the Food and Drug Administration (FDA; www.fda.gov), the Environmental Protection Agency (www.epa.gov), the Centers for Disease Control and Prevention (CDC; www.cdc.gov), and the Department of Agriculture (www.usda.gov) all have websites devoted to various aspects of HAB education/outreach. Many state agencies have also developed websites to both educate and warn constituent groups regarding local HAB problems. The main problem with web-based resources is the ability of an agency to keep the information current and to keep the website operational. An example of a long-lasting, federal government–funded educational web resource is the "Bad Bug Book" at the FDA site.

The "Bad Bug Book" is produced as a web-based resource for pathogens, bacteria, viruses, parasites, and natural toxins, which can contaminate food and cause human illness. The second edition of the "Bad Bug Book" provides current information in an abbreviated and general form, and is not intended to be a comprehensive scientific or clinical reference. Examples of outbreaks with links to the CDC's *Morbidity and Mortality Weekly Report* (MMWR) are also found in this publication. This resource is updated by researchers at the FDA regularly.

The educational CD-ROM *Phytopia: Discovery of the Marine Ecosystem* is a data-rich resource with interactive tools, high-quality graphics, and movie clips designed for users of various skill and interest levels (de Charon *et al.*, 2016). The main components include "Phyto files" with a virtual microscope with images, and "Phyto factors" containing a bloom activation tool and demonstration of how environmental factors affect blooms. The CD-ROM is supported by the Bigelow Laboratory Phytopia website (http://www.bigelow.org/phytopia/). This website presents a series of special educational materials on various topics, including understanding toxic and nontoxic HAB, where they occur in U.S. waters, HAB effects on the food web, how specific toxins affect humans, and the species of phytoplankton that cause HAB.

11.4 Citizen Science

Citizen science is scientific research conducted by amateur or nonprofessional scientists. Citizen science and crowdsourcing projects are powerful tools for providing students with skills needed to excel in STEM. It has become increasingly important in conservation science, as resources for monitoring fail to match the scale of the questions at hand (Tulloch *et al.*, 2013). For citizens, the motivation is to contribute to scientific understanding and conservation decisions. For scientists, citizen science provides an opportunity to gather information that would be impossible to

collect due to limited time and resources (Dickinson *et al.*, 2010). Volunteers in citizen science gain hands-on experience doing real science and, in many cases, take that learning outside of the traditional classroom setting.

Citizen science volunteers may be retired individuals or students in a classroom; however, quality training is critical and must be performed regularly if the collected data are to be considered viable. MacKenzie *et al.* (2016) noted in their quality assurance protocol that volunteers who were not familiar with an area misidentified plants. They suggested that a small number of highly trained volunteers worked best for their Mountain Watch plant identification program (Mackenzie *et al.*, 2016).

The data collected by volunteers in various projects deserve to have a "place at the table" of science and research. Long-term data trends on species population changes, composition, and migratory patterns, and on climate, would not be possible without volunteers. Data collection by citizen scientists for various projects is often of great magnitude and is too vast for researchers alone with limited resources. Often, the data provided by trained volunteers give information about subjects from a diverse geographic area, which would be an impossible undertaking for scientists (Dickinson *et al.*, 2010).

11.4.1 Contributions of Citizen Science

Citizen science benefits communities by educating the public about science through hands-on involvement; it also addresses the lack of data and identifies pollution problems. In addition, citizen science projects offer opportunities to educate youth, provide information for natural resource management, and answer research questions (McKinley *et al.*, 2016).

It is important to let volunteers know that what they do is important and that they are contributing to genuine databases and scientific reports. If nothing occurs because of their data collection efforts, there will not be a long-term commitment to citizen science programs. For example, the Georgia Adopt-A-Stream/Wetland coordinators and trainers will provide training for volunteers to collect water quality data (pH, dissolved oxygen, temperature, turbidity, and salinity) at their adopted site. All data are added to the Coastal Georgia Adopt-A-Stream/Wetland Program. The website includes an interactive database housing all data collected by volunteers since the 1990s. Reports were published about the Coastal Georgia Adopt-A-Wetland Program groups, and an analysis of the data was provided for each site on the Georgia coast (Sweeney-Reeves *et al.*, 2009). These reports were important for volunteers to know they were contributing to a body of knowledge. The data sets can be downloaded by school groups, the public, researchers, and other scientists.

Programs exist in various natural history projects, especially projects for monitoring animal populations. The main database that houses bird population data collected by citizen science groups is the eBird program. Volunteers can keep track of bird populations in their own yards or at local parks. The data are available to the public online at: http://Ebird.org. This program has proven to be a useful tool in observing trends in declines or increases of bird populations, mostly due to habitat loss. It is also interesting that the data can be accessed to look at migration patterns. Data from two of the most popular birding events (the Annual Christmas Bird Count and the Backyard Bird Count) are now housed at eBird.org, making the database a singular data source. Reports and trends are provided on their website. Another outstanding source of bird identification workshops and training is the Cornell Laboratory of Ornithology. Since birding is rapidly becoming a popular activity, there are numerous resources provided to increase the level of bird identification expertise. The main website is at: http://www.birds.cornell.edu/Page.aspx?pid=1478. One important feature of the Cornell Lab of Ornithology is the Project Feeder Watch Monitoring Program. Every year, participants become certified in monitoring backyard feeders, and then provide long-term trend reports.

In the United States, there are a number of state and federal citizen science programs that range from collecting water samples to recording environmental conditions and identifying HAB species. The number of citizen science programs is too vast to list in this chapter; however, the National Water Quality Monitoring Council, sponsored by the EPA, the U.S. Geological Survey (USGS), and the Advisory Committee on Water Information, hosts an online catalog of volunteer monitoring programs across the United States (http://acwi.gov/monitoring/vm/index.html).

The Florida Fish and Wildlife Conservation Commission established the Red Tide Offshore Monitoring Program in 2000 to assist researchers in the monitoring of the toxic dinoflagellate *Karenia brevis*. Citizen volunteers expand the spatial coverage of the state monitoring program by

collecting water samples from routine collecting points from offshore and coastal waters of the Gulf of Mexico and the Atlantic Ocean. The integration of citizen science programs such as the NOAA Phytoplankton Monitoring Network (PMN), the California Phytoplankton Monitoring program, the Maine Phytoplankton Monitoring program, and SoundToxins is outlined in Chapter 10.

With the modern technological features of the smartphone, there are hundreds of thousands of apps on the market designed for education and outreach. As more schools bring tablets into the classroom, the need for educational apps will increase. Developers have created easy-to-use programs that serve as learning platforms for students and as tools for teachers. HAB education and outreach, like most fields, have a few smartphone applications available in both the IOS and Android platforms.

The smartphone application Phyto was developed to assist in helping volunteers with the NOAA PMN learn to identify phytoplankton, and as a reference guide to use when analyzing a water sample (Morton and Gano, 2016). The IOS version of the app has been downloaded approximately 6700 times, and the Android version about 3200 times. Both applications have been downloaded globally. The second version of Phyto is currently under development for both IOS and Android, and will include many new features. The app has updated the index of common species to 46 (from the original 28 species), including those typically associated with coastal HAB as well as freshwater species. The index includes a detailed listing of each species with multiple photos and a pronunciation sound clip (Figure 11.7). The app also includes a guide, which was designed to help aid in identification of different species by shape and other visually observable characteristics. A newly released (August 2016) crowdsourcing application called BloomWatch allows citizen scientists to track cyanobacterial blooms using smartphones. Users will use their smartphone to take pictures of water bodies, which will be uploaded to the CitSci.org website (www.citsci.org) for data visualization.

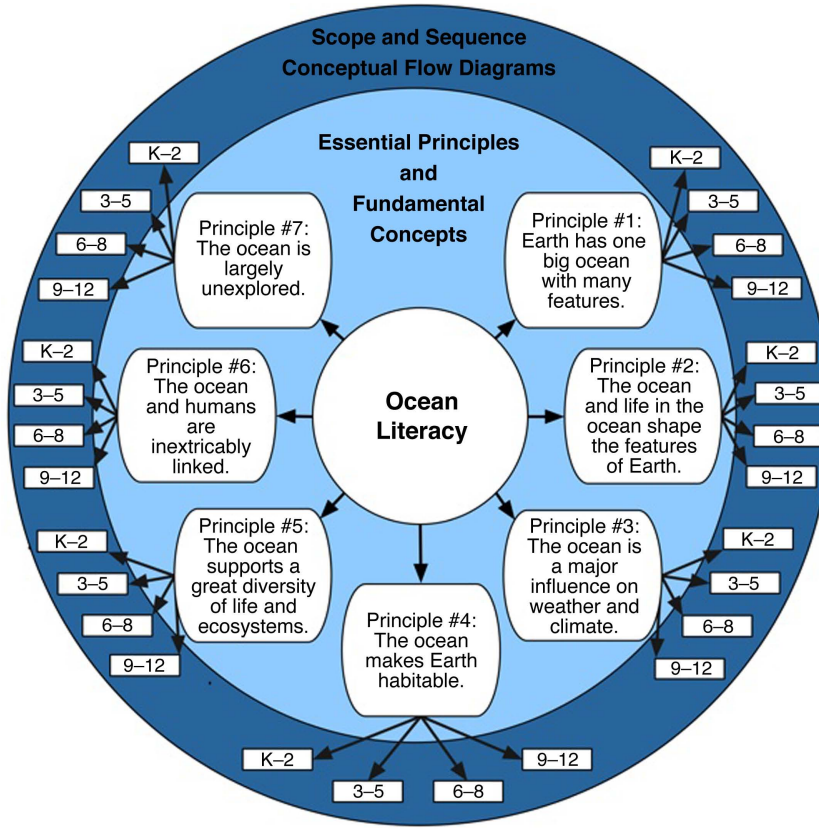


Figure 11.6 The principles and concepts of ocean literacy.

Figure 11.7 Phyto smartphone application.

11.4.2 Connecting Citizen Science to Ocean Learning

In 2001, the NOAA PMN began with three volunteer groups monitoring phytoplankton in Charleston, South Carolina. Over the next few years, Margaret Olsen with University of Georgia (UGA) Consortium for Ocean Science Exploration and Engagement (COSEE) in Savannah, Georgia, and Terri Kirby-Hathaway of the University of North Carolina (UNC) and UNC COSEE collaborated with PMN, resulting in a rapid expansion of the program in the southeastern region of the United States (Morton *et al.*, 2015). Marine educators at the UGA Marine Extension and Georgia Sea Grant (MAREXGASG) added PMN to their volunteer program activities. There is a consistent number of interested volunteers participating in the PMN. Several of them have been involved for almost 14 years.

Volunteers in Savannah, Georgia, meet to sample the Skidaway River each week. They measure water and air temperature, salinity, and tidal flow, and tow a three-minute, mini plankton net. Samples are brought into the plankton lab, slides are prepared, and the search for potential toxic species begins. Results are entered into a NOAA database, and if a bloom is suspected, volunteers send a fixed sample to the NOAA facility in Charleston (Morton *et al.*, 2015).

11.4.2.1 Safety

Safety is a priority when sampling in coastal waters in the southeastern United States, especially in areas such as Georgia and South Carolina where there are strong tidal currents. When collecting water samples to monitor phytoplankton from a dock on the Skidaway River, it is important to enforce the "buddy system." There must be at least two volunteers available to sample from a floating dock. Often, there are Georgia Sea Grant interns tasked with assisting the volunteers. The interns learn a tremendous amount of information from the PMN volunteers, especially those who have been monitoring phytoplankton for so long.

11.4.2.2 Training Sessions

Trainings for the PMN are offered annually at the UGA MAREX and Georgia Sea Grant office in Savannah. Training begins in a classroom setting

using a PowerPoint presentation. Key identification features of each local species are discussed, followed by a "hands-on" lab session where participants sample water and test for salinity, temperature, and dissolved oxygen. Participants learn about microscopes while observing the plankton "catch of the day." For the Marine Science Day offered annually, the entire Skidaway Island science community opens their doors to the public. It is on this day that the public learns about phytoplankton and marine invertebrates. Volunteers in the PMN program share their expertise for 4–5 hours with children and adults. Visitors often stay in the plankton lab for hours to listen and learn about the coast.

11.5 Conclusion

In conclusion, the need for action to improve communication and education about HAB risk and exposures was one of the five recommendations for addressing HAB and hypoxia in the 2016 *Harmful Algal Bloom and Hypoxia Comprehensive Research Plan and Action Strategy* (Gould *et al.*, 2016). Although communication is key to helping society become more scientifically literate, effective communication is difficult since methods to communicate science to the public are constantly changing. It is critical that research results be delivered accurately and in a timely manner to increase public confidence and the utility of the results to resource managers. Education represents a long-term delivery tool to increase public understanding of extreme environmental events such as HAB and their health effects on humans and aquatic ecosystems. Outreach approaches work best when technical support and educational materials are provided by a centralized state or federal agency that is required to update website information and other key essential public resources such as brochures to keep information current and accurate. These agencies should hold workshops, webinars, and professional conferences, which include stakeholders, to disseminate current HAB conditions and risks. Moreover, these activities should be recognized and encouraged by governmental management offices and funding agencies as critical components of monitoring, research, and educational needs.

Outreach education in all of its forms and formats is key to an educated and healthy public, and there is an almost unlimited number of platforms available to accomplish the effort. Scientists, educators, and citizens should all be encouraged to collaborate in the outreach efforts to secure an informed population.

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12

Prevention, Control, and Mitigation of Harmful Algal Bloom Impacts on Fish, Shellfish, and Human Consumers

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12.1 Introduction

With the increasing demand for quality seafood protein for the global population, it is critical to alleviate as many environmental limitations as possible to maximize commercial harvest of fish and shellfish from both freshwater and marine environments and aquaculture facilities. Worldwide, aquaculture production of aquatic food in inland waters exceeds that of marine waters (FAO, 2014), but use of the sea for fish and shellfish mariculture is rapidly increasing in many coastal oceans.

Harmful algae (HA) and their toxins present increasing obstacles to routine protein production in aquatic systems. Additionally, these same accumulations of algae and cyanobacteria can also pose severe health risks to consumers, both wildlife and humans, as sources of toxins debilitating or deadly to those ingesting, drinking, or recreating in systems dominated by the harmful taxa. Less obvious, but still a concern in some regions, is the loss of habitat, through bloom-induced hypoxia/anoxia, shading and mortality of aquatic plants, or modified food webs as well as aesthetics that can alter property values or local perceptions of water body health. Hence, preventing, controlling, or mitigating harmful algal blooms (HAB) and cyanobacterial HAB (cyanoHAB) have become major foci for many geographic areas and systems of the world, from freshwater ponds to open-ocean fish cages, problems potentially exacerbated with continued anthropogenic loading and a changing climate selecting for some bloom-forming taxa (see Paerl and Huisman, 2009; Fu *et al*., 2012).

In the 1970s and 1980s, HAB initially threatened to curtail or destroy the developing marine fish cage industry in several regions worldwide. Fish mariculture in net cages was relatively new throughout the world, and the initial response to major fish-killing HAB events was often chaos as fish farmers were unprepared and unable to protect their fish. Through trial-and-error experimentation, it was found that some locations within a single region have frequent HAB events, while others do not. Industry collaboration with phytoplankton experts led to training for bloom monitoring and the establishment of industry- or agency-sponsored monitoring programs. Fish growers and aquaculture associations began experimenting with different means of HAB mitigation at the farm sites. Large fish-farming companies participated as insurance companies began to demand that farms have viable mitigation equipment and strategies or face losing coverage. The initiation of long-term HAB monitoring programs, such as the Harmful Algae Monitoring Program in British Columbia, Canada, continue to the present in areas with a critical mass of fish culture (Haigh and Esenkulova, 2014). A critical topic germane to this discussion is the increasing prevalence of cultural (human-caused) eutrophication and water column hypoxia in coastal oceans.

12.2 HAB Prevention

12.2.1 Aquaculture Site Selection or Relocation

As discussed in detail in Section 12.7, "Fish Mariculture," site selection is one of the most important criteria for successful finfish and shellfish

Harmful Algal Blooms: A Compendium Desk Reference, First Edition. Edited by Sandra E. Shumway, JoAnn M. Burkholder, and Steve L. Morton.

mariculture, both in terms of the success of a project economically and for environmental protection. Judicious selection of fish farming location is also a primary method for avoiding HAB events and a major consideration to limit nutrient loading to sensitive inland or near-coastal waters. Fish farmers planning large fish farms should consider candidate sites with regard to prevention of eutrophication and hypoxia, although arguably this should be the responsibility of government to provide guidance and regulatory control. In the pragmatic world of fish farming, there are many important socioeconomic and logistic siting and permit considerations that also require attention. Few countries presently have stated policy or enforcement of rules to protect inland or coastal oceans from eutrophication; a few case studies are detailed later in this chapter. Inland freshwater habitats have been remediated through nutrient source controls, with examples frequently discussed in the literature. Large-scale fish mariculture with individual sites producing up to 5000 MT or more is concentrated in just a few countries in the Western Hemisphere (e.g., Norway, Scotland, Canada, and Chile) with the exception of Australia. This is mostly salmon aquaculture, well capitalized, evolving quickly, regulated closely for benthic effects, and trending toward locating in better and sustainable sites near or in open-ocean locations. More numerous but much smaller fish mariculture farms owned by individuals, family groups, or small companies dominate fish mariculture in most of the Eastern Hemisphere (e.g., Thailand, Malaysia, Indonesia, Singapore, Vietnam, Japan, South Korea, mainland China, and Hong Kong). Freshwater fish aquaculture remains dominant with a preponderance of lower food web-feeding fish such as carp, but fish mariculture in these areas is expanding despite coastal waters already adversely affected by cultural eutrophication via riverine and atmospheric sources (Halwart *et al.*, 2007; FAO, 2014). Most of these fish farmers operate with limited means and locate in shallow, nearshore water bodies that are, in many cases, nutrient sensitive, but the fish production is presently much smaller than occurs in the West, as described later in this chapter.

12.2.2 Nutrient Load Reductions

Although some HAB are "natural" increases or accumulations of autotrophic plankton and macroalgae (Sellner *et al.*, 2003), some HAB arise from anthropogenic nutrient inputs (Anderson *et al.*, 2002, 2008; Heisler *et al.*, 2008) that, when combined with physical conditions such as vertically stratified, calm, and warm waters, provide ideal niches for these organisms. Numerous researchers have documented global nutrient enrichment of headwaters, estuaries, and coastal seas (e.g., Bricker *et al.*, 2008; Howarth *et al.*, 2011), and it is the unique behavioral (motility enabling diurnal light harvesting in surface waters and nocturnal nutrient assimilation at depth) and physiological attributes (production of allelopathic and toxic compounds) of many HA that allow proliferation and "blooms." Hence, eliminating conditions that open niches that favor growth and accumulation of the HA taxa is the primary prevention strategy that needs to be pursued in most developed areas. Unfortunately, sufficiently reducing nutrient loading to aquatic systems (Figure 12.1(1)) remains largely unattainable due to a common perception that receiving waters have a huge assimilatory capacity or simply due to ignorance or disregard of the problem. As a result, freshwater and near-coastal nutrient loads that have increased through time are only perceived as a threat when local conditions lead to fish or shellfish mortalities; harvest closures from fecal contamination or HA toxin accumulation; "dead zones" that develop or expand; restricted use of routinely accessible lakes, rivers, estuaries, and beaches; or odor or aesthetic issues that appear. *Reactive* nutrient management is now the norm globally, typically starting late in a eutrophication trend by attempting to reduce nutrient loading, but more commonly trying to manage around it. This often leads to challenges for government officials, industries, established land uses, and cultural beliefs that delay implementation of often expensive best management practices (BMPs) to reduce loads. In the Eastern Hemisphere, relocation of the mostly small fish farms or shellfish raft culture toward or into open-ocean conditions may be extremely difficult to achieve as the types of cages or rafts and infrastructure required demand the resources of large, established companies. Lack of international cooperation is a major factor preventing progress to reduce eutrophication in the Asian Pacific region, where the discharge of neighboring countries regularly affects marine water quality (several chapters in Wolanski, 2006). Anthropogenic point source nutrient load reductions are more readily undertaken, even when expensive, through discharge permitting. Wastewater treatment plants in some developed nations now include tertiary treatment (denitrification) that has made huge reductions of nitrogen load

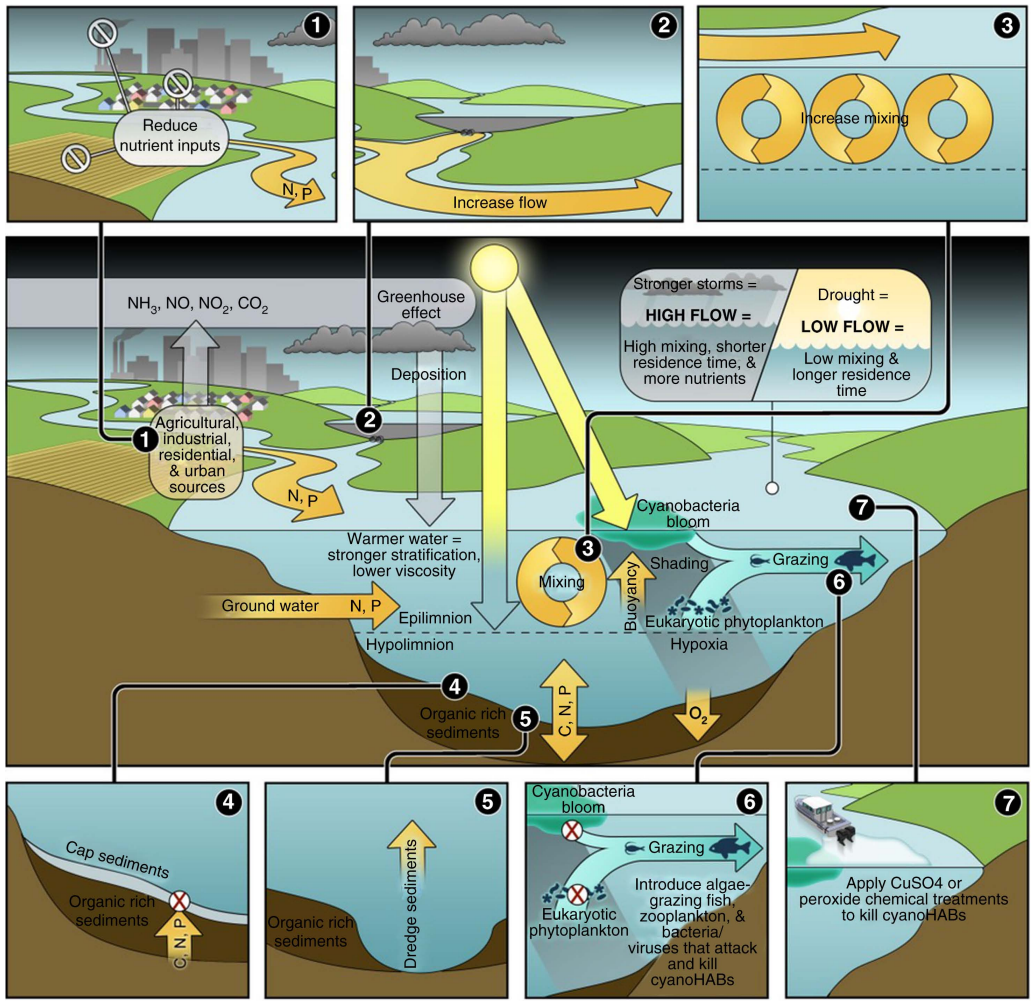


Figure 12.1 Comprehensive PCM (Prevention, Control, and Mitigation) approaches for limiting cyanoHAB (and some marine HAB as well). *Source*: Paerl *et al.* (2014). Panel descriptions are: (1) Reducing nutrient inputs; (2) decreasing residence times to prevent slow-growing HA or cyanoHA from accumulating; (3) increasing mixing to select for non-motile or non-buoyant HA or cyanoHA taxa; (4) capping sediments to limit nutrient fluxes, overwintering cyanobacteria populations, or excystment of dinoflagellates or emergence of cyanobacteria from akinetes in bottom sediments; (5) dredging to remove resting stages or stored nutrient pools; (6) biocontrol options for HA and cyanoHA; and (7) use of chemical biocides to kill HA and cyanoHA. *Source*: From Paerl (2014), http://www.mdpi.com/2075-1729/4/4/988/htm. Licensed under CC BY 4.0.

practical in many highly populated cities. Industry is also regulated through permitting (in the United States, National Pollutant Discharge Elimination System [NPDES] permits), limiting amounts of most pollutants discharged from these point sources. A large problem is the loads from diffuse sources (without a pipe, aka non-point sources), which are more difficult to control, such as agriculture runoff, impervious surfaces (highways, rooftops, and parking lots), and fossil fuel combustion and subsequent atmospheric deposition of nitrogen. Glibert *et al.* (2006) have documented fertilizer (primarily urea-based) use and correlations with coastal eutrophication, with N and P from applied fertilizer and manure entering waterways through surface runoff, with N also moving in substantial quantities as subsurface (groundwater) contamination (Figure 12.1). Excessive application of fertilizers and manures is common, with excess nutrient applied to cropland to ensure that nutrients would not be the reason for failure to obtain maximum crop yields, with any excess available for runoff as particle-bound phosphorus or soluble nitrate over and through surface and subsurface soils.

Figure 12.2 Floating wetland effective in nutrient removal and reducing algal blooms in Wagga Wagga, Western Australia. *Source*: Courtesy of www.FloatingWetlands.org and www.Aquabiofilter.com.

Prevention of excess aquatic nutrient loading could be accomplished through altered land uses to reduce or trap nutrients, and these land use modifications are grouped into BMPs (for an extensive list of science-based BMPs, see http://casttool.org/Documentation.aspx). These land use changes can be implemented through voluntary actions or through enforced regulatory limits for N and P (in the United States, a Total Maximum Daily Load allowed), which partitions N and P load reductions between point and non-point sectors. Unfortunately, *enforcement* of load limits in many countries remains minimal for diffuse sources, perpetuating nutrient enrichment of most waterways and the coastal ocean and accompanying HA proliferation. Also in many countries, no load limits exist for mariculture of fish or shellfish either, including some of the largest aquaculture production countries in the world.

Once in receiving waters, several natural and human-constructed systems and technologies can reduce ambient nutrient levels. Wetlands and marshes are excellent nutrient and sediment traps and have been used to reduce flavor-producing compounds in fishponds with dense cyanobacteria (Zhong *et al.*, 2011). Hence, preserving existing natural filters should be a high priority, particularly in eutrophic systems typified by HAB where shellfish culture is anticipated. Human-made structures can also perform an important role in nutrient assimilation. In-water nutrient uptake (e.g., floating beds; Tao *et al.*, 2014; Figure 12.2) or diversion of ambient waters through channelized systems and across slightly inclined screens can also be used to lower available nutrient stocks for cyanobacteria or other brackish HA. These systems, often referred to as algal flow-ways (Figure 12.3), have been used in streams, rivers,

Figure 12.3 Algal flow-way used to reduce nutrient concentrations in ambient waters through colonization of inert screens by natural periphyton assemblages. *Source*: Courtesy of P. Kangas and L. Smite, UMD.

estuaries, and large aquaria as preventative measures where natural flora and their associated nutrient demand reduce nutrient concentrations in natural systems (e.g., Adey *et al.*, 1993; Mulbry *et al.*, 2010), thereby potentially lowering nutrients for use by HA or nuisance taxa. The systems are usually near-surface, slightly inclined channels or structures where ambient waters flow across supported nylon, Teflon, plastic, or other inert netting, allowing the natural periphyton to colonize the mesh and grow. The accumulating biomass can then be harvested periodically for landfill deposition, composting, or, in the future, commercially important products in biofuels or fatty acids (Adey *et al.*, 2011), removing assimilated nutrients and lowering nutrient concentrations below the flowway discharge; the mesh can be reused, with multiple harvests scheduled dependent on temperatures, flow rates, light availability, and incoming nutrient concentrations. These are increasingly likely practices for eutrophic systems where flow can be diverted across these surfaces and will likely be profitable, particularly after development of the infrastructure required for biofuel production.

Another option used in freshwaters is shading, whether through addition of colored compounds to the water column to limit light availability (e.g., Aquashade®; Boyd *et al.*, 1982) or through deployment of leafy plants that spread across the surface to reduce light to underlying depths. Boyd and colleagues (1982), however, reported no differences in water quality between Aquashade-treated and nontreated channel catfish ponds, so the benefits of the shading compound in fish ponds remains unknown. In Frederick, Maryland, United States, an urbanized shallow creek through the city center has routinely experienced large algal blooms, primarily chlorophytes but with a concern for a shift to toxic cyanobacteria. A local scientist has deployed multiple oriental plants throughout the creek in the last 3 years (http://www.coloronthecreek.com/home.html) with substantial reductions in chlorophyll-*a* below the planted area (D. Ferrier, personal communication); little bloom algae is now apparent, and introduced carp are thriving. Use of this approach on a scale conducive to fish farms would likely be less effective, due to problems associated with harvesting any cultured fish from the mass of plants that would interfere with capture.

Another practice is one form of integrated multitrophic aquaculture (IMTA) (Chopin *et al.*, 2001a,b; Soto, 2009; Figure 12.4) that utilizes cultured seaweed downcurrent or in the vicinity of fish or shellfish farms that will assimilate fish or shellfish ammonia or urea excretion that otherwise might foster elevated autotrophic production in the ambient environment, including HAB. Bouwman *et al.* (2013a, 2013b) have predicted that intensifying fish and shellfish aquaculture/mariculture may lead to huge eutrophication issues for freshwaters and coastal areas of the world, including HAB proliferation. By combining animal culture with seaweed culture (reviewed in Neori *et al.*, 2004), however, nutrients generated from fish or shellfish upcurrent could be partially assimilated by several possible

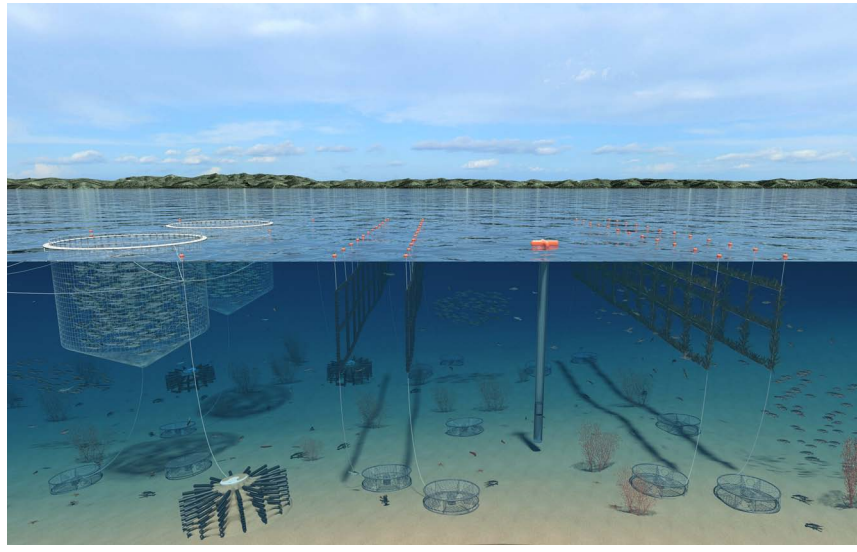


Figure 12.4 Conceptual diagram of possible polyculture (see also *integrated multi-trophic aquaculture* in the text): raising fish, bivalves, crabs, and macroalgae. *Source*: Courtesy of OceanForest, Inc.

Figure 12.5 Removal of mass *Ulva* accumulations in Qingdao, China, prior to the 2008 Olympics. *Source*: Courtesy of M. Zhou and R. Yu, Chinese Academy of Sciences, Qingdao, China.

commercially important green, red, or brown macroalgae on or immediately below the animal cages, pens, or areas. Algal harvest would remove the incorporated N and P from the coastal ocean. Another advantage of this approach is that some of the macroalgae also produce allelopathic compounds that inhibit several of the HA taxa (Tang and Gobler, 2011; Chowdhury *et al.*, 2014; Tang *et al.*, 2014; see below). Multi-step linear food webs have also been used, with fish, shellfish, phytoplankton, macroalgae, and abalone. Polyculture is common to Asia, with a history over 1500 years in China (Parker, 2002), but it can lead to huge macroalgal blooms or biomass accumulation even further downcurrent (e.g., *Ulva* in China; Keesing *et al.*, 2011; see also Figure 12.5 and Chapter 15) if not routinely and carefully harvested.

There are limits to the effectiveness of downcurrent macroalgae for assimilating fish farm wastes (i.e., seaweed aquaculture may offer significant mitigation potential for nitrogen sequestration near fish farms only under certain conditions). Ammonia excretion from the gills of fish can be detected at shallow, seaweed-appropriate depths near fish farms (Sanderson *et al.*, 2008); however, the amount of seaweed required to remove the valued algal growth substrate is dauntingly large. At comparably sized fish farm and seaweed culture areas, *Saccharina latissima* kelp provided only 0.4% of the nitrogen remediation necessary to compensate for the 5000 MT fish farming in place in the area (Broch *et al.*, 2013), suggesting that seaweed culture area would need to be 250 times larger than the spatial footprint of the fish farm. Continental shelf areas of the global oceans are available for very large seaweed farms in some regions, but usually not inshore or in populated and developed areas due to user conflicts from commercial shipping, fishing, and recreation. If considered in an ecoregion approach (Rensel *et al.*, 2006), however, nutrient sequestering need not be done immediately downstream of the fish or shellfish farm, provided that sufficient ambient dissolved inorganic nitrogen is available relative to the nutrient requirements of the seaweed species being cultivated. Greater detail and case studies for fish mariculture are provided later in the chapter (see Section 12.7.2, "Best Management Practices for Fish Mariculture Siting, Including HAB and Eutrophication Issues").

12.2.3 Phytoplankton Mixing, Increasing Turbulence, and Decreasing Residence Times (Mostly Freshwater Systems)

Because many freshwater ponds, lakes, and reservoirs and estuarine embayments experience

seasonal thermal or salinity stratification, cyano-HAB and HAB prevention can be facilitated by increasing turbulence, water column mixing (e.g., Visser *et al.*, 1996; Jungo *et al.*, 2001; Figure 12.1(3)), or flushing rates (low residence times; Paerl, 2008, 2014; Figure 12.1(2)). In general, vertical mixing (Figure 12.3) selects for diatom-dominated plankton communities. Except for *Pseudo-nitzschia* and the robustly spiniferous *Chaetoceros convolutus* and *C. concavicornis* and related subgenus *Phaeoceros* in marine systems, diatoms only rarely pose problems for freshwater, natural, or cultured fish and shellfish; however, the "rock snot" epiphytic diatom *Didymosphenia geminata* prefers running water of streams and rivers. Hence, destratifying freshwaters with seasonal thermoclines (Visser *et al.*, 1996; Chorus and Mur, 1999) or brackish embayments with seasonal haloclines or pycnoclines is a viable but costly option to reduce growth of most motile or buoyant HAB species on a small scale. Technologies that mix surface waters (aerators, bubblers, and mixers, e.g., solar-powered circulation; Hudnell *et al.*, 2010) can be located upcurrent of natural or cultured fish and shellfish stocks, creating conditions that favor growth of rapidly dividing non-motile diatoms rather than the generally slower growing motile or buoyant HAB species. For the solar-powered circulators (SPCs), deployment is estimated at 1 unit for each 0.15 km^{-2} (Hudnell *et al.*, 2010), and at costs of $10,000–100,000 per unit (SolarBee®), capital costs are not low. Furthermore, local bathymetry is a major consideration for likely effectiveness of these mixing technologies. In U- or V-shaped systems, mixers might work well to create needed turbulence to support diatoms; but if the water bodies have heterogeneous bathymetries with multiple small embayments or shallows, these side pools/areas may become low-turbulence refuges for the HA species, essentially reseeding areas for HAB, requiring multiple placements of the mixing technologies and added expense. Barkoh *et al.* (2011) noted, as well, that the SPC was not effective in controlling blooms of *Prymnesium parvum* in Texas ponds.

In a similar manner, ponds, lakes, or reservoirs that can regulate hydraulics through dams or weirs (e.g., Mitrovic *et al.*, 2011) can decrease residence times in the systems (Figure 12.1(2)), thereby flushing slower growing HA taxa into suboptimal growth conditions downstream. Sellner and colleagues (2015) used a manually adjusted wooden weir to flush overwintering vegetative *Microcystis aeruginosa* populations into a downstream reach and, ultimately, the brackish Choptank River estuary to reduce likely resuspension of the cyanobacterium in the lake the following spring and summer. The lake was drained to one-third of its normal volume in 8 h in the fall, with bottom shear flushing *M. aeruginosa* from the lake's surficial sediments, documented from characterizing emergent autotrophic plankton free of the taxon from nearshore cores. Furthermore, only a small increase in *M. aeruginosa* and its toxin microcystin was detected in the lake in late summer, sufficiently late and at a size that permitted recreational use of the lake for 2012–2013, continuing into 2017.

12.2.4 Reducing HA Introductions

Novel taxa identified in areas where the species have never been reported are increasingly common, perhaps due to increasing observations through expanding monitoring as well as actual introductions from distant locations. Most notorious introductions include the attribution (later refuted) of dinoflagellates from Japan in Tasmanian waters (Hallegraeff and Bolch, 1992; Bolch and de Salas, 2007) that opened global debate on potential transfer of HAB taxa via ship ballast. This concern has led to the development of open-ocean ballast exchange (the International Convention for the Control and Management of Ships' Ballast Water and Sediments in 2004) as a means to reduce transfer of noxious species into naïve environments. Beyond open-sea exchange, biocides appear to be the most practical approach to minimize ballast introductions (Hallegraeff, 2015). Individual organism transfers from one site to others may also introduce HA taxa to new, optimal growth areas. Springer *et al.* (2002), Hégaret *et al.* (2008), and Rosa *et al.* (2013) have documented the potential introduction of HA taxa in the relocation of bivalves and benthic ascidians that biofoul shell and uncleaned aquaculture gear. Other examples abound, such as the dramatic expansion of *Didymosphenia geminata* (rock snot) in New Zealand (Kilroy and Unwin, 2011) and that national program to prevent its transfer from the South Island to the North Island (MAF, 2007). Similar efforts are in place in British Columbia, Canada; Chile; and Maryland, United States. A key prevention component is chlorinating fishing gear, "killing" any attached or embedded *D. germinata* before the gear is used in other waterways (Figure 12.6). Another example is the macroalga *Caulerpa taxifolia* that was introduced into the Mediterranean Sea from public or private aquaria and has rapidly expanded to eliminate native macroalgae (Meinesz

Figure 12.6 New Zealand's didymo cleaning advisory, one approach to slowing expansion of the invasive alga. *Source*: Ministry for Primary Industries, http://www.biosecurity.govt.nz/cleaning. Licensed under CC-BY 4.0.

et al., 1993; de Villèle and Verlaque, 1995); it has also been identified off California, United States, where algal beds were quickly eradicated using application of chlorine bleach (Anderson and Keppner, 2001).

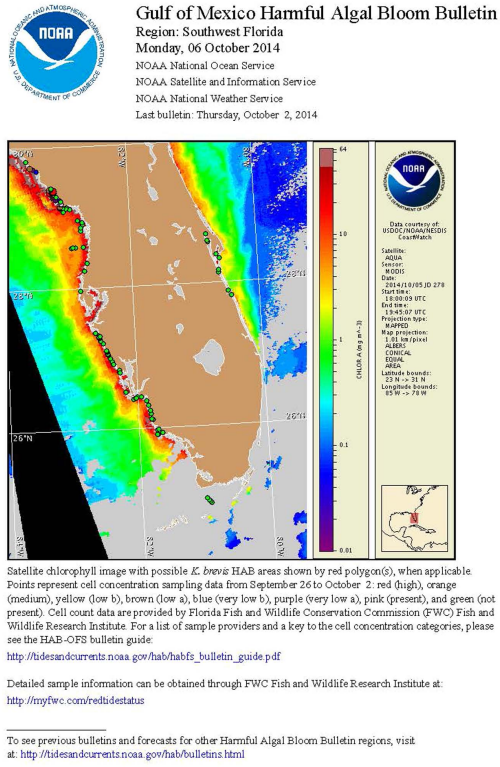
12.3 Preventing and Reducing HAB Impacts on Shellfish and Fish

12.3.1 Preventing Human and Animal Exposures

Human exposures to toxins are generally through consumption of fish and shellfish containing the toxins or analogs and, more infrequently, through aerosolization of these compounds (e.g., Smith *et al.*, 2008; Zaias *et al.*, 2008; Ettoumi *et al.*, 2011; see Figure 12.7). Humans can also be exposed to off-flavors and odors (e.g., methylisoborneal and geosmin) in fishponds (Paerl and Tucker, 1995; Crews and Chappell, 2007), jeopardizing siting of the culture operation as well as harvests. Attempts to minimize exposures are often through posting of advisories, closures, harvest and sales restrictions, and occasionally product recalls, derived from rigorous monitoring of phytoplankton species to identify toxin-producing taxa and cells or toxins in fish and shellfish tissues. Human exposure still occurs, and mitigating toxin effects through medical intervention is possible, although often not implemented due to inadequate recognition of the toxin-induced symptoms that frequently resemble those of other common illnesses (see Chapter 9 for more details).

12.3.1.1 Shellfish and Finfish Monitoring

There are extensive shellfish monitoring programs in the North American, European Union, and Asian countries (see Chapter 10, this volume). The U.S. program is administered by the National Shellfish Sanitation Program (NSSP), which has published a *Guide for the Control of Molluscan Shellfish, 2013 Revision* (http://www.fda.gov/downloads/Food/GuidanceRegulation/FederalStateFood Programs/UCM415522.pdf; NSSP, 2013) and implemented by numerous coastal state government agencies. The document outlines requirements for establishing growing areas as well as monitoring and reporting toxin exposures; countries or firms exporting to the United States are required to meet the NSSP toxin monitoring requirements for shellfish. These and the programs elsewhere lay out guidelines on approaches to subsample harvested product and test for known toxins that accumulate in animal tissue that can cause serious illness or death if consumed. Extensive monitoring, advisories, closures, and harvest and sales restrictions can minimize public exposure to intoxicated shellfish. In the United States, the Food and Drug Administration (FDA) has tried to minimize public exposure to toxin-containing fish and fishery products through



Gulf of Mexico Harmful Algal Bloom Bulletin
Region: Southwest Florida
Monday, 06 October 2014
NOAA National Ocean Service
NOAA Satellite and Information Service
NOAA National Weather Service
Last bulletin: Thursday, October 2, 2014

Satellite chlorophyll image with possible *K. brevis* HAB areas shown by red polygon(s), when applicable. Points represent cell concentration sampling data from September 26 to October 2: red (high), orange (medium), yellow (low b), brown (low a), blue (very low b), purple (very low a), pink (present), and green (not present). Cell count data are provided by Florida Fish and Wildlife Conservation Commission (FWC) Fish and Wildlife Research Institute. For a list of sample providers and a key to the cell concentration categories, please see the HAB-OFS bulletin guide:
http://tidesandcurrents.noaa.gov/hab/habfs_bulletin_guide.pdf

Detailed sample information can be obtained through FWC Fish and Wildlife Research Institute at:
http://myfwc.com/redtidestatus

To see previous bulletins and forecasts for other Harmful Algal Bloom Bulletin regions, visit at: http://tidesandcurrents.noaa.gov/hab/bulletins.html

Conditions Report

Not present to high concentrations of *Karenia brevis* (commonly known as Florida red tide) are present along- and offshore portions of northwest and southwest Florida from Bay to Citrus counties. *K. brevis* concentrations are patchy in nature and levels of respiratory irritation will vary locally based upon nearby bloom concentrations, ocean currents, and wind speed and direction. The highest level of potential respiratory irritation forecast for alongshore southwest Florida Monday, October 6 through Thursday, October 9 is listed below:

County Region: Forecast (Duration)
Dixie: Low (M, Th), Moderate (Tu-W)
Levy: Very Low (M, Th), Low (Tu), Moderate (W)
All Other SWFL County Regions: None expected (M-Th)
NWFL County Regions: Visit http://tidesandcurrents.noaa.gov/hab/#nwfl

Check http://tidesandcurrents.noaa.gov/hab/beach_conditions.html for recent, local observations. Health information, from the Florida Department of Health and other agencies, is available at http://tidesandcurrents.noaa.gov/hab/hab_health_info.html. Over the past several days, reports of dead fish were received from Dixie and Levy counties.

Analysis

Dixie to Citrus counties: Recent samples collected along- and offshore southwest Florida over the past several days continue to identify not present to 'high' concentrations of *Karenia brevis*. Samples collected last Wednesday identified several 'very low a' to 'high' *K. brevis* concentrations alongshore Dixie County, with the highest concentrations collected off Big Pine Island, Shired Island, and Seven Sisters Reef (FWRI; 10/1). Background to 'low a' concentrations were also identified alongshore Levy County (FWRI; 9/30), while all samples collected along- and offshore Citrus County indicated that *K. brevis* is not present (FWRI; 10/1). Over the past several days, reports of dead fish were received from along- and offshore Dixie and Levy counties (FWRI; 10/3-4). No respiratory irritation associated with *K. brevis* has been reported along the coast of southwest Florida (MML; 10/2-10/6).

In Recent MODIS Aqua imagery from 10/5 (shown left) and 10/4 (not shown), elevated to very high chlorophyll (5 to > 20 μg/L) is visible in patches along- and offshore Dixie to Citrus counties, with the highest chlorophyll patches visible along- and offshore Dixie and Levy counties, consistent with areas in which *K. brevis* concentrations have been identified. Elevated chlorophyll in this region is not necessarily indicative of the presence of *K. brevis*; due to the optical characteristics that are typical in the area, some elevated chlorophyll may also be due to the resuspension of benthic chlorophyll and sediments along the coast.

Observed winds and surface currents over the past several days may have promoted southerly transport of *K. brevis* concentrations. Winds and surface currents forecasted over the next several days may promote northerly transport of surface *K. brevis* concentrations. Forecasted winds over the next several days may decrease the potential for respiratory irritation at the coast.

Figure 12.7 The use of satellite imagery for detecting chlorophyll-*a* anomalies associated with Florida's *Karenia brevis* blooms, and the use of local winds to forecast bloom landfall in NOAA's *Florida HAB Bulletin*. *Source*: Courtesy of M. Tomlinson, NOAA-NOS.

its requirement for processors and importers to develop and implement Hazard Analysis Critical Control Point (HACCP) systems (http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Seafood/ucm176892.htm). The FDA requirements cover fresh and frozen molluscs imported into the United States and transported between states, and these products are controlled by the NSSP, which, as noted above, oversees state-specific programs (http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Seafood/ucm176892.htm#xi). A Memorandum of Understanding must exist between the exporting country and the FDA before the NSSP will permit receipt of the product. Canned shellfish requires processing in accord with the FDA HACCP mandate. The FDA can also test fish tissue for toxin content, generally in response to reported human illnesses or to identify whether newly identified toxins or other compounds might pose health risks to consumers.

The agency also has set "action levels" (see Chapter 10) for common toxins to be used by "State, tribal, local, or foreign authorities . . . to decide whether to issue local advisories to consumers recommending limits on consumption of all or certain species of locally harvested fish (some of which may be commercially important) or to close waters for commercial harvesting of all or certain species of fish" (HHS-FDA 2011). These may also be used for local consumption guidelines for shellfish/molluscs. Internationally, the FAO has published toxin levels for bivalve molluscs that would limit harvest, sale, or transport (see Chapter 10). In the European Union, legislation states that ciguatoxin (CTX) has to be absent in fish placed on the market; furthermore, tetrodontidae (puffer fish) may not be placed on the market in order to prevent tetrodotoxin (TTX) problems in consumers (P. Hess, personal communication). Degrasse and Diaz-Martinez (2012) have tabulated toxin action levels for all countries of the Americas, while Hess (2012) has summarized EU discussions and considerations for toxin monitoring, detection/assays, and concentrations.

The World Health Organization (WHO; 1998) also has published a tolerable daily intake (TDI) for

microcystin-LR in humans at 0.04 μg kg^{-1}. Mulvenna *et al.* (2012) also have estimated TDI levels for several cyanobacteria toxins common to Australian waters (see Chapter 10). Considering that some of the largest freshwater natural fisheries in the world (e.g., Uganda) yield fish with body burdens up to 1917 μg equiv kg^{-1} (Poste *et al.*, 2011), there is deep concern for human illness including cancers. Similarly, consumers of product and operators of freshwater pond culture systems (carp, mullet, tilapia, and prawns) face these toxins, with operators also exposed to aerosolized toxins (Cheng *et al.*, 2007). Shellfish harvesters in coastal areas receiving advected populations of toxic *M. aeruginosa* (Miller *et al.*, 2010) as well as consumers of the marine shellfish should also use caution. Fish and shellfish harvesters, producers, operators, and processors should examine detailed local and international regulations for HA toxins for each product they consider.

12.3.1.2 Depuration and Detoxification

Moving cultured fish and shellfish to non-HAB areas has been suggested to either minimize exposure or, alternatively, displace ingested toxin from culture tissue. Some shellfish taxa rapidly (days) clear toxin from body tissues, others require extended periods of time, and it is all dependent on the toxins and shellfish species under consideration. Examples include the clam *Mesodesma donacium* (Álvarez *et al.*, 2015) and the mussel *Mytilus edulis* for paralytic shellfish toxins (Bricelj and Shumway, 1998), while others require months (e.g., 6 months for *M. edulis* with okadaic acid compounds [Lembeye *et al.*, 1993] and 3–9 months for *Patinopecten yessoensis* with paralytic shellfish poisoning [PSP] toxins [Kaga *et al.*, 2015]), and others years (e.g., *Placopecten magellanicus* [Cembella *et al.*, 1994] and *Spisula solidissima* [Shumway *et al.*, 1994] for paralytic shellfish toxins). For other examples, see Fernández *et al.* (2003, table 24.2), Bricelj and Shumway (1998), and below. In most cases, however, relocating exposed populations to depurate toxins is rarely economically or biologically successful, or, in most cases, even feasible (Bricelj and Shumway, 1998).

There appears to be some reduction in microcystins in fish tissue after removal from microcystin-rich diets (i.e., depuration or degradation occurs within the vertebrates). For example, after 47 d periods of growth on low (26.60 μg MCs/g), medium (78.82 μg MCs/g), and high-microcystin (201.03 lg MCs/g) diets, Dong *et al.* (2009) noted that microcystins declined in hybrid sturgeon (*Acipenser baeri* ♀ and *A. gueldenstaedtii*) following 43 d exposure to microcystin-free food. Feeding rates as well as specific growth rates for low and medium toxin-fed fish and control food fish were similar after the microcystin-free period; fish receiving the highest microcystin doses during their feeding period fed and grew significantly faster than either control or low- and medium-dosed fish. As in shellfish, toxin loss rates are very likely species- and toxin-specific. In general, however, depuration of shellfish and fish is left to natural processes and overseen with monitoring programs.

12.3.1.3 Food Processing

Evisceration of shellfish, particularly the hepatopancreas, has proven effective in reducing toxin levels in some bivalves (Reboreda *et al.*, 2010) but not others (e.g., Álvarez *et al.*, 2015). It is particularly effective in the domoic acid (DA)-intoxicated scallop *Pecten jacobeus*, and even more reduction occurs in combination with thermal processing or freezing/thawing (Reboreda *et al.*, 2010).

Shellfish canning has been a common practice used to reduce toxin levels in contaminated tissue, although little degradation occurs for the lipophilic species (see references in Blanco *et al.*, 2015). The canning heating process has been observed to lower PSP toxins in the clam *Ruditapes decussatus* and the softshell clam *Mya arenaria*, the mussels *M. edulis* and *Mytilus galloprovincialis*, scallops, and the cockles *Acanthocardia tuberculatum* and *Cerastoderma edule* (Cembella and Todd, 1993; Noguchi *et al.*, 1980; Berenguer *et al.*, 1993; Reboreda *et al.*, 2010), but it increases diarrhetic shellfish poisoning (DSP) toxins in mussels (Blanco *et al.*, 2015). In contrast, Reboreda *et al.* (2010) reported no change in total DSP levels with thermal heating and hermetic container sealing (analogous to canning). Toxin derivatives may result (e.g., OA to DTX-3 [Reboreda *et al.*, 2010] and AZA3 increases [McCarron *et al.*, 2009]) as well as toxin partitioning between meats and packing fluids so caution in the use of heating as a routine prevention technique is encouraged.

In some cases, freezing can lower toxin content, but usually not to levels below regulatory limits. Leira *et al.* (1998) noted declines in DA in the canned scallop *Pecten maximus*, but only to the surrounding packing fluid. Reboreda *et al.* (2010) noted decreases in DA in *P. jacobeus* and PSP in cockles (*C. edule*) (but not in the clam *R. decussatus*) following freezing but to levels still above regulatory limits; freezing with thermal processing was very effective in toxin reduction for all molluscs tested. Critical point extraction (dehydration)

for DSP toxins from molluscs is also feasible (González *et al.*, 2002).

Rinsing or washing contaminated tissue can also lower toxin levels in some molluscs, most notably abalone. For example, washing the black epithelium of the foot of the abalone *Haliotis laevigata* significantly decreased PST levels below regulatory limits (Homan *et al.*, 2010). Lassus *et al.* (2007) reported that a 15-min tap water washing of DA-rich *Pecten maximum* gonads reduced mean toxin levels below the statutory safety level of 20 μg DA g^{-1}, and after 120 min, DA in gonad tissue decreased below the minimum detectable limit for the toxin.

12.3.1.4 Cooking

Cooking has limited impact on toxin reduction in tissues. As noted above, the lipophilic toxins (okadaic acid and analogs, and spirolides) are not degraded (and may increase on a weight-specific basis) with normal cooking treatments employed in shellfish consumption, while levels for other toxins vary with molluscan and toxin species present (Hess *et al.*, 2005; Picot *et al.*, 2012; Blanco *et al.*, 2015). In a recent review, Gutiérrez-Praena *et al.* (2013) summarized results from the literature and concluded that cooking bivalves does not reduce microcystin levels, whereas boiling can reduce microcystins in fish fillets with some of the toxin moving to the surrounding water. Hence, boiling may lower toxin intake to levels below the tolerable daily intake levels for humans, but pose even more serious problems for making soups from the concentrated broth. In an examination of five molluscs, Picot *et al.* (2012) noted decreases in DA in cooked razor clams (*Ensis* and *Solen* spp.) and cockles (*C. edule*) and increases in mussels (*M. edulis*), carpet shell clams (*Ruditapes* sp.), and a *Donax* sp. Vidal *et al.* (2009) noted similar decreases for the cockle after boiling or steaming, but no change in DA in the manila clam *Ruditapes philippinarum*. Okadaic acid and analogs as well as spirolides increased on cooking *M. edulis* (Hess *et al.*, 2005; Picot *et al.*, 2012). Although 51% of PSP in the scallop *Patinopecten yessoensis* leaked from steamed tissue (Wong *et al.*, 2009), consumption would still pose serious health hazards for the consumer.

12.3.1.5 Aerosols

Some HAB toxins can affect humans through the atmosphere, as aerosols from coastal bloom areas. Respiratory distress is common in beach visitors in Florida, from inhalation of brevetoxin from *Karenia brevis* (Backer *et al.*, 2003, 2005; Fleming *et al.*, 2005). Florida has set up a beach-monitoring program (lifeguard-based) for warning and caring for exposed beachgoers (Kirkpatrick *et al.*, 2008), with a preliminary model derived to forecast respiratory impacts (Kirkpatrick *et al.*, 2015). Aerosolized toxin from *Pfiesteria* spp. has also been noted, potentially leading to flulike symptoms in exposed individuals (Haselow *et al.*, 2001). CyanoHAB have similar issues caused by aerosolization (Cheng *et al.*, 2007), posing acute and chronic problems for lake users. These examples indicate potential risks to watermen, mariculture operators, and harvesters of natural and cultured shellfish and fish.

12.3.1.6 Medical Treatments

Unfortunately, many toxin-induced illnesses are misdiagnosed due to symptoms that resemble those of many common sicknesses and a lack of physician familiarity with symptoms of these maladies (e.g., Zaias *et al.*, 2010). If identified, however, there are some therapies that can reduce several severe characteristics of the intoxication (see Chapter 9), mitigating debilitating and even deadly outcomes. Common toxins from the cyanobacteria include neurotoxins like anatoxin-a (a neuromuscular blocking agent), anatoxin-a(s) (an anti-acetylcholinesterase), and saxitoxins, the PSP toxins that block nerve sodium channels. Other common toxins of these procaryotes are hepatotoxins such as microcystin and nodularin, both implicated in liver injury and tumor growth, and cylindrospermopsin, which causes injury to the gastrointestinal lining, liver, and kidneys (Falconer, 1998). PSP from the neurotoxin produces symptoms that include tingling, numbness, headaches, weakness, and difficulty breathing, and, in mammals, paralysis and death from respiratory failure. Medical treatment is to provide respiratory support, without which the prognosis can be fatal.

12.4 HAB Controls

12.4.1 Protections

As noted above, with regard to human exposure to HAB and toxin levels, the NSSP guidance document outlines specific requirements for permitted harvest of natural and cultured shellfish (Approved Growing Areas). Additionally, the NSSP guidance document lays out specific sampling frequencies and procedures for harvest closures and reopenings following HAB exposure and shellfish

intoxication. Hence, the NSSP attempts to control distribution of toxin-rich shellfish tissue, thereby limiting threats to human health from consumption of tainted shellfish.

12.4.2 Biomass Removal

Some HAB accumulate in surface waters to form scums or thick layers of buoyant species. For example, many cyanobacteria are buoyant due to gas vesicles (aerotopes) or vacuoles and become trapped at the air–water interface due to accumulation of buoyant cells below or even gasses from heavily photosynthesizing populations trapped in or immediately below the surface biomass. UV in sunlight can oxidize the accumulated cells, kill these trapped populations, and destroy ballast-driven nocturnal descent, leading to "scum" formation. The accumulated biomass poses large problems if toxic or as a threat to local levels of dissolved oxygen. Surface dwelling cyanobacteria, as living or "scum" populations, have been removed with surface-skimming technologies (e.g., Brearley, 2005, fig. 2.18; Atkins *et al.*, 2001), with the skimmed biomass then transported to landfills or composted. Macroalgae, such as mass accumulations of *Ulva* off China during 2008, have also been manually removed for disposal (e.g., Wang *et al.*, 2009; see also Figure 12.5 and Chapter 15).

12.4.3 Capping

In some systems, regeneration of nutrients from remineralization processes in the sediments (Malone *et al.*, 1986; Seitzinger, 1991) can provide needed N and P for HAB development and persistence. Control of this nutrient release has been suggested in several sediment-capping or nutrient-binding techniques (Figure 12.1(4)), usually coupled to attempted removal of nutrients and the bloom from the overlying waters with another technology. In freshwater systems dominated by cyanoHAB, Pan and coworkers (2012) have advocated spraying of coarse sand over a lake surface following flocculation of a cyanobacterial bloom (see Section 12.5.3, "Flocculation in Mitigation") like *M. aeruginosa*, effectively sealing the sedimented, flocked cyanobacteria (and, to some extent, flocculated toxin) into the benthos for decomposition. In sufficiently shallow water where light reaches the bottom, the sand can be mixed with seeds of submersed grasses for subsequent growth of underwater grasses (Pan *et al.*, 2006b, 2011b), excellent habitat for other biota and a nutrient sink. For aphotic depths, once buried, only severe storms with sufficient shear stress or resuspension could release the cyanobacteria for possible recolonization of surface waters. Coarse-grain capping of the bottom would also minimize some future blooms due to burial of HA akinetes or cysts already present; but without prevention of new blooms advecting into an area, excystment and growth of new resting stages deposited after the capping might still occur.

12.4.4 Nutrient Trapping in Sediments

In a similar approach, alum (see Section 12.5.2) and the commercial product Phoslock®, a lanthanum-substituted bentonite, is dispersed over bloom areas, in both fresh and salt waters, to bind soluble P in the water column and transport it to the bottom for retention in the sediments. Some of the bloom biomass can also be bound and carried to the bottom, but the primary role of Phoslock® is P removal and retention. It has been successfully used in Southwest Australian cyanobacteria blooms (Robb *et al.*, 2003), cyanobacteria blooms in a Netherlands lake with the addition of polyaluminum chloride (Lürling and van Oosterhout, 2013), and *Prymnesium parvum* blooms in North Australia (Body, 2011). It appears to have some limitations, however, as Sellner *et al.* (2013) noted enhanced benthic flux of N following Phoslock treatment and a return of P flux after several weeks; furthermore, Seger *et al.* (2015) noted efficient removal of *P. parvum* and phosphorus at pHs of 7 and 9, but much less efficient removal of compounds responsible for fish gill toxicity at the higher pH typical of blooms. If P loads into a system continue, repeated Phoslock applications will be necessary to ensure absence of the cyanoHAB. At U.S. \$3 kg^{-1}, and an application rate of 30 kg kgP^{-1} (Body, 2011), large doses of the compound would be expected for most eutrophic open systems, but it could be practical in shallow fish and shrimp farms.

12.4.5 Reductions of Algal Resting Stages (Cysts)

Burying cysts (or akinetes) further into bottom sediments has also been suggested as a control strategy (D. Anderson and D. Kulis, unpublished data), thereby reducing abundances of more physiologically active resting stages in surficial sediment and minimizing numbers of excysting cells for

subsequent development of the next surface bloom. In another example, Australia has implemented regulations that require a risk assessment of the impact of redistributing cysts from dredging of bottom sediments (Figure 12.1(5)) to control possible reseeding of new areas for HA development (G. Hallegraeff, personal communication). The concern for resting stages in bottom sediments for subsequent growth and impacts is also evident in Japan. Imai and coworkers (2014) have attempted to use mixing in a very different way to discourage blooms of *Chattonella* and *Karenia* in Saiki Bay, Seto Inland Sea, Japan. Using a large-volume pump, bottom sediments were brought to the surface and dispersed to encourage germination of the diatom resting stage and population increases in September and May. In both cases, diatoms (*Chaetoceros* spp. and *Skeletonema*) proliferated, with the authors speculating that the diatoms outcompeted the HAB taxa for N and P. All of these methods attempt to control HA development through minimizing likely growth opportunities for recurring threatening taxa.

12.5 Mitigation of HAB

As noted above, HAB are common to all water bodies, from fresh to oceanic waters, coincident with the culture of fish and shellfish. Freshwater blooms of cyanobacteria, *P. parvum*, *D. geminata*, and two species of *Euglena* (Zimba *et al.*, 2004) have been shown to impact pond fish aquaculture. A wide spectrum of dinoflagellates, raphidophytes, diatoms, pelagophytes, cyanobacteria, and macroalgae may threaten shellfish, fish, other invertebrates, and algal culture in estuaries, coastal waters, and open-ocean areas. Detecting these harmful algae prior to contact with cultured or natural stocks of commercially important commodities and subsequent mitigation is therefore critical to minimizing loss of harvest or transfer of toxins to human or other consumers.

12.5.1 Detection

There are a number of methods used in the detection of ambient phytoplankton and HAB species. For individual farms, water samples can be collected and analyzed microscopically. Classical identification and enumeration for Lugol's iodine-treated samples and light microscopy (see Sournia, 1978) has been a standard protocol for over a century, and many nations (Canada, Croatia, Denmark, Finland, France, Ireland, Japan, Philippines, Portugal, Slovenia, and Spain) and local jurisdictions (e.g., California, Florida, Maryland, Massachusetts, New York, Texas, Virginia, and Washington in the United States) maintain active plankton monitoring in aquaculture areas. Newer automated methods that identify taxa (e.g., FlowCam: Fluid Imaging, Inc., Edgecomb, ME, USA; and Imaging FlowCytobot, McClane Research Laboratories, Inc., East Falmouth, MA, USA) are now deployed in some locations for routine characterization of local populations (see Chapter 2). Toxins can be detected using mouse bioassays (Stewart and McLeod, 2014) or, more commonly, enzyme-linked immunosorbent assay (ELISA; e.g., Biosense Laboratory and Mercury Science kits for DA; Abraxis kits for saxitoxin, okadaic acid, brevetoxin, and microcystin; and complement-ELISA [cELISA] for microcystin). Other methods use dipsticks (e.g., Jellett Rapid Testing, Ltd., Nova Scotia, Canada), high-performance liquid chromatography (HPLC), or mass spectrometry (MS). In-water techniques include HPLC (e.g., for DA; G. Doucette adaptation from Pocklington *et al.*, 1990) and MS (Short *et al.*, 2001). Other technologies detect pigments, usually chlorophyll-*a,* but accessory pigments are also possible. For example, fluorescence of chlorophyll-*a*, phycocyanin, and phycoerythrin are routinely used to determine contributions of all autotrophic plankton, cyanobacteria, and cryptophytes, respectively, using handheld or deployed fluorometers manufactured by many companies (e.g., Turner, Turner Designs, YSI, HydroLab, WetLabs, Satlantic, bbe Moldaenke GmBH, and others). Other technologies employ unique pigment spectra for in-water detection of species-specific pigments, such as the brevebuster for *Karenia* spp. (Kirkpatrick *et al.*, 2000). Solvent extraction for these pigments in grab samples can also be employed, either for single pigments like chlorophyll-*a* or a large spectrum of pigments using chromatography (e.g., ChemTax; Mackey *et al.*, 1996).

Molecular detection of phytoplankton groups or individual species using unique antibody–antigen complexes or nucleic acid or lectin content is also increasingly common using kits (see above) or deployed platforms (see Chapter 2; Anderson *et al.*, 2017). These techniques are highly specific and rapid, ranging from simple color changes in some handheld kits to LED readouts of a suite of species using microarrays and other small technologies.

For large geographic areas, regional distributions of pigment detected through remotely

deployed technologies are available (CZCS, Landsat, SeaWiFS, MODIS-Aqua, and MERIS; Blondeau-Patissier *et al.*, 2014a). Thereafter, species within chlorophyll fields can be determined through field collections and the analytical methods noted above. Some areas use satellite or aerial detection of pigment, most often chlorophyll-*a*, coupled to regional wind fields or hydrodynamic models to transport the autotrophic plankton in an area. These distributions and landfall forecasts can be delivered as routine bulletins to regional stakeholders (Figure 12.7), including shellfish growers, such as those experiencing blooms of *Karenia brevis* along Texas and Florida, the Gulf of Maine *Alexandrium* blooms, cyanobacteria, principally *Microcystis aeruginosa* in Lake Erie, *Nodularia* and *Aphanizomenon* blooms in the Baltic, and several toxic dinoflagellates and *Pseudo-nitzchia* in Ireland and Scotland. A pilot forecast system is being developed in the United Arab Emirates (D. Anderson, personal communication). As needed, non-operational capacities for bloom detection via satellite remote sensing (and, in some cases, linked to hydrodynamic modeling) is common in the U.S. inland seas and coastal zones (e.g., Stumpf *et al.*, 2003; Wynne *et al.*, 2010; Eberhart *et al.*, 2012; Anderson *et al.*, 2017), the United Kingdom (Holligan *et al.*, 1983; Miller *et al.*, 2006), Ireland (Raine *et al.*, 2001), Northern and Southern Europe (e.g., Kahru 1997; Barale *et al.*, 2008; Gomez *et al.*, 2011; Vilas *et al.*, 2013), the Middle East (Banse and English, 2000; Zhao *et al.*, 2015), Korea (Ahn *et al.*, 2006), China (Yu *et al.*, 2007; Hu *et al.*, 2010; Shang *et al.*, 2015), Australia and New Zealand (Allan *et al.*, 2015; Blondeau-Patissier *et al.*, 2014b), and the Atlantic, Pacific, and Indian Oceans (e.g., Brown and Yoder, 1994; Subramaniam and Carpenter, 1994; Dupouy *et al.*, 2013). Industry representatives are encouraged to check with national officials to identify availability of distributed satellite imagery for chlorophyll-*a* or other derived products for specific regions and water bodies.

Chlorophyll-*a* accumulated at the surface also absorbs light, resulting in reflectance in the visible band and increasing temperatures detectable with near-infrared and thermal channels for satellite detection of blooms by AVHRR (advanced very-high-resolution radiometer). This is most apparent in the historical descriptions of Baltic blooms of *Nodularia spumigena* and *Aphanizomenon flos-aquae* (Kahru, 1997), but its use in routine short-term forecasts of landfall and hence near-shore shellfish or farmed animals has not been found. Cyanobacterial blooms in ponds >1 km and lakes may now also be detected via a phycocyanin-adjusted algorithm for the spectral shape of the chlorophyll peak (Tomlinson *et al.*, 2015), perhaps useful in future mitigation of blooms in freshwater aquaculture ponds.

Fish farmers in Washington State conduct aerial surveys by fixed-wing aircraft over marine waters to identify locations of *Heterosigma akashiwo* blooms (Rensel and Whyte, 2003; Rensel, 2007). These blooms are often monospecific with a characteristic visible color and appearance that can be visually tracked as they are advected through the area by strong tidal currents and winds. Aerial surveys combined with *in situ* routine collection and identification at farm sites provide early warning to prepare or apply mitigation techniques (discussed below).

12.5.2 Chemical Additions

The most commonly employed additives (Figure 12.1(7)) to reduce or eliminate HAB and cyanoHAB include alum, chlorine (sodium hypochlorite), copper sulfate ($CuSO_4$), ammonium chloride, and potassium permanganate (K_2MnO_4), with varying degrees of success and possible ancillary environmental issues. The compounds are generally added to closed systems, as dilution rapidly reduces the biocide levels to non-inhibitory concentrations. For a list of other aquaculture biofoulants and their limitations, see Guardiola *et al.* (2012).

Alum is a common water additive to enhance removal of phosphorus by adsorption and flocculation in drinking and wastewater treatment facilities (e.g., Omoike and VanLoon, 1999). It is a common additive to turbid eutrophic ponds and lakes rich in inorganic phosphorus and suspended sediments, carrying bound P to bottom sediments for long-term storage (e.g., Boyd, 1979). Lam *et al.* (1995) indicate alum and lime are effective flocculants for sedimenting *M. aeruginosa*, with most intracellular microcystin remaining inside the settled cells. Effective alum concentrations for earthen fish ponds rearing Nile tilapia fingerlings (*Oreochromis niloticus*) approximate 10 mg L^{-1}, with no impact on the cultured fish (Dahwah *et al.*, 2015), far below the 92 mg alum L^{-1} used for treating blooms in a walleye pond (*Stizostedion vitreum*) by Bandow (1974, cited in Boyd, 1979).

Chlorination is frequently used for drinking water supplies where cyanobacterial blooms are found in source waters. Unfortunately, even though cyanobacteria are lysed by added chlorine, intracellular toxins can be released, with some more susceptible to chlorine-induced oxidations than others (saxitoxin > cylindrospermopsin > microcystin; Zamyadi *et al.*, 2012), permitting

some toxins to remain in treated drinking water (Carmichael, 2008). Additionally, strongly oxidizing by-products (residuals) may be a threat to many biota and processes (e.g., Brungs, 1973). Because freshwater fishpond aquaculture (tilapia, carp, catfish, and prawns) is common in many areas (Asia and southeastern United States), chlorination through sodium hypochlorite addition may be a mitigation option and hence care in amounts added should be a concern. For marine harmful taxa, Jeong *et al.* (2002) suggested effective doses for minimizing HAB exposures would be 300–500 ppb NaOCl for 10 min or 200–400 ppb for 1 h.

McKnight *et al.* (1983) have identified copper as the most effective agent in controlling HAB in lakes and reservoirs, and several marine HAB taxa are sensitive, but there are numerous and serious side effects associated with its use that have discredited its application in many aquatic habitats (e.g., Kuiper-Goodman *et al.*, 1999; Deeds *et al.*, 2004; Qian *et al.*, 2010; Ebenezer *et al.*, 2014). It is sometimes used for cyanobacteria control in catfish ponds (e.g., Jacob *et al.*, 2015; Figure 12.1(7)). The same authors reported, as others have for years, that copper sulfate treatments reduced the biomass of Cyanophyta, but increased biomass of more tolerant taxa among the Chlorophyta and Chrysophyta. Copper sulfate induces cell lysis and release of intracellular toxins into surrounding waters (Lam *et al.*, 1995), often inducing gastro- or hepato-enteritis in local citizens and particularly children, as well as individuals with liver cirrhosis, toxic liver injury from other sources, hepatitis, or kidney damage (Kuiper-Goodman *et al.*, 1999). As a follow-up to $CuSO_4$-induced fish mortalities (<2 mg L^{-1}) in a striped bass fish farm in Maryland, United States, Deeds *et al.* (2004) noted elevated hemolytic activity in *Karlodinium veneficum* cultures treated with copper sulfate as a result of biocide lysis of the cells, but little effect on the released ichthyotoxin. There has been one field exposure using copper sulfate for a marine HAB, *K. brevis* off west Florida (Rounsefell and Evans, 1958); immediate declines in cell abundances were noted, but bloom levels returned after 10–14 d. Control of biofouling on fish farm nets in the past has included antifouling paints containing copper that has raised concerns about contamination of fish tissue or nearby sediments (Fitridge *et al.*, 2012) although they have not been observed in practice. For example, in routine NPDES monitoring at Puget Sound fish farms since sediment metals were monitored in 2007 there have been no occurrences of copper concentrations in sediments failing to meet State of Washington sediment standards (Rensel, 2008 and later similar reports to government). In recent years in the marine fish aquaculture industry copper treated nets are much less common while semi-automated net cleaning *in situ* is increasingly common as are robotic systems for detection of holes in netting (Rundtop and Frank, 2016).

Ammonium chloride with copper sulfate has been shown to be effective in reducing populations of *Prymnesium parvum* in Texas aquaculture ponds, with potential toxicity to some fish via production of ammonia (Barkoh *et al.*, 2003, 2004). Ammonium chloride with phosphoric acid was somewhat effective for the prymnesiophyte in limnocorrals, but non-target species were also impaired (Kurten *et al.*, 2007; Grover *et al.*, 2013). Wyatt *et al.* (2013) reported that hybrid striped bass hatcheries in Texas employed NH_3-N in *P. parvum* control. A concern beyond these mitigation issues is the impact of diluted ammonia, as ammonium, as a substrate to enhance phytoplankton growth downcurrent of any application site; ammonium is a preferred N substrate for many algal species (phytoplankton and macoalgae), and stimulation of algal productivity could lead to other blooms in many freshwater and coastal areas.

Potassium permanganate (K_2MnO_4) is used in wastewater treatment plants for reducing biological oxygen demand and in drinking water facilities to reduce soluble manganese and iron (Cherry, 1962; Fan *et al.*, 2013a). It is an effective biocide for *Microcystis aeruginosa* (e.g., Fan *et al.*, 2013b) and has been used in the temporary elimination of *Planktothrix prolifica* from a spring-fed pond in Walkersville, Maryland (K. Sellner, unpublished). Rodríguez *et al.* (2007) compared permanganate, chlorination, and ozonation in toxin removal and found permanganate effective in oxidizing cylindrospermopsin and microcystin-LR. In contrast to copper sulfate, it also proved effective in lysing *K. veneficum* at 4 mg L^{-1} and reducing hemolytic activity of intracellular toxins released on cell lysis (Deeds *et al.*, 2004); these results complemented field observations of increased survival of hybrid striped bass in fishponds treated with potassium permanganate versus copper sulfate in Maryland.

Hydrogen peroxide has been effective in reducing cyanobacteria and microcystins in laboratory studies and wastewater ponds (Figure 12.1(7)), with cyanobacteria replaced by eucaryotes (e.g., Barroin and Feuillade, 1986; Barrington *et al.*, 2013). Compounds derived from substituted peroxides that produce free radicals have also been used against *Planktothrix perornata*, a cyanobacterium that causes musty flavor in freshwater catfish (Nanayakkara and Schrader, 2011). In natural

systems, *Planktothix agardhi* was removed from Lake Anita Louise, Maryland, USA, and Lake Koetshuis, the Netherlands, using 3 (Mattheiss *et al.*, 2017) and 2 mg H_2O_2 L^{-1} (Matthijs *et al.*, 2011), respectively, with little impact on other biota; for the latter, the cyanobacterium did not return for 7 weeks. Barrington *et al.* (2013) reported about two-thirds of the cyanobacteria and all microcystins were removed from a wastewater pond after the addition of 44–95 mg H_2O_2 L^{-1}; the initial populations returned after 3 weeks. In contrast to results in Matthijs *et al.* (2011), however, these concentrations threatened ambient crustacean zooplankton populations. In a similar treatment approach in the brackish Ouwerkerkse Kreek, the Netherlands, vegetative cells and pellicle cysts of *Alexandrium ostenfeldii* were killed by the addition of 50 mg H_2O_2 L^{-1}; PSP toxins were also reduced to <15 μg L^{-1}, the local regulatory limit, with minimal impact on macroinvertebrates and fish and some susceptibility in zooplankton (Burson *et al.*, 2014). The dinoflagellate did return, but at lower levels the next year. Ichikawa *et al.* (1992 in NOWPAP CEARAC, 2007) reported rapid declines in *Cochlodinium* sp. in Kagoshima Bay, Japan, after spraying with 30 ppm hydrogen peroxide; in accompanying laboratory cyst experiments, it was noted that 10–100 ppm and >10 ppm H_2O_2 severely depressed excystment of *Polykrikos shwartzii* and *Alexandium catenella* cysts, respectively. Murata *et al.* (1989; in NOWPAP CEARAC, 2007) reported *Chattonella marina* lysis at peroxide concentrations between 15 and 150 ppm. Hence, peroxide shows promise for use in HAB mitigation, but caution must be exercised as detrimental effects on ambient zooplankton (and hence the food web) are possible. Furthermore, permitting restrictions that can prevent its use without special training and extensive safety precautions exist in some countries (e.g., the United States). Additionally, for most effective application, depth distributions of the bloom should be known. If surface blooms are prevalent, surface application with local meteorological dispersion will be effective (Barrington *et al.*, 2013); if, however, the harmful algal population is dispersed throughout the water column, application should be at all depths (e.g., Matthijs *et al.*, 2011), requiring capital investment for specially designed equipment.

Other less known compounds for HA control have also been explored. Freshwater cyanobacteria *Microcystis aeruginosa* Kützing and *Anabaena affinis* Lemmermann can be inhibited by sorghum root extracts, specifically sorgoleone added at 0.5 and 4 mg L^{-1}, respectively, with little impact on fish until concentrations >1.5 mg L^{-1} (Uddin *et al.*, 2012). Garlic extract added at >0.04% inhibits several cultured *Alexandrium* species (*A. tamarense*, *A. satoanum*, and *A. catenella*) and *S. trochoidea* (Zhou *et al.*, 2008). Bacillamide, an extract from *Bacillus* SY-1, is also effective in inhibiting *C. polykrikoides* (Jeong *et al.*, 2003b). Another surfactant from *Pseudomonas aeruginosa* was found to kill four of five species (marine *Alexandrium minutum*, *Karenia brevis*, *Pseudonitzschia* sp., and freshwater *Gonyostomum semen*) when added at 50 μg L^{-1} (Gustafsson *et al.*, 2009). Several antimicrobial peptides (1–8 μM of HPA3 [19-mer] and HPA3NT3 [15-mer] peptides) extracted and modified from another bacterium *Helicobacter pylori* are very effective at killing the raphidophytes *Heterosigma akashiwo*, *Chattonella* sp., and *C. marina* as well as the dinoflagellates *Cochlodinium polykrikoides*, *P. micans*, and *P. minimum* with no apparent effect on diatoms (*Skeletonema costatum*) or black rockfish red blood cells (Park *et al.*, 2011). 10–20 mg L^{-1} of sophorolipid inhibited motility of about 90% of *A. tamarense*, *H. akashiwo*, and *C. poykrikoides* and specifically induced *H. akashiwo* lysis, ecdysis of *A. tamarense*, and swelling of *C. polykrikoides* (Sun *et al.*, 2004). Li *et al.* (2014) noted that ε-polylysine, betaine, stachydrine, and berberine exhibited selective inhibitory effects against *C. marina* Hara et Chihara, *A. tamarense* Balech, and *Karenia mikimotoi* (Miyake & Kominami ex Oda) G. Hansen & Ø. Moestrup; as discussed below in barley straw, flavonoids may be important in inhibiting photosystems and cell membrane integrity (e.g., Huang *et al.*, 2015). A polar, water-soluble algicide, from the bacterium *Shewanella* sp. IRI-160, was lethal to *Karlodinium veneficum*, *K. brevis*, *Gyrodinium instriatum*, *C. polykrikoides*, *Heterocapsa triquetra*, *Prorocentrum minimum*, *Alexandrium tamarense*, and *Oxyrrhis marina*, but had no or little effect on *Dunaliella tertiolecta* or *Rhodomonas* sp. (Pokrzywinski *et al.*, 2012). Macroalgal compounds can also inhibit several HA taxa (Alamsjah *et al.*, 2007; Tang and Gobler, 2011; Chowdhury *et al.*, 2014; Tang *et al.*, 2014), posing additional positives for growth of these seaweeds in multi- or poly-culture with fish and shellfish (see Section 12.2.2 above). For *Prymnesium parvum*, the addition of the herbicide flumioxazin (200 μg L^{-1}) resulted in significant decreases in cell abundance, with large and small decreases in rotifers and adult copepod densities, respectively (Umphres *et al.*, 2012), but no apparent effect on fish (Umphres *et al.*, 2013). Two antifungal compounds, bifonazole and terbinafine hydrochloride, were effective at altering cellular

structure in *Chattonella marina* and *Heterocapsa circularisquama* when added to cultures at 1 g L^{-1} (Nakashima *et al.*, 2008). And the addition of 0.3–0.4 mg $Ca(NO_3)_2$ L^{-1} cleared a *Gymnodinium splendens* bloom in Tokyo Bay in the mid-1960s (Sugawara and Sato, 1966).

12.5.3 Flocculation

There has been considerable focus on the use of fine-grain sediments, particularly certain clays, in flocculating harmful algae and toxins in many areas, from fresh to marine systems (Table 12.1). Sediments alone can be distributed across blooms, surfactants as well, but the combination of sediments and surfactants appears to be most effective in HA removal. There can be two main goals for cell removal: a reduction in cell abundances to reduce potential toxic or DO (dissolved oxygen) effects on caged fish, shellfish, and their consumers, or near-complete removal to prevent re-emergence of the bloom and associated toxin(s) from very low post-flocculation populations: the former is fairly easily attained, while the latter is quite difficult. In freshwater systems, removal of toxic cyanobacteria is best described in work by Pan and associates (Pan *et al.*, 2006a, 2006c; Zou *et al.*, 2006; Shi *et al.*, 2016), where clays and eventually coarse-grain sands have been combined with surfactants like chitosan (β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine) and cationic starch to floc and settle *M. aeruginosa*. Their work generally suggests that very low sediment additions approximating 100 mg L^{-1}, with 10% additions of acidified chitosan solutions (10 mg L^{-1}), result in cell removal efficiencies exceeding 90% in several hours. By combining the sediment slurry with seeds of submersed grasses, settled flocculated *Microcystis aeruginosa* can subsequently decompose, and the remineralized nutrients can support growth of the bottom grass (Pan *et al.*, 2006b, 2008, 2011b; Certner *et al.*, 2011). An alternative treatment for non-seed applications was the dispersion of coarse sand over the treated area, thereby capping the previously flocculated and settled bloom (Pan *et al.*, 2012). There is, however, considerable variability in reproducing these results, particularly for the low concentrations of both sediment and chitosan effective in the work above. In two hypereutrophic lakes in Maryland, Sellner *et al.* (2015) found that sediment and chitosan additions needed to be 3–50 times larger than noted in China, posing substantial constraints on routine use of the technique due to quantities of materials to be added that were well above permitted sediment-loading rates. Additional concerns with the methods above include possible chitosan toxicity to fish (Bullock *et al.*, 2000), bottom deposition potentially harmful to the benthos (e.g., Shumway *et al.*, 2003), time of day for application (likely attributable to pH effects on flocculation; e.g., Body, 2011; Seger *et al.*, 2015), growth stage/physiology of the HA (Sengco *et al.*, 2005; Pan *et al.*, 2006c; Certner *et al.*, 2011; Tian *et al.*, 2014), possible sediment particle size differences (larger particles are less effective; e.g., Park *et al.*, 2013), and costs (sediments, surfactant, transport, preparations, dispersion, and monitoring). In recent work, nanosilica has been found to be effective in removing several cyanobacteria, in the laboratory and field, but field populations returned after 20 days (Xiong *et al.*, 2017).

Flocculation of brackish-to-marine HAB is also well studied and used routinely in some waters (Sengco and Anderson, 2004; Yu *et al.*, 2017). Many clays (loess, yellow clay, phosphatic clay, kaolinite, montmorillonite, and bentonite), sand, slaked and quick lime, and surfactants (chitosan, PAC, sophorolipids, cocamidopropyl betaine, and sulfobetaines), as well as many natural plant extracts (larch tannin, *Moringa olifeira*, and Chinese herbs), individually and together, have been used to remove HA populations. Rensel *et al.* (2004) found high removal efficiency (84%) of *Heterosigma akashiwo* in replicate mesocosms treated with only 0.12 g L^{-1} phosphatic clay treatment in calm and vertically stratified but deep waters of East Sound, Washington. "Ball" clay (20–80% kaolinite, 10–25% mica, and 6–65% quartz) can remove >90% of *Pyrodinium bahamense* var. *compressum* and *Gymnodinium catenatum*, both marine dinoflagellates posing problems for Philippine coastal waters (Padilla *et al.*, 2007; Rivera, 2015). Clays rich in titanium dioxide appear to be perhaps 20% more effective, generating reactive oxygen at light levels >2300 μmol m^{-2} s^{-1} versus dark rates (Kim *et al.*, 2001). A number of the compounds discussed above have also been investigated, alone and in combination with sediments, including poly-aluminum chloride (PAC; Sengco *et al.*, 2001; Hagström and Granéli, 2005; Ghafari *et al.*, 2009; Lu *et al.*, 2015) commonly used in wastewater treatment plants as a flocculant. As an example, PAC-clay was effective in removing 90% of a cultured *A. tamarense* (A3) population; it also resulted in TP and TN reductions of 43–60% and 17–30%, respectively, as well as some of the dinoflagellate-produced gonyautoxins. Pan *et al.* (2011a) recently suggested that any sediment could flocculate cells, following the addition of PAC and chitosan. Extracts of many

Table 12.1 Sediments, surfactants, and other compounds used in effectively flocculating and removing HAB in the environment (also see Park *et al.*, 2013, table 4).

Environment, organism	Additive(s)	Condition	Reference
Freshwater, *M. aeruginosa*	0.023 g clay L^{-1} + 0.002 g acidified chitosan L^{-1}	Field	Pan *et al.* (2006c)
Freshwater, *M. aeruginosa*	0.011 g clay L^{-1} + 0.001 g L^{-1} acidified chitosan	Field	Zou *et al.* (2006)
Freshwater, *M. aeruginosa*	~0.031 g local soils L^{-1} + ~0.003 g acidified chitosan L^{-1}	Field	Pan *et al.* (2008)
Freshwater, *M. aeruginosa*	~0.025 g local soil L^{-1} + ~0.003 g acidified chitosan L^{-1}	Field	Pan *et al.* (2011b)
Freshwater, *M. aeruginosa*	0.156–5 g kaolinite or sand L^{-1}, 0.03–0.25 g acidified chitosan L^{-1}	Field mesocosms	Sellner *et al.* (2013, in prep.)
Freshwater, cyanobacteria	0.075 g nanosilica L^{-1} + 0.0025 g diallyldimethylammonium chloride L^{-1}	Field (pond)	Xiong *et al.* (2017)
Freshwater, *A. circinalis* and other cyanobacteria	Phoslock® at 0.5–1 mm thickness	Field	Robb *et al.* (2003)
Brackish-polyhaline, *P. parvum*	30 kg Phoslock® $kgPO_4^{-1}$	Field	Body (2011)
Chattonella antiqua	360 t modified clay 86 km^{-2}	Field	Yu *et al.* (2017)
Cochlodinium sp.	200 g montmorillonite and/or kaolinite m^{-2} and 110–400 t km^{-2}	Field	Shirota (1989) in Sengco and Anderson (2004) and Yu *et al.* (2017)
Cochlodinium sp.	0.11–0.4 g Iriki montmorillonite L^{-1}	Field	Kagoshima Prefectural Fisheries Research Institute (1980–1982) in NOWPAP CEARAC (2007)
C. polykrikoides	10 g yellow loess L^{-1}	Field	Choi *et al.* (1998)
C. polykrikoides	400 g yellow loess (kaolinite) m^{-2}	Field	Na *et al.* (1996)
C. polykrikoides	0.005 g sophorolipid L^{-1} + 1 g yellow clay L^{-1}	Field	Lee *et al.* (2008)
C. polykrikoides	24 and 42 g dredged coastal sediment (52% clay) m^{-2} + 2–10% slaked lime, quicklime, aluminum sludge, bentonite, zeolite	Field	Song *et al.* (2010)
K. brevis	0.25–0.5 g phosphatic (montmorillonite) clay L^{-1}	Field mesocosms, cultures	Sengco *et al.* (2001) and Sengco and Anderson (2004)
Heterocapsa triquetra	0.25 g phosphatic (montmorillonite) clay L^{-1}	Field mesocosms, flumes	Sengco and Anderson (2004)
Multiple marine HA spp.	4–10 t modified clay km^{-2}	Field	Yu *et al.* (2017)
Noctiluca scintillans	Unknown concentration of Iriki montmorillonite	Field to lab	Kagoshima Prefectural Fisheries Research Institute (1980–1982) in NOWPAP CEARAC (2007)
N. scintillans	100 t modified clay (several 100 km)$^{-2}$	Field	Yu *et al.* (2017)
Mesodinium rubrum	7.5 g Iriki montmorillonite L^{-1}	Field to lab	Kagoshima Prefectural Fisheries Research Institute (1980–1982) in NOWPAP CEARAC (2007)
Phaeocystis globosa	210 t modified clay (nuclear plant intake)$^{-1}$	Field	Yu *et al.* (2017)
Prorocentrum sigmoides	2 g Iriki montmorillonite L^{-1}	Field to lab	Kagoshima Prefectural Fisheries Research Institute (1980–1982) in NOWPAP CEARAC (2007)
Prorocentrum sp. and *Gymnodinium* sp.	15 or 30 g coal ash derivative L^{-1}	Field (pond)	Lin *et al.* (2002) in NOWPAP CEARAC (2007)

Note: There is a comprehensive list of additional treatments summarized in NOWPAP CEARAC (2007), but access to most of the original articles was not possible; only those in NOWPAP CEARAC (2007) with identified field concentrations of treatment materials are noted; see also Anderson *et al.* (2001).

angiosperm leaves and fruits (e.g., Li and Pan, 2013; Wang *et al.*, 2013; Tian *et al.*, 2014) have also been proposed as local, natural control agents in flocculation, with routine use limited due to impracticalities of mass extraction, distribution, and application. Li and Pan (2013), Li *et al.* (2015), and Yu *et al.* (2017) provide universal guidance that the addition of any compound (usually an organic residue) that reduces zeta potential of added sediment and the alga or cyanobacterium results in surface charge neutralization and small floc formation; subsequent addition of a flocculant (e.g., PAC and chitosan) leads to organic bridging across the flocs to create larger flocs and sedimentation.

The most extensive and effective broad-scale clay treatment is in South Korean coastal waters, frequented by *Cochlodinium polykrikoide*s blooms that kill penned fish. Park *et al.* (2013) have summarized technological advances in clay flocculation for those areas, with the latest approach using electrolysis of ambient seawater to produce sodium hypochlorite and other residuals; subsequent mixing with local clays and dispersal across local blooms quickly remove the HAB (Figure 12.8). Short-term post-dispersal sediment concentrations near the fish pens approximate 20 mg L^{-1} (K. Sellner, unpublished data), well below the sediment concentrations employed in the freshwater treatments noted above. Yu *et al.* (2017) have reviewed current use of modified clays in Chinese waters and report that their recent clay-flocculant mixtures are hundreds of times more efficient at removing cells than other procedures and at doses of only 4–10 tons km^{-2} (4–10 g m^{-2}), much lower than previously reported applications; if these concentrations can be duplicated in many locations and for many taxa, this would be a huge improvement for bloom removal at modest impacts on the fauna below. Rensel and Anderson (2004) deployed 54 kg (dry weight) of phosphatic clay within the confines of a commercial fish farm in Puget Sound, Washington. Goals were to assess removal efficiencies of different taxa of phytoplankton, effects on turbidity, dissolved nutrient concentrations, sea bottom grain size composition, and the behavior of cultured Atlantic salmon that were within a large pen equipped with surrounding impervious skirts to retard flushing of the clay out of the cage. High removal rates of microflagellates and reasonably good removal of dinoflagellates were noted, but diatoms were removed at a much lower rate. Short-term increases in turbidity were also found inside the cage, but not downstream or beneath it. There were no significant or persistent effects on nutrient concentration or sea-bottom percentage of clay. The method was judged potentially useful for occasional use, but other methods such as upwelling-aeration (see below) have since been adopted as simpler for within-pen use.

Figure 12.8 Dispersion of fine clays following electrolysis for the flocculation and sedimentation of *C. poykrikoides* in Korean fish culture area. *Source*: Courtesy of R. Kudela, UCSC.

12.5.4 Barely Straw (*Hordeum vulgare*)

For closed systems or those with long residence times, deployment of barley straw *H. vulgare* several months before a bloom is expected can lead to substantial reductions in blooms of some taxa, most often cyanobacteria (see references in Brownlee *et al.*, 2003; Spencer and Lembi, 2007; Sellner *et al.*, 2015). Bales of barley straw (approx. 1 m × 0.5 m × 0.5 m, ~22.7 kg) or straw dispersed in mesh bags have been used effectively in the United States and United Kingdom. For the former, the application rate ranged from 30 to 43 g m^{-2} of lake surface area (30–43 mg L^{-1}), resulting in 10^3 lower abundances of *M. aeruginosa* very late in the summer and microcystin declines from >1000 to <6 ppb, with all but one concentration <1 ppb (Sellner *et al.*, 2015; Figures 12.9a and 12.9b). Effective barley straw concentrations for field deployments have been reported from 6 mg L^{-1} for UK lake treatments (Newman and Barrett, 1993; Barrett *et al.*, 1999), to 50 mg L^{-1} in one UK reservoir (Everall and Lees, 1996), to 400 mg L^{-1} for a freshwater canal, the latter likely due to a short residence time therefore requiring high doses of the inhibitory materials (Welch *et al.*, 1990). The addition of straw requires time for decomposition, as there is no decline in autotrophs over several days on mixing with dry straw (Brownlee *et al.*, 2003; Xiao *et al.*, 2010). Decomposition of the straw over time yields polyphenolic compounds that inhibit the growth of the planktonic autotrophs (Pillinger *et al.*, 1994; Ridge and Pillinger, 1996; Xiao *et al.*, 2013; Huang *et al.*, 2015). Some of these compounds, (e.g., flavonoids) are most effective at inhibiting *M. aeruginosa* via damage to the cell membrane (Xiao *et al.*, 2013; Huang *et al.*, 2015). Concentrations as low as 10–25 mg L^{-1} of several barley straw compounds (apigenin, luteolin) caused >90% mortality in *M. aeruginosa* cultures in 3–5 d (Huang *et al.*, 2015). Straw decomposition is facilitated by an active microbial community including fungi (Gibson *et al.*, 1990; Newman and Barrett, 1993; Sellner *et al.*, 2015), and Sellner *et al.* (2015) documented increased potency of fungi-inoculated barley straw extracts to cultured *M. aeruginosa*, thereby indicating that fungicide-free straw should be deployed as the most effective algicide. Note, however, that brackish and marine HAB may not be as susceptible to these compounds (Terlizzi *et al.*, 2002; Hagström *et al.*, 2010), which may even stimulate growth in some taxa (*Prorocentrum minimum*, Brownlee *et al.*, 2003; *Gyrodinium instriatum* and *P. micans*, Terlizzi *et al.*, 2002). In contrast, however, no algal growth was reported in a saltwater pond in the United Kingdom/Ireland after barley straw treatment (Newman and Barrett, 1993, table 3). It also should be noted that rice straw may inhibit cyanobacteria (Park *et al.*, 2006) while still maintaining high Nile tilapia production in freshwater ponds (Shahabuddin *et al.*, 2012). Hence, species-specific responses of marine HAB to aged *H. vulgare* (or rice straw) should be examined prior to use in both fresh and saline waters.

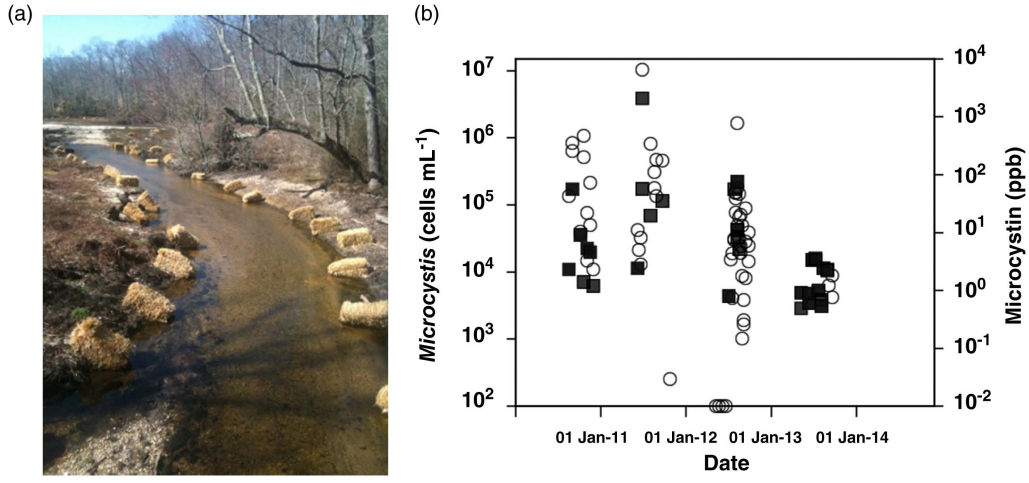


Figure 12.9 (a) Barley straw (*H. vulgare*) bales in Lake Williston, Maryland, USA, during winter drained condition. *Source*: Courtesy of A. Place, UMCES-IMET. (b) *M. aeruginosa* densities and microcystin levels in Lake Williston, Maryland, USA, 2010–2013. *Source*: Sellner *et al.* (2015). Reproduced with pemission of The International Society for the Study of Harmful Algae.

12.5.5 Other Treatments

Ozonation is used in oxidation of organic matter, bacteria disinfection, and water treatment (see Gonçalves and Gagnon, 2011) and has been employed for HAB, cyanoHAB, and toxins. The use of ozone followed by flocculation to remove cell debris or charcoal filters for toxin by-products is common in drinking water facilities. Ozonation is effective in destruction of cyanobacteria cells (41–80% of *Anabaena*, *Aphanizomenon*, *Microcystis*, and *Pseudanabaena*; Xie *et al.*, 2013a, 2013b; Zamyadi *et al.*, 2015). The toxins microcystin LR and LA, anatoxin-a, and cylindrospermopsin are also removed (Rositano *et al.*, 2001; Rodríguez *et al.*, 2007; de la Cruz *et al.*, 2013), with the latter two research teams indicating more effective oxidation with ozone versus treatment with chlorine or permanganate. Ozonation followed by chlorination is very effective at cell removal, but strong oxidants can result (e.g., Xie *et al.*, 2013a, 2013b), leading to oxidative stress for other biota. Duplicating results from Blogoslawski *et al.* (1973), Schneider *et al.* (2003) noted complete lysis of cultured *K. brevis* after 60 s exposure to 25 mg ozone, with brevetoxin significantly reduced (but not eliminated) at 135 mg ozone, a substantially higher oxidant level than would be found in commercial ozonation. Ho and Wong (2004) found that injection of $1\,gO_3\ m^{-3}$ was effective in killing cultures of *Prorocentrum triestinum*, *Scrippsiella trochoidea*, and *Karenia digitata* in 15 min. Oemcke *et al.* (2005) noted effective control of *Amphididinium* sp., while ozone was 100% effective in inactivating *S. trochoidea* (Yang *et al.*, 2015) as a possible treatment in ship ballasts. Use of ozone in shellfish detoxification is summarized in Fernández *et al.* (2003), and these authors conclude that ozone is not effective in detoxifying shellfish, nor is it economically feasible; a recent publication is consistent with these conclusions, as toxin remains in mussels following ozonation (e.g., Louppis *et al.*, 2011). Importantly, for open systems, ozone treatment for arriving blooms is likely impossible.

12.5.5.1 UV Exposure

Most planktonic autotrophs are susceptible to UV-induced injury, through UV damage to cell organelles or via UV production of ozone as a by-product; however, use of UV exposure in open-water systems for minimizing HAB or toxin exposure in fish or shellfish is virtually nonexistent. UV–ozonation or UV–strong oxidizer treatment (e.g., chlorine dioxide or persulfate) have been suggested as viable treatments in ballast systems (e.g., Gregg *et al.*, 2009; Wu *et al.*, 2011a; First and Drake, 2014; Yang *et al.*, 2015), but general use for natural blooms is doubtful.

12.5.5.2 Cavitation

This process rapidly produces bubbles that, upon bursting, effectively destroy planktonic algae. Effective in ballast systems (Gregg *et al.*, 2009), cavitation might be useful in small systems and particularly closed systems. Using cultures and field bloom samples, Jančula *et al.* (2014) report that hydraulic jet cavitation may destroy gas vesicles in *Microcystis aeruginosa*, leading to 99% removal of the population; however, neither cell membranes nor metabolic activity was affected, with the former explaining the absence of any intracellular toxin release. A planktonic green alga was not impacted by the treatment, suggesting that cyanoHAB might be removed, leaving non-HAB taxa to persist and govern ecosystem and food web dynamics. Li *et al.* (2015) reported that a combination of ozone and cavitation decreased total chlorophyll by 78.8% in a eutrophic system pilot study, versus 62.3% with ozone only. Use in open systems, however, seems unlikely. High-power transducers used in ultrasound units for ballast tanks can induce cavitation, with resulting cell and toxin reductions (see details below and Lürling *et al.*, 2015).

12.5.5.3 Ultrasound

In contrast to chemical control/biocide methods in closed systems (such as seawater reverse-osmosis systems), ultrasound has been proposed as an environmentally friendly mitigation option through the sonication of algal biomass. After treatment with ultrasound, the addition of a flocculant increases organic matter removal (e.g., Hakata *et al.*, 2011). Wu *et al.* (2011b) suggested that ultrasound (~20 kHz) may be quite effective at controlling bloom growth and removing toxins, with removal rates of *M. aeruginosa* as high as 93.5% (Zhang *et al.*, 2009); intermediate frequencies appear to be the most effective at degrading toxins such as microcystin (Song *et al.*, 2005; Wu *et al.*, 2011b). In a recent study of two non-HAB populations (Wang *et al.*, 2014), higher frequency focused ultrasound (3.2 MHz, 40 W) was found more effective than in non-focused 20 kHz, 100 W treated cultures, perhaps indicating a better treatment option in the future. These positive results are likely due to free radical formation from the bursting of bubbles produced in cavitation (Lürling *et al.*, 2015) from the high-power

transducers employed. Results from commercially available ultrasound units with much lower power transducers (e.g., 7.9×10^{-4} W mL^{-1} vs. 22.7 W mL^{-1} in Song *et al.*, 2005) resulted in no reduction of cyanobacteria or microcystins, and some *Daphnia* mortality was noted (Lürling *et al.*, 2015). In contrast, however, using a 3-W power source, 200 kHz for 30 sec sunk *M. aeruginosa* scum, while 600 sec exposures reduced microcystin, geosmin, and 2-methylisoborneol by 81, 76, and 88%, respectively (Srisuksomwong *et al.*, 2011). Recent deployments of a LG-Sonic MP3 Buoy system in Emmitsburg, Maryland, USA, appear to be effective in reducing drinking water facility back-washing, indicating lower cells and cellular debris accumulations on facility membranes (Rada, 2017). Unfortunately, proprietary restrictions from the company on ultrasound power, frequencies, or durations prevent ascertaining specifics of the effective ultrasound attributes or the details on the taxa controlled by the unit. Hence, the HAB community remains skeptical of its routine use in most systems, particularly in large, open waters or at floating aquaculture facilities.

12.5.5.4 Electrolysis

Passing electric current into aquatic systems can prove lethal to planktonic organisms, likely through generation of strongly oxidizing free radicals (see Gregg *et al.*, 2009). In a particularly effective treatment for Korean coastal waters (Park *et al.*, 2013), an electric current is passed into seawater to produce sodium hypochlorite, which is then mixed with yellow clay and dispersed into *Cochlodinium* blooms, killing cells and sedimenting them to the bottom (see above). It also appears that many HAB taxa are more susceptible to this treatment than non-HAB and other plankton (Park *et al.*, 2013), thereby proving modestly effective for routine use and low ecosystem impact.

12.5.5.5 Hydraulics and Mixing

As stable water columns provide one critical environmental condition for HAB expression, disrupting water column stability and shortening residence times are the most effective strategies to shift from HAB taxa to less disruptive and threatening species (Figures 12.1(2) and 12.1(3); see Section 12.2.3, "Phytoplankton Mixing, Increasing Turbulence, and Decreasing Residence Times"). Hence, if a HAB is approaching or has developed in an important area, initiating rapid flushing (lower residence times) through dam or weir controls can effectively flush HAB from an impoundment (e.g., Sellner *et al.*, 2015), ideally to less favorable/suboptimal conditions below the embayment or lake (e.g., salinity extremes, lower nutrient concentrations and supply, or well-mixed or turbulent systems). Inducing mixing in the upper water column via bubblers, diffusers, mixers, and so on (e.g., Visser *et al.*, 1996; Hudnell *et al.*, 2010; Figure 12.10) also destroys the HA niche. As will be described in Section 12.7.4 ("HAB Mitigation Methods for Fish Mariculture"), coastal ocean fish farmers in several countries dilute blooms by pumping water from depth, hence displacing the HA-ladened surface water with deeper, cell-free water (Rensel and Whyte, 2003; Park *et al.*, 2013). Hayden *et al.* (2012) used pumped deep water to reduce densities of surface populations of *Prymnesium parvum* in Lake Granby, Texas, and continuous pumping of deep water at 16.3×10^4 m^3 d^{-1} into adjacent coves was predicted to shorten hydraulic residence times to 0.88–1.41 days, leading to a likely bloom decline of 65–80% (Lundgren *et al.*, 2013).

12.5.5.6 Biological Controls

There are many suggestions of biological control or mitigation of HAB (Figure 12.1(6)), but most methods are largely impractical due to the spatial extent of most blooms and, importantly, require major infrastructure investment for routine use in bloom control. Hence, only a brief summary of options that have been proposed will be addressed. Several investigators have suggested that the introduction of organisms that infect and lyse harmful algae might be excellent candidates for routine use. A dinoflagellate, *Amoebophyra*, is a species-specific parasite of several HA dinoflagellates and can induce mass population lysis in laboratory cultures as well as field populations (see Park *et al.*, 2004; Kim *et al.*, 2008). Species-specific viruses have also been identified (e.g., for *Aureococcus anophagefferens*; Milligan and Cosper, 1994) and nominated as control agents (Nagasaki *et al.*, 1999; Tomaru *et al.*, 2008). There has been considerable excitement associated with bacteria-induced lysis (Figure 12.6), with water reed–associated bacteria capable of lysing *M. aeruginosa* (Imai *et al.*, 2012), and sea grass– and macroalgae-associated bacteria killing *H. akashiwo* and *A. tamarense* (Onishi *et al.*, 2014), indicating potential natural controls in several Japanese bays (see Imai *et al.*, 2014) and Puget Sound (Inaba *et al.*, 2015). Bacteria have been shown to kill *K. brevis* (Doucette *et al.*, 1999), *A. tamarense* (Wang *et al.*, 2010; Su *et al.*, 2011), *Gymnodinium catenatum* (Lovejoy *et al.*, 1998), *Chattonella* spp. (Imai *et al.*, 1998; Lovejoy *et al.*, 1998; Pokrzywinski *et al.*, 2012),

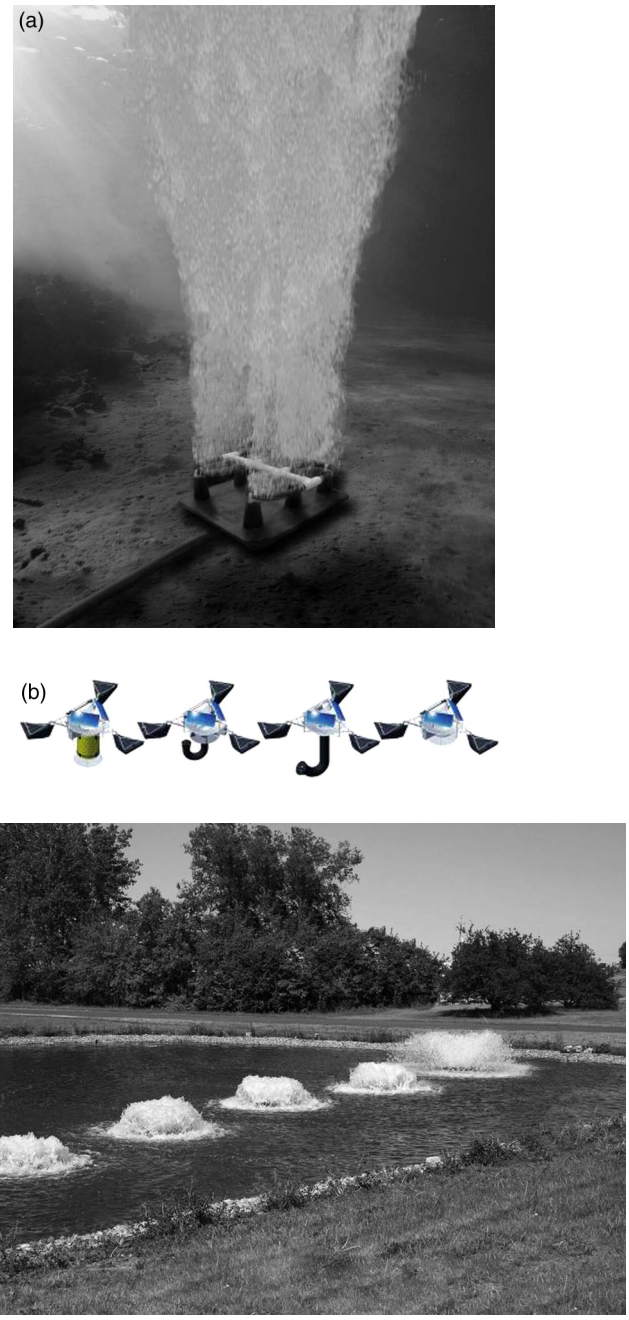


Figure 12.10 Technologies to routinely destratify water columns: (a) diffuser, (b) Solar Bee, and (c) aerator. Reproduced with permission of Kasco Marine and Moderca, Inc.

Noctiluca scintillans (Keawtawee *et al.*, 2011), and *H. akashiwo* (Lovejoy *et al.*, 1998). Heterotrophic and mixotrophic dinoflagellates and ciliates have also been identified as HAB predators and potential bloom controls in small systems (Jeong *et al.*, 2003a, 2008; see Park *et al.*, 2013, table 6); Jeong *et al.* (2001a, 2001b, 2008) propose mass culture of these grazers as effective HAB biocontrols. Planktonic metazoans have not, or have rarely, been suggested as HAB biocontrols in brackish-marine waters, although ingestion of HA taxa, at bloom levels, does occur (see Turner, 2006). CyanoHAB are ingested by freshwater planktonic grazers (e.g., Sellner *et al.*, 1993; Hogfors *et al.*, 2014), but

generally at insufficient grazing pressure to remove the blooms. There are a few exceptions (e.g., Wilson and Chislock, 2013), but use of freshwater planktonic grazers in bloom removal, at least after reaching bloom magnitudes, seems unlikely.

Shellfish can also clear bloom taxa from the water column effectively and could be biocontrols under low cell densities, but may shut down due to high cell abundances or, after toxin exposure, become moribund (MacQuarrie and Bricelj, 2008), suffer hemocyte deformities and activities (Galimany *et al.*, 2008), or die (e.g., Luckenbach *et al.*, 1993; Brownlee *et al.*, 2008). There are shellfish populations that are physiologically resistant to toxin exposure that might also be effective in HAB removal, but use in controlling or resisting blooms has not been attempted and may be unrealistic at the spatial scales required. Bricelj *et al.* (2005) have documented physiological resistance to paralytic shellfish toxins from *Alexandrium* species in some populations of the soft-shell clam *Mya arenaria*. This pattern has also been observed in copepods (Colin and Dam, 2002), but may be temporary (Jiang *et al.*, 2011). Resistance may also be present in some fish exposed to ciguatera (Jiang *et al.*, 2012) and domoic acid (Lefebvre *et al.*, 2012). Whether these resistant populations could be established and effectively clear HA taxa before bloom densities are reached remains to be determined.

12.6 Shellfish

Harmful and toxic algal blooms can have devastating impacts on individual shellfish, commercial shellfisheries, and aquaculture, and these are summarized elsewhere (see Chapter 4; Shumway, 1990; Shumway *et al.*, 1995; Landsberg, 2002; Matsuyama and Shumway, 2009). Mitigation of these impacts on the shellfish industry and the environment is mentioned regularly in numerous publications, and there is an ongoing discussion and recognition of the importance of identifying mitigation strategies. The reality is, however, that there are few realistic means available to industry members by which mitigation is possible. Comprehensive monitoring programs (see Chapter 10) protect public health, but with regard to the industry, in most situations the response is to "wait it out" (i.e., let the shellfish depurate naturally after the bloom has subsided, and resume harvest).

Early warning systems (see earlier in the chapter and Chapters 3, 12, and 13) can provide industry with information that allows them to alter their harvesting schedules when blooms are imminent, and furthermore, forecasts of trajectory of events by plankton monitoring can provide valuable information with regard to the expected length and path of the bloom.

Safe marketing of shellfish relies upon a strict regulatory regime, and the most effective means of quality control during outbreaks of toxic algae is either by blanket closure during certain times of the year, or by instituting a shellfish toxicity-monitoring program (see Shumway *et al.* [1995] and the earlier description of the U.S. NSSP).

It is important for growers and harvesters to be aware of the potential for outbreaks of toxic algae even in areas not previously impacted. As summarized earlier and in the fish section that follows, careful selection of shellfish aquaculture sites can provide reduced risks for the growers; and, if aquaculture sites can be located in areas with little or no history of toxic blooms, there are fewer public safety issues and minimal disruption of harvesting. Often, successful shellfish aquaculture and commercial fisheries take place in areas regularly plagued by algal blooms, and the presence of monitoring programs makes production and harvest of a safe product possible and economically feasible.

Clay flocculation is increasingly used (see Section 12.5.3, "Flocculation") as a means of removing algal blooms in some regions utilized by fish cages and shellfish harvests, such as South Korea and China, and the process continues to be refined. Shumway *et al.* (2003) demonstrated potential detrimental effects of clay on filtration in bivalve molluscs at specific dosages of 1.0 and 10.0 g^{-1}. As noted previously, 100 mg L^{-1} is now recommended for general application in bloom removal (e.g., Zou *et al.*, 2006) although Yu *et al.* (2017) suggest even lower applications. Note, however, to the best of our knowledge, clay flocculation has not been used on shallow, extensive ground culture of shellfish. This method should be viewed with caution depending upon the local environmental situation, the bivalve or other sensitive species being cultured or occurring in natural populations, the accuracy of doses in spreading techniques, and the algal species and toxins involved.

Rapid detection test kits for algal toxins (see earlier in this chapter and Chapter 2) have made it possible for shellfish harvesters (wild and cultured) to reduce the time, associated labor costs, and lost product associated with harvesting unmarketable product.

Dispersal of harmful and toxic algal species has been investigated, and most discussions have focused on the role of ship ballast waters (see Hallegraeff, 1998; Smayda, 2007). More recently, several Shumway studies have assessed the role of

shellfish and biofouling materials as potential vectors of transport (see Honjo *et al.*, 1998; Shumway *et al.*, 2006; Hégaret *et al.*, 2008; Rosa *et al.*, 2013). Transport of infected shellfish (cells and/or cysts) can result in infection of a clean area wth cysts or motile cells. This has been documented in several species (see Matsuyama and Shumway [2009] for review). While there are regulations in place at the International Maritime Organization (IMO) and other agencies that regulate the transfer of harmful aquatic organisms by ships, mitigation via transfer of shellfish and other organisms is left to best management practices by the industry participants.

Hégaret *et al.* (2008) demonstrated that some HAB species can be rendered non-viable if shellfish are held out of water for short time periods (24 h) and that, in many cases, this mitigation strategy coincides with standard operating procedures and shipping practices. Shellfish transplants should be monitored until toxic algal species are not detected either in the seawater from the cultivation area or in the shellfish being transported. It was suggested by Matsuyama and Shumway (2009) that a global framework to reduce the potential dispersal risk of toxic algal species via shellfish transport, as well as potential dispersal risk of pathogenic microbes, should be considered.

More recently, Rosa *et al.* (2013) assessed the potential for transfer and expanded distribution of toxic and harmful algal species via biofouling materials removed from aquaculture gear. This study demonstrated that several toxic and harmful algal species were able to pass through the digestive systems of ascidians and were excreted as viable cells demonstrated as capable of restablishing algal cultures. These findings were formally incorporated into a new cost-share program developed to help shellfish producers prevent the further spread of ascidians and associated HAB species (Getchis *et al.*, 2012).

Depuration of algal toxins has been investigated in many algal–shellfish species combinations (see Matsuyama and Shumway [2009] for summary). The depuration rates are species-specific and depend upon the shellfish–algal paring. Shellfish species have been classified as fast- and slow-depurators (see Bricelj and Shumway [1998] for review). In general, mussels and oysters are rapid detoxifiers, in comparison with other species (e.g., butter clams, surfclams, and scallops). Most bivalves will eliminate the accumulated toxins once the algal blooms have receded or if they are tranferred to a bloom-free site. Caution should be noted, however, as the transfer of cultivation pens or rafts during the occurrence of toxic algal blooms may cause expansion of the bloom. In general (as discussed in this chapter), cooking does not deactivate toxins, and thus shellfish should only be consumed from areas known to be monitored regularly by an authorized public health agency.

There are to date no cost-effective means of depuration in shellfish.

12.7 Fish Mariculture

12.7.1 HAB Mitigation for Fish Mariculture

After several decades of experimentation and method refinement, it is clear that a combination of judicious fish farm site selection and mitigation methods can significantly reduce HAB-induced mariculture fish kills to economically acceptable levels. Examples are numerous, especially in the salmon farming industry, which represents the most rapidly evolving form of net cage fish mariculture.

Physiological causes of fish mortality, affected organs or tissues, and exposure thresholds of harm for several HA species were reported by Rensel and Whyte (2003). In general, the etiology of fish death is somewhat understood for most HA species, but not all. A variety of physiological mechanisms, singly or in combination, may lead to fish mortality from HAB. The mechanisms can be attributed to at least four categories that may overlap depending on the species of fish or HA: (1) physical damage or irritation of gill tissue leading to mucus production, blood hypoxia, and possibly bacterial infection; (2) toxigenic reactions to ichthyotoxic agents such as reactive oxygen species (ROS) and by-products of polyunsaturated fatty acids (PUFA) that may function together with some HAB species to injure fish gill tissue. In other cases, ROS alone or brevetoxins or karlotoxins are linked to fish kills via gill damage from specific HAB species (e.g., Dorantes-Aranda *et al.*, 2015; Mardones *et al.*, 2015), nerve systems or the operation of primary organs, such as heart and liver; (3) blood hypoxia from environmental oxygen depletion caused by senescence of large algal blooms that are not necessarily toxic; or (4) gas bubble trauma and gill lesions associated with high-density blooms of any kind of phytoplankton that may cause oxygen supersaturation at unsafe levels. For more details, see Rensel and Whyte, (2003) and numerous recent studies completed using microplate assays of fish gill cell lines.

Mitigation of HAB at fish farms is usually not a mariculture priority in a region until a major fish-killing bloom or series of blooms occur. When such events first occur within a region, they generally cause a degree of chaos for the fish farmers, processors, government regulators, and food safety agencies, but adaptations occur as discussed below. Fish mariculture is relatively new in most coastal seas of the world, and therefore it is just a matter of time before many fish farms experience either naturally occurring or human-induced or exacerbated HAB events resulting in fish kills. Although some have ascribed HAB to excess nutrients and eutrophication (Anderson *et al.*, 2002; Heisler *et al.*, 2008) and question the role of bivalve mariculture to mitigate eutrophication (Bouwman *et al.*, 2013b), the linkages between eutrophication in all its various manifestations and HAB events are not consistent (Anderson *et al.*, 2008; Davidson *et al.*, 2014). HAB events also occur in pristine locations with no cultural eutrophication effects (e.g., PSP in western Alaska and the associated Aleutian Islands) and are frequently advected from non-eutrophied areas (Sellner *et al.*, 2003). Where significant abatement of wastewater discharge effects has occurred, the types of HAB species may change, but HAB events continue (e.g., Seto Inland Sea in Japan; Davidson *et al.*, 2014). These same reviews point out that where links between algal blooms and nutrients have been demonstrated, high-biomass nontoxic blooms rather than biotoxin-producing blooms are more common.

With climate change occurring at a rapid rate, increased seawater temperatures and water column stratification may exacerbate the occurrence and severity of some species and harmful algae events, and increasing frequencies of HAB impacts on cultured fish and shellfish are likely and pose a serious future threat to the expanding industry.

12.7.2 Best Management Practices for Fish Mariculture Siting, Including HAB and Eutrophication Issues

Due to the lack of published HAB avoidance and eutrophication-related BMPs for fish mariculture, the following siting and operation recommendations are provided. These BMPs should supplement risk assessments and literature surveys on bloom occurrences that combine HAB avoidance and optimization of fish-growing conditions for environmental and economic sustainability. Candidate mariculture sites and regions should be evaluated initially according to a matrix of considerations ranging from practical business and logistical factors to environmental sustainability and suitability of fish culture conditions. In most locations, compromises among the site consideration factors may be necessary, but several are important for all aspects of fish farm success and environmental protection. The history of fish mariculture in the 1970s and 1980s is replete with examples of HAB events that were unexpected and caused massive losses to fish resources as net pen technology was relatively new (Nash, 2011). In retrospect, these events could have been avoided through some basic monitoring and management steps, discussed herein.

12.7.2.1 Local Land Use

For nearshore or shallow ocean sites, current and future local land uses should be identified and nutrient loads estimated in watersheds or areas upstream of potential fish or shellfish stocking or harvest locations. If progressive BMPs are implemented between agricultural farmlands and impervious surfaces and receiving waters, much or the surface runoff of soluble N and particle-bound P can be intercepted before reaching local waters. Aquaculture and mariculture industries should work with local planners to identify locations with likely lowest nutrient loads currently and into the future, thereby narrowing possible culture areas to locales with a low probability for HA habitat or niches.

12.7.2.2 Plankton Monitoring and Water Quality Assessments

Hydrographic monitoring or data collection and analysis of a proposed fish mariculture project should be conducted to assess site suitability for the intended size and fish species, as well as to assess the risks of HAB events and the nutrient sensitivity of the waters to particulate and dissolved wastes. Variables collected in vertical profiles such as water temperature, salinity, DO, turbidity, chlorophyll-*a*, and Secchi disc or other measures of light penetration can be surrogate indicators to predict the risks of HAB and sensitivity to eutrophication; concurrently collected samples for phytoplankton identification and enumeration are very desirable, permitting elucidation of HA species, life histories, and growth requirements. Hydrographic and phytoplankton data from prior studies may be available from universities, government agencies, or other sources to help evaluate HAB and eutrophication risks in lieu of any governmental regulations or guidance.

12.7.2.3 Physical Hydrographic Considerations

Surface to near-bottom current velocities and directions should be determined for a candidate site and may be displayed as current vector rose diagrams to indicate intensity and frequencies of directional flows in a given area. Strong tides or ocean currents are necessary to disperse and aerobically assimilate waste products from large-scale mariculture as well as continuously deliver oxygen-rich waters and plankton and detritus as potential food. In a shallow area, high velocities may preclude the presence of a soft bottom substrate and deposition of vegetative overwintering populations (e.g., *Microcystis aeruginosa*), cyanobacterial akinetes (e.g., *Nodularia spumigena*), diatom spores, and dinoflagellate cysts, as these all accumulate in muddy sediments, not sandy or rocky sea bottoms. A surrogate for bottom current measurements would be determining bottom substrate (particle size) composition as all of the populations above would only be found in fine particle sediments; if fines were found, all of the above could be enumerated as well, but this is tedious and expensive, and the populations are often sparse.

Again, consistent with what has already been stated, hydrographic and phytoplankton data from prior studies by universities, government agencies, or anecdotal observations of other groups frequenting the subject area may be available to help evaluate potential HAB risks. If these are not available, field surveys should be conducted during the appropriate algal bloom season(s). Alternatively, another approach is to operate only small-scale or test facilities while hydrographic, phytoplankton, fish growth, and survival data are gathered.

Once completed, an experienced analyst can use these data to evaluate site suitability with available or recently collected data, and apply indices such as the degree of vertical stratification during neap tides and clement/inclement weather to quantify site suitability. Concurrently, these data can be configured into GIS layers and used to help identify special habitats of concern such as coral reefs, sea grass meadows, and essential wild fish habitat or near-critical wildlife and marine mammal habitat where mariculture should not be located.

12.7.2.4 Vertical Mixing Considerations

Strong vertical water column mixing is useful to prevent HAB from occurring or to help disperse them if they are advected into an area, as many dinoflagellates and microflagellates prefer tranquil waters and generally dominate when the surface mixed layer is well established and separated from the deep, stratified layer by strong density discontinuities caused by salinity or temperature differences. Increasing turbulence selects for non-motile and non-buoyant taxa such as diatoms (Margalef, 1997) and may destroy cells or reduce growth of some microflagellate and dinoflagellate species (White, 1976). The fish-killing diatom *C. concavicornis* and related subgenus *Phaeoceros* species that are harmful to fish at very low concentrations (Albright *et al.*, 1992; Rensel, 1993) may, however, be present to 40 m or more throughout a well-mixed water column and recur annually (Rensel *et al.*, 1989).

For protection of nearshore habitat and essential ecosystem services, fish mariculture farms should not be located in poorly flushed bays or estuaries with weak circulation and extreme vertical water column stratification during the algal growing season. Alternatively, if the candidate site is well mixed with very high turbidity, the site may be less suitable as net cage cultured fish are visual feeders and will not be able to see the feed, resulting in increased organic loading to the botttom. For salmon mariculture, alternating moderate turbidity from freshwater rivers and low turbidity from ocean water input combine to provide some of the best fish-growing areas in the Salish Sea, with no HAB events over many decades of operations in some cases (Rensel *et al.*, 2010).

12.7.3 Mitigation of HAB at Fish Mariculture Facilities

Despite all precautions or planning, there remains the risk of HAB occurring near, or being advected into, a usually safe area from adjacent waters. A few examples where blooms occur in one location, but affect marine resources in remote locations through advection, include *Gymnodinium aureolum* blooms along the coast of Norway (Dahl and Tangen, 1990), *K. brevis* fish kills and other problems that have occurred on the west coast of Florida for over 100 years (Magaña *et al.*, 2003), shellfish toxin-producing *Gymnodium catenatum* blooms on the northern Atlantic coast of Spain (Fraga *et al.*, 1988), and fish-killing blooms of *H. akashiwo* in the Salish Sea of Washington State and British Columbia (Rensel *et al.*, 2010). In such cases, having prepared plans and equipment to address fish-killing HAB is a prudent measure that should have a high benefit-to-cost ratio over time.

The concentration of HAB that will adversely impact or kill fish is a subject of considerable interest

to fish farmers, fishermen, and management authorities. HAB concentrations may vary tremendously depending on many factors, including the ecoregion of occurrence, fish species considered, and conditions for growth and toxin content of the subject HAB. In some cases, one HAB species may kill fish routinely in one region, but not in another. Rensel and Whyte (2003) provided a table that describes what was known at that time regarding threshold effects or killing concentrations of HAB for some species of fish. These data are very approximate, but new studies are rare because the data sources are often uncontrolled field observations, and laboratory assays may have difficulty recreating field bloom conditions. Similarly, the cause of fish stress and mortality associated with some important HAB species remains either poorly described or unknown, and this limits the ability to design and operate mitigation methods.

12.7.4 HAB Mitigation Methods for Fish Mariculture

Fish farm techniques for HAB mitigation previously or presently used are addressed below, excluding flocculation with clay and other materials that were previously discussed. The following review represents an update of a prior effort by Rensel and Whyte (2003). It is often desirable to plan and utilize more than one technique because recurring blooms of the same or different HA species may behave uniquely with variable hydrographic conditions and weather.

12.7.4.1 Feeding and Handling Practices

Fish culture practices should be altered at the onset of a HAB event occurring near a fish farm. Fish feeding should be suspended if no other reasonably efficient mitigation method is available. Fish oxygen demand increases after feeding, and fish respiration could be difficult if the gills become damaged by exposure to HAB cells, as gill damage is common during many HAB events (Rensel and Whyte, 2003). Over prolonged periods of several weeks, withholding feed causes increased physiological stress, including reduced liver glycogen, catabolism of tissues with associated weight loss, and an increased susceptibility to chronic diseases such as bacterial kidney disease, all of which may endanger fish survival or pose an economic hardship to the fish farmer. Thus, withholding feed is not a very satisfactory method and no guarantee for fish survival but is recommended for short-term events.

Cessation of all fish handling and restricted human activity on the farm site comprise a complementary strategy that further limits fish stress by reducing oxygen demand and increasing metabolic demand for food. These strategies are usually inadequate by themselves to protect fish during major HAB events.

12.7.4.1.1 Aeration

Aeration in aquaculture is the introduction of air into water with the intent of increasing DO to sustain cultured organisms. Information regarding aeration requirements for normal operation of net cage fish mariculture or for mitigation of HAB events at fish farms is relatively scarce in the published literature. Many sources of information exist, however, for freshwater ponds, tanks, and recirculating aquaculture operation during normal operations (e.g., Kepenyes and Váradi, 1983; Boyd and Tucker, 2014; Tucker, 2005).

Aeration is widely practiced in marine fish farms, usually to offset high loading densities of culture organisms or low ambient levels of ambient DO. The largest fish mariculture farms in the world are mostly for salmonids, especially Atlantic salmon (*Salmo salar*), and aeration systems are now widely used in this industry to prevent stress from low DO that can lead to disease or reduced growth. In some cases, the aeration systems are configured to start automatically when monitoring equipment detects within-cage oxygen concentrations have dropped below a given threshold.

Aeration may also be useful in some cases for mitigating the physiological effects of HAB that cause environmental hypoxia or anoxia (i.e., large-biomass blooms of noxious or normally benign microalgae that may be respiring or have become senescent). Aeration is considered more effective if used in combination with other techniques discussed below, such as airlift upwelling or bubble curtains.

There are three broad categories of aeration equipment to consider:

1) *Pumped water systems* are principally those that utilize venturi nozzles to aspirate air into the flow of water that is directed to the fish. Any type of pump (volume, pressure, submerged, etc.) may be used to create water flow, but some types are more economical and efficient for differing discharge depths. There are several designs of venturi nozzles to accommodate different and varying flow rates. Electricity or fuel-powered engines may power pumps. The most dependable performance is from shore power with diesel-powered

generator backups, but these are not commonly available for floating or submerged fish mariculture installations.

2) *Pumped air systems* include air pumps, compressors, and blowers to force air through distribution devices, usually diffusers such as airstones or porous pipes and hoses, using the same power sources discussed above. This category could include airlift pump systems; this category is so important and prevalent in mariculture that it is discussed separately below. Pumped air systems also include subsurface and hypolimnetic destratificaion (artificial vertical circulation) devices that could have some application in mariculture and HAB treatment but are usually used in lake restoration efforts.
3) *Surface agitators*, *fountains*, *splash aerators*, *vertical pumps*, and *paddlewheels* are designed to disturb the surface of the water and to enhance the transfer rate of oxygen from the atmosphere to the water. Boyd (1998) judged paddlewheel aerators to be the most efficient in transferring oxygen and circulating water in large but shallow ponds. These surface-oriented systems have been used in fish mariculture in coastal ponds but are not used in net pens due to their limited depth penetration and the common perception that they are better suited for aquaculture in freshwater or brackish ponds.

Many types of harmful algae adversely affect fish gills by different mechanisms (Rensel and Whyte, 2003), and to the extent that aeration can increase the concentration and saturation of DO in the net cages, the fish may be assisted with their ability to acquire oxygen. In cases where there is extensive diel fluctuation of DO due to nocturnal algal respiration, aeration can be very effective in maintaining the fish, unless the gills are too impaired with tissue damage or mucus from the gill holocrine cells. Holocrine cell cytoplasm is released by the rupture of the cell membrane after these cells migrate from subdermal areas to near the epidermis. The resulting mucus secretion onto the gill filaments and lamellae acts as a coating to protect and lubricate the gill epidermal cells, but also retards gas exchange of the gills. If many holocrine cells discharge in a short period of time, some species of fish will react by a coughing reaction in an attempt to reduce mucus loads on the gills or dislodge phytoplankton that becomes lodged in the secondary lamellae. This results in reduce blood oxygen concentrations, and eventually leads to blood hypoxia, asphyxiation, loss of homeostasis, and death (Rensel, 1993).

Although the large-bodied and hollow spines with sharp setae of *Chaetoceros* subgenus *Phaeoceros* are usually associated with such occurrences, large concentrations of a variety of usually benign, chain-forming pennate and other types of diatoms can induce similar reactions (Kent *et al.*, 1995) as well as silicoflagellates such as *Dictyocha speculum* (Henriksen *et al.*, 1993). Many toxic microflagellates and dinoflagellates can induce toxicosis in fish due in some cases to gill damage or paralysis leading to fish death from blood hypoxia (Noga, 2010). Simply raising the percent saturation of DO in the fish cages is inadequate to avoid fish injury or death. Moreover, once fish are exposed to harmful algae that cause physiological damage to the gills or other organs, future growth may be compromised and the fish may also be more susceptible to diseases. This has been the case with some groups of commercially raised salmon exposed to and surviving major *Heterosigma akashiwo* blooms (K. Bright, Cooke Aquaculture Pacific Inc., personal communication). Thus, prevention of fish contact with HAB is a better strategy that can be accomplished by the following mitigation technique if sufficient depth beneath the cages is available.

Airlift Aeration Airlift aeration is one of the most widely used and efficient methods to pump deeper water to displace surface-oriented HAB cells from fish aquaculture net cages. Concurrently, airlift aeration can potentially increase DO saturation that may further protect fish gills from exposure to toxin or mechanical damage from fish-killing HAB species.

For cultured Atlantic salmon (*S. salar*) in net cages, the upwelling movement of water creates strong, radial, horizontal water flows from the center of the fish cages. The flow also allows the fish to orient into the water current and passively allow the flow of water over their gills (K. Bright, Cooke Aquaculture Pacific Inc., personal communication), without signs of branchial pumping resulting in reduction in respiratory demand that is critical to fish survival during a HAB event. This process is known as *ram jet ventilation*, reducing respiratory energy loss by about 10% in another member of the genus, rainbow trout (*Oncorhynchus mykiss*; Steffensen, 1985; Farrell and Steffensen, 1987; Hughes and Nyholm, 1979). No prior record of this behavior for *S. salar* was found, although it has also been documented for sockeye salmon (*S. nerka*; Smith *et al.*, 1967). The fish achieves a reduction in respiratory demand by expending less energy in the process of respiring, critical to their survival during a HAB event.

The method is similar to lake de-stratification, except that well-located net pen cages are not

found over hypoxic sea bottoms and the goal is to replace the algal-populated surface water with relatively cell-free deep water as well as increased DO through the airlift–aeration process. The surface-oriented algal cells are initially displaced horizontally by the deep water that mixes with the surface water. As the bubbles reach the atmosphere, the horizontally displaced water may begin to sink if the source deep water was cooler than the surface waters. The sinking water advects algal cells downward that are then exposed to less light and cooler temperatures that tend to slow algal growth. Many HAB species are not tolerant of even minor amounts of turbulence, so the combined effect is to remove them from the fish cage but also reduce viability of the affected algae. In some regions, vertically hung perimeter skirts, discussed below, are arrayed around the fish cages to retain the upwelled water and prevent horizontal advection of HAB cells from surrounding waters to the fish.

Airlift aeration is sometimes used to rehabilitate eutrophic lakes or other slowly flushing water bodies by adding DO to an otherwise hypoxic deep layer (i.e., the hypolimnion). The airlift system introduces air bubbles in the deep layer that rise to the surface, entraining and aerating the deep water that results in reduced vertical stratification.

There are at least three reasons for some potential adverse effects of airlift aeration if a net cage site is poorly located in a highly stratified aquatic region. First, in such an environment, deep water may have much lower DO saturation compared to surface waters, and cultured fish and any other aerobic animals may be stressed due to the lower DO. This could arise if coarse bubble aeration is employed or if no diffuser is used; both generate upwelling but provide insufficient aeration. Second, if bottom waters or sediments are rich in H_2S, mixing of this natural "poison" into surface waters densely packed with fish could be devastating. Finally, the deep layer of the water column may be enriched with nitrogen or phosphorus from normal oceanic processes such as upwelling or from benthic remineralization of natural or anthropogenic waste materials. Introduction of those deep-layer dissolved nutrients to the surface layer during the algal growing season could promote more algae production, even HAB (e.g., Sellner *et al.*, 1990).

The solution to the above situation is similar to other issues in fish mariculture: initial site selection should be conducted to find suitable areas that do not compromise the environment and other biota, and the farm should be designed to limit biomass of fish to carrying capacity estimates. Fish farms should be operated to follow published sustainability practices. It is relatively simple to assess the degree of vertical stratification and probable habitat sensitivity from simple temperature, salinity, and oxygen profiles collected during the algal growing season(s) or by the use of worldwide satellite imagery (e.g., IFREMER, 2015) for open coastal waters. Fish mariculture net cages should generally not be located over highly stratified deep layers in inshore waters because such habitats are susceptible to or already affected by water column and sediment eutrophication and hypoxia. Furthermore, particulate organic matter deposition and assimilation in such conditions can shift sediment conditions from aerobic to anaerobic biogeochemistries with resulting hypoxia enhancing nutrient flux out of the sediments. At net cage sites with active vertical mixing or recently mixed conditions during the algal growing season, the deep water is often similar to the shallow (mixed) layer in DO and nutrient content, and the above potential limitations are not a concern.

A necessary prerequisite for airlift aeration to function properly for HAB mitigation is that there is sufficient depth to provide deep water with reduced microalgal abundance below the cages. Depending on water clarity, some shallower sites may contain algal populations throughout the water column. Airlift aeration may not be suitable for all types of HAB, particularly those that may migrate deeply or cluster at subsurface depths (Whyte *et al.*, 2001), as discussed in Section 12.7.4.1.2, "Perimeter Skirts."

For large-scale mariculture net cage applications, a series of commercial-sized compressors or other types of air pumps are required to force air through a hose to a sufficient depth where it may be discharged with or without a diffuser. Use of micropore diffuser tubing manifolds to produce extremely small bubbles can elevate DO saturation, depending on ambient DO concentrations. Newer types of micropore air tubing diffusers are designed to open only during use, reducing biofouling in the diffuser pores when not in use that otherwise would result in loss of air flow efficiency. The openings on these diffusers are uniformly small to increase oxygen transfer compared to the use of other types of airstones and diffusers.

Airlift aeration is a primary method used in British Columbia and Washington State salmon farms to prevent fish kills of *H. akashiwo* (Haigh and Esenkulova, 2014). Although this alga is capable of vertical migration, it often concentrates near the water surface in high densities during fish mariculture kills in the region. It is not uncommon, however, to have fish-killing concentrations of cells

Figure 12.11 Aerial photograph of fish cages surrounded by *Heterosigma akashiwo* and resulting bloom dispersion inside cages from airlift upwelling in British Columbia, Canada. *Source*: Courtesy of German Campos, General Manager of Operations, Cermaq Canada, Ltd.

distributed to ≥10 m depth at other times in well-mixed channels and passages (Rensel *et al*., 2010). In either case, airlift aeration of deep (e.g., >15–40 m) seawater to net cages can be very effective in excluding *H. akashiwo* cells from the cages, resulting in reduction or near elimination of fish mortalities. From a low-flying airplane during some blooms, the contrast of the deeper, blue water within the pens stands out next to the surrounding brown-red, bloom-affected surface water as a testament to how effective this system can be (Figure 12.11). This technique may also perform well in relatively high-current velocities, depending on the amount of upwelled water provided or if the cages are configured side-by-side in a row parallel to the dominant current directions to allow adjacent cages to share upwelled water.

If air compressors or suitable air blowers are already available, capital costs for airlift aeration will be restricted to air lines and diffusers. In regions where HAB are typically seasonal events (spring to early fall), fish farmers may utilize rental compressors to further reduce capital costs.

Venturi Nozzle Aeration These are cost-effective means to aerate water that involve the use of a venturi, defined as a gradually narrowing restriction in a pipe or tube that is reversed on the downstream side. The restriction creates a vacuum to aspirate air into the water, allowing for gas transfer to the water (Baylar and Ozkan, 2006). Venturi nozzle oxygen transfer efficiency is rated the highest of all available systems, from 2.0 to 3.3 kg O_2 KW^{-1} hr^{-1} (Lawson, 1995). There are known engineering requirements for such units, and several designs on the market with varying characteristics, efficiencies, and size. Larger venturi nozzles must be mounted on rigid surfaces to counteract the jet-like force of the discharge; however, they can be mounted in opposing groups of 2 or 4 to counteract the physical thrust produced.

Venturi nozzles as shown in Figure 12.12 have depth limitations: the deeper the nozzle is placed in the water, the more restriction on pumped water flow and air aspiration. However, with increasing

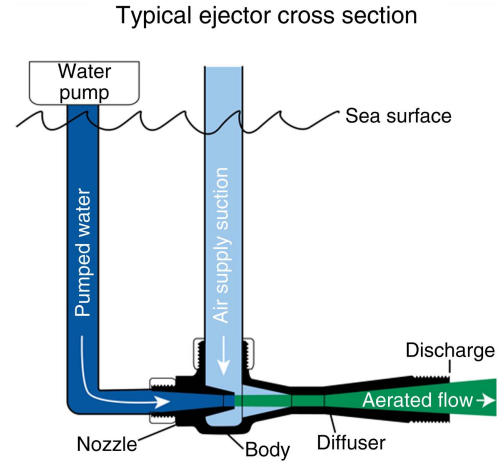


Figure 12.12 Schematic diagram of a Venturi nozzle system using pumped seawater from any source and an airline to the surface to allow rapid mixing of air and water via suction of air. *Source*: Adapted from http://www.northvalekorting.co.uk/images/fluidjetworks.jpg by J. Rensel.

depth, although the aspirated air supply rate decreases, oxygen transfer efficiency increases due to exposure time of the bubbles to seawater. Available trials (e.g., Colt and Tchobanoglous, 1981) indicate that venturi systems have competitive oxygen transfer rates compared to other types of aeration units. Venturi nozzles may also be operated as plunging water jets (Baylar *et al.*, 2006), but they do not penetrate far into the water column and are thus not typically useful for mitigation of HAB at fish net cages. They are used to provide aeration or oxygenation for fish that may be temporarily crowded together or within perimeter skirts for therapeutant baths, as described in the next section.

12.7.4.1.2 Cage Perimeter Skirts

Airlift upwelling with aeration has been used in the past with a companion technique referred to variously as cage perimeter skirts, shielding tarpaulins, fabric skirts, or tarps (Figure 12.13). The skirts are suspended vertically from the surface around the perimeter of fish net cages. These are used in some locations within Norway, British Columbia, and other fish mariculture countries (Haigh and Esenkulova, 2014). A major advantage of this technique is that the upwelled water is less likely diluted by surrounding surface or subsurface waters with high concentrations of harmful algae. Perimeter skirts have worked well with *H. akashiwo* in some fish farms of the Salish Sea of the North American west coast as this species is often concentrated in surface waters (Whyte, 1997, 1999). Perimeter skirts were less efficient with *Cochlodinium* sp. blooms in British Columbia, apparently because this species has a deeper diel vertical migration, to 25 m; however, a strategy of raising the tarps in the evening to flush any residual cells from the pens during the night, then lowering the tarps when cell density increased on the surface in late morning, proved to be useful (Whyte *et al.*, 2001).

Perimeter skirts may require extensive effort for installation, deployment when needed, and cleaning, and they can produce significant hydrodynamic drag that is a stress on net cages and associated moorings, especially in regions of fast-flowing tidal waters. The skirts are often rolled up alongside the cages and may retain some saltwater, become biofouled, and may interfere with normal management practices. In other cases, where tarps are required to surround cages during fish health treatments such as freshwater baths to treat for amoebic gill disease, the tarps may be very desirable for a dual-purpose role. Perimeter skirts of fine mesh may also be used at some fish farms to screen out post-larval *Cancer* spp. crab that have a sharp spine on their rostrum that may injure fish gills. The post-larvae are surface-oriented and may be flushed from the net cages by airlift aeration without perimeter skirts (as previously discussed).

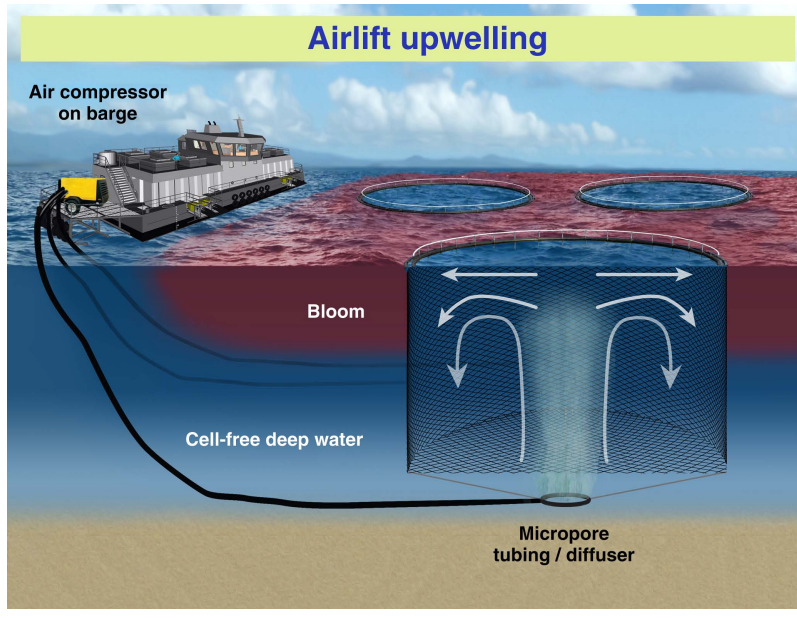


Figure 12.13 Diagram of airlift-upwelling system used to displace surface-oriented algal blooms from within fish cages using cell-free deep water; shown here with a vertical perimeter skirt around the cage, but often used with no skirting.

12.7.4.1.3 Bubble Curtains

Similar to upwelling aeration, this method uses porous tubing with a flexible lead weight to counteract buoyancy. Rather than being beneath the middle of each cage, tubing is arrayed around the bottom perimeter of each net cage. At least one fish farm company in British Columbia, Canada, is able to operate in Jervis and Sechelt Inlets where regularly occurring *H. akashiwo* blooms occur and other companies no longer remain. The method has also been used in South Korea for *C. polykrikoides* (Kim, 2012) and most probably in other regions.

Some companies use a combination of mitigation methods that include bubble curtains around all sides of the fish cages. When a bloom occurs, the pumping commences and results in significantly better survival than in non-treated cages. There are no published studies or technical reports detailing how this system can be effective, but the fact that it has been used by large commercial fish farmers in Canada for many years indicates that it must have some degree of efficacy. It is possible that the bubbles entrain the *H. akashiwo* cells to the surface, preventing exposure of the fish to the alga. *Heterosigma* cells are delicate, and it is possible that the turbulence causes them to rupture. This method may not be effective with other species that are more robust or have persistent toxins that cell rupture would discharge into the water and affect the cultured fish.

12.7.4.1.4 Oxygenation

Although not widely used in the past due to costs and equipment corrosion in net cage operations, addition of pure oxygen has been conducted at fish mariculture farms as a primary or backup means to maintain oxygen saturation, typically by using bottled oxygen and airstone diffusers but also with on-site oxygen generation equipment. In recent years *in situ* oxygen production has become more reliable and affordable due to simplifications in the process, and some large fish mariculture companies are now experimenting or relying upon it to replace aeration with atmospheric air. Systems are now available that do not require air compressors, dryer mechanisms, or feed buffer tanks that were formerly necessary. Manufacturers maintain that fewer moving parts and reduced maintenance contribute to improved efficiency and lowered costs.

Volume of oxygen gas required to achieve desired dissolved oxygen saturation will be much less per unit volume of net cage with oxygenation versus aeration. Oxygenation also provides for easily achieved supersaturation that may help mitigate common problems with gill damage due to HAB exposure; however, it is better and more humane to prevent than to mitigate HAB exposure to fish, as simply keeping the fish alive is not sufficient to insure continued fish health and normal subsequent growth. Despite these advantages with pure oxygen, the methodology may not replace the use of airlift-upwelling aeration. The large volume of air being pumped with airlift upwelling enhances replacement of cage water with airlifted cell-free deep water. Oxygenation to achieve the same volume of airlift capacity would introduce about five times as much oxygen gas and could risk causing harmful levels of oxygen supersaturation unless a mixture of air and pure oxygen was used. Oxygenation to moderate supersaturation levels may be useful in mitigating HAB events where gill clogging with algae, excessive gill mucus production, or physical gill damage occurs, with or without toxins. Moderate levels of oxygen supersaturation have repeatedly proven beneficial to fish species most susceptible to hypoxia, such as all salmonids and yellowtail (*Seriola* spp.) subjected to compromised water quality conditions (Boyd and Watten, 1989; Okaichi *et al.*, 1989). *Moderate* in this case means <300% of air saturation concentration of oxygen without supersaturated nitrogen gas for 190 g rainbow trout (Boyd and Watten, 1989). Colt and Tchobanoglous (1981) recommend up to 400 mm Hg oxygen pressure for high-density salmonid culture. Supersaturated oxygen in fish-culture water has been used with success in freshwater fish hatcheries and is often referred to as *oxygen supplementation*. Oxygenation is also increasingly being used during fish parasite treatment. For example, hydrogen peroxide is sometimes used as an efficient treatment and safely dissociates into water and oxygen, but it can damage fish gills and double oxygen demand by the fish during the short-term treatment due to necessary fish crowding and resulting stress (Bergheim *et al.*, 2013).

Compared to oxygenation, aeration *without airlift upwelling* is not recommended to sustain marine fish exposed to and stressed by HAB because it is often only marginally effective in increasing the ambient DO concentration during blooms. This is because the transfer rate of dissolved oxygen to the water is proportional to the difference between ambient and desired concentrations with aeration, and it is not possible to exceed 100% oxygen saturation in most cases. Aeration may be very effective in situations where high-density blooms occur that in some cases may result in nocturnal hypoxia due to algal respiration and cell decay. This is rare or nonexistent at

modern fish mariculture sites with good site conditions.

12.7.4.1.5 Submerging Net Cages

In the formative years of fish mariculture, submersion of net cages to avoid HAB (or waves from storms) was generally not recommended because it was technically difficult with existing designs not equipped to deal with structural stresses and fish containment issues. Additionally, physostomous fish such as salmonids must occasionally imbibe air for their float bladders to stay neutrally buoyant, which would not be possible during continuous submersion. Physoclistious fish that lack a connection between the gas bladder and the gastrointestinal tract generally do not have this limitation except when they are small juveniles. Submersion-designed, large-scale cages for open-ocean environments have been developed and refined (e.g., SeaStation and AquaPod cage designs by InnovaSea Systems, Inc.) and are being used in a number of locations around the world (Olivares, 2003; Loverich, 2010; Page, 2013).

Open-ocean mariculture fish farm technology is rapidly improving at present, and some locations such as near the leeward side of the island of Hawaii near Kona have had submersible cages for over a decade. No pelagic HAB events have occurred at or near these fish mariculture cages. Blue-water tropical waters of extremely low chlorophyll-*a* and dissolved inorganic nitrogen content and relatively strong current velocity (Rensel *et al.*, 2015) indicate that this is an unlikely habitat for HAB events to occur. With regard to ciguatera, the limited evidence available indicates that any linkages between eutrophication and increased incidents of ciguatera food poisoning remain inconclusive (Anderson *et al.*, 2008). Ciguatera occurs in a variety of locations, including remote and pristine reefs of the southeast Pacific Ocean, without aquaculture or any anthropogenic source of nutrient discharge nearby.

12.7.4.1.6 Deep Net Cages

In large-scale net-pen mariculture, some companies that have normal surface cage structures have fitted them with very deep net pens as a means of increasing rearing volume, lowering stocking density, and avoiding surface-oriented HAB problems. This was practiced in the past in British Columbia with chinook salmon, but most of the production is now with Atlantic salmon (Nash, 2001). There is no evidence that cultured fish will purposely seek refuge in deeper water during an algal bloom. Cultured salmon in cages that are suffering from stress or environmental problems including HAB events often orient themselves at the surface of the water and are accordingly referred to as *finners*. These fish stay at the surface despite having access to deeper waters beneath. Fish that are not fed during HAB events also may be more surface-oriented when humans are present, as they may be conditioned to expect feed when humans walk by an individual cage, if the feeding is not automatically controlled. Therefore, workers should stay away from the fish as much as possible during blooms to prevent the fish from reacting to human activity. There are no published reports on the use of deep net pens solely for HAB mitigation, but there is increasing interest in attracting the fish to deeper water with cages for other reasons, described below.

Controlling Fish Depth If a HAB is surface-oriented, another option involves controlling fish depth by excluding them from surface layers with horizontal nets lowered within each cage. Such techniques are technically feasible, but possibly cumbersome particularly in fast currents or in wave-swept locations. The technique is useful to avoid other surface water quality problems (Dempster *et al.*, 2009), gas supersaturation that occurs in shallow water (Colt, 2012), and, in some cases, sea lice infestation associated with fish that congregate near the surface in some locations and seasons (Jones and Beamish, 2011). In the mid-Columbia River of Washington State, nitrogen gas supersaturation from water spilling over the Grand Coulee Dam and other upstream dams may exceed safe limits for wild fish that are surface-oriented or net-caged steelhead trout during late-spring peak river discharge (Elston and Rensel, 1996). The nets are stored on the upstream side of each cage and deployed at short notice if conditions warrant. Stien *et al.* (2016) describe trials of a similar system of horizontal netting but with a "snorkel" (large-diameter vertical pipe) that allows salmon to rise to the surface to imbibe air to maintain their swim bladders for correct flotation and minimize infective-stage sea lice (copepodids) that are found in some locations near the surface. In this case, the purpose was to restrict fish from the top 4 m of the water column where sea lice are much more prevalent in the fall in Norway. The snorkel arrangement would not be necessary for many marine fish that do not imbibe air.

Most effective at night has been subsurface lighting that has been used experimentally to attract caged fish to deeper layers (Frenzel *et al.*,

2014). These authors used subsurface (5 m deep) feeding to further attract fish to deep layers, but the fish returned to the surface layers after satiation. This work was done for prevention of sea lice infection, not for HAB mitigation. It must be considered that some microflagellates and flagellates may be attracted to light sources as well, but in locations with strong tidal or ocean currents, the horizontal advection of water would dominate over the ability of microalgae to vertically migrate. It should not be assumed that a species of HAB will concentrate at the same depths or layers in different locations. Rather vertical sampling is recommended to confirm the distribution before and during treatments.

Moving Net Pens Towing net pens from an area affected by a HAB to a known refuge area may be an effective mitigation measure and is a preferred method in some regions (Anderson *et al.*, 2001; Rensel and Whyte, 2003). It does, however, present a considerable risk and expense for larger systems, although a fish-farming insurance company may offset part of the expense. Towing involves the risks of structural damage to facilities, fish escape, and fish mortality by crowding and stress when lower parts of the net pens collapse in currents if not towed properly. Interference with commercial shipping and other navigation is another potential issue. To be used effectively, a towing contingency plan should be devised in advance that includes protocols for addressing anchoring systems, timing of movement with regard to tides, and approval by government agencies if necessary. Practice towing exercises may be warranted, and in most cases, a permit from government would likely be required.

Towing of net pens has been used for preventing cultured fish losses due to *H. akashiwo* in Puget Sound, Washington State and British Columbia (Horner *et al.*, 1997; Whyte, 1997) as well as with the harmful dinoflagellate *Karenia mikimotoi* (as *Gyrodinium aureolum*) in Norway, and *Chattonella antiqua* in the Seto Inland Sea of Japan and Hong Kong (Anderson *et al.*, 2001). Aerial surveys by small aircraft or visual surveys by boat can be useful for detecting possible refuge areas, particularly when conducted in the morning before land breezes may disperse HAB cells from surface water. For HAB species that kill fish at low concentrations or are known to cluster at subsurface depths, using aerial surveys to find a true refuge area may not be feasible. In such cases, another strategy is to examine vertical cell distributions (via cell counts or a fluorometer) in possible refuge areas where strong vertical mixing inhibits growth and dilutes surface concentrations of HAB species.

Preemptive Harvest Although often considered as a HAB mitigation tool, preemptive harvest is rarely a useful option for fish mariculture as practiced in the Western Hemisphere. This is simply because the stock of fish at any modern fish farm is too large to be quickly harvested prior to a bloom, even if several days or a week's notice of an impending bloom was provided. Moreover, processing facilities are geared for a specific and limited size range of fish, and harvesting much smaller fish would not be possible or financially feasible. The ability to source and mobilize a large fleet of ships, tenders, and large fishing vessels to haul the fish is unlikely in temperate areas as these same vessels are often at sea for the summer fishing season. Additionally, processing facilities cannot handle orders of magnitude more fish on short notice. Thus, preemptive harvest has never been used successfully to capitalize on a HAB-caused fish mariculture kill. In many cases, fish can die rapidly, and the major task is to remove and dispose of the fish before the fish cages are overstressed with the weight of the dead fish. This was the case in the northern region of the Chilean Inland Sea in the austral summer of 2016, when nearly a billion dollars of fish were lost due to a HAB event fueled by much warmer than normal weather and water temperatures, and the fish had to be disposed of offshore in the Pacific Ocean (Clément *et al.*, 2016). In Asian Pacific waters, where small-scale fish mariculture is practiced, individual family farmers may be able to harvest most of their fish prior to a bloom, but markets are not structured to accept massive amounts of fresh fish at once, particularly when the public knows that they were killed by a toxic HAB species.

Turbulence: Incidental Partner to Other Methods Although not considered a standalone method, turbulence resulting from some other methods discussed herein may contribute to reduction of HAB events when fundamentals of phytoplankton ecology are considered. Kinetic turbulent energy in the upper ocean has long been thought largely responsible for controlling microalgal physiological performance and production in part due to inability of the cells to rapidly adapt to rapidly varying irradiance while entrained in mixing (Lewis *et al.*, 1984). It is also well known that many HAB species, excluding noxious diatoms such as *Chaetoceros* (subgenus *Phaeoceros*), generally prefer quiescent water conditions as described

in Margalef's conceptual mandala that involved nutrient supply and turbulence (Margalef, 1997). The mandala has been updated several times to reflect the fact that HAB causes are more complex than just the two factors (e.g., Glibert, 2016). Microflagellate and dinoflagellate blooms typically dissipate when winds and wave action increase, and species composition may change to species tolerant of and dependent upon vertical mixing such as the diatoms (White, 1976; Smayda and Reynolds, 2001).

Turbulence as a HAB mitigation agent has not been tested in a commercial application and has no other obvious direct advantage for cultured stocks of fish. Fish mariculture farms are typically not connected with a terrestrial electrical power grid and that limits the use of some types of equipment that could create turbulent vertical mixing.

Turbulence is induced with other mitigation methods such as airlift upwelling and bubble curtain application, although the relative contribution to the effectiveness of these methods for preventing or mitigating HAB effects on fish has not been systematically studied *in vivo*. In a laboratory, vigorous aeration has been shown to reduce chain length and adverse blood hypoxia effects of *C. concavicornis* cultures on Atlantic salmon compared to normal, longer chains grown in non-aerated cultures that were gently swirled for mixing once per day (Rensel, 1993; Rensel and Whyte, 2003). For toxin-containing species of HAB, if a persistent toxin is released from cell lysing related to turbulence, this could have an undesirable and adverse effect on fish and other biota. Enhanced toxicity of *Cochlodinium* sp. on Atlantic salmon smolts was observed in bioassays by Whyte *et al.* (2001) when the dinoflagellate was oxygenated or aerated, but the authors opined that fish mortality might have been a function of agitation caused by the aeration, leading to increased release of lethal reactive oxygen species or other toxins. Blooms of many HA species are known to dissipate with abrupt change of calm to stormy weather, but it is not known if this creates a more toxic environment for short periods due to release of toxins or if combined physicochemical, biological, and water circulation processes mitigate the possible effect. It is possible because lysis of some HAB species' cells can increase damage of fish gills in the laboratory (e.g., for some Chilean strains of *Alexandrium catenella*; Mardones *et al.*, 2015).

Blooms of delicate HA species such as *H. akashiwo* are known to terminate rapidly with winds and surface water mixing associated with wind and storm fronts (Rensel, 2007).

12.8 Conclusions

Rapidly expanding fish and shellfish aquaculture must involve considerations of threats from harmful algae and cyanobacteria to maximize yield while balancing the need for environmental sustainability.

For freshwater aquaculture, Figure 12.1 identifies several critical strategies that could be used in controlling cyanoHAB, likely important for use of local surface water for fish or prawn culture. Additionally, within the pond or lake culture facility, blooms in the aquaculture operation itself may be another issue. If water is pumped into constructed fish ponds or other structures, minimizing introduction of cyanoHAB (e.g., vegetative populations or akinetes) or their toxins from local water is imperative. Hence, operations using local surface sources would want to consider cooperation with local users to ensure the lowest possible levels of cyanobacteria or their toxins (Table 12.2A); many of the listed approaches are ranked from most to least effective, followed by practicality or likelihood of implementation. The second portion

Table 12.2 Strategies for potential control of cyanoHAB and toxins for (A) harvest of stocks maintained in open waters of a pond or lake, as well as source water conditions for use in a fish/prawn facility; (B) approaches for in-farm minimization of cyanoHAB and toxins; and (A.B) items that overlap A and B.

A. Issue	Strategy	Effectiveness (H,M,L)	Implementation practicality (H,M,L)
Elevated nutrient loads	Watershed-wide implementation of BMPs effective in nutrient and sediment control, wetlands, flow-ways	H	L due to very high cost, land use rights, lack of public/political will
Long residence time	Increase flow	H	M–L, dependent on creek, river, pond, or lake size and the presence/absence of flow control

Table 12.2 (*Continued*)

A. Issue	Strategy	Effectiveness (H,M,L)	Implementation practicality (H,M,L)
Stratification	Mixing with aerators, diffusers, circulators	M–L	M–L due to direct relationship between system size and units needed (cost) and bathymetry and shape of the system
Large cyanoHAB	Chemical additions	M–L	L because of direct relationship between system size and quantity of chemical to disperse (costs), ancillary deleterious impacts
Large cyanoHAB	Barley straw deployment months prior to bloom	H–M	H due to low cost for straw and deployment
Large cyanoHAB	Flocculation, flocculation + capping	M–L	M due to relatively low costs, but strong storms may erode sediments in shallow systems requiring additional treatment
Large cyanoHAB due to high P	Alum, Phoslock® additions	M–L	L because of water body size-dependent costs, new P loads, and storm-induced resuspension for shallow systems

B. Issue	Strategy	Effectiveness (H,M,L)	Implementation practicality (H,M,L)
Stratification	Mixing with aerators, diffusers, circulators	H–M	H–M with small farms easily and cheaply able to mix systems, but as farm size increases, costs increase
CyanoHAB	Skimming	H–M	H–M for small ponds if frequently implemented
CyanoHAB	Chemical additions	M–L	M for permanganate, chlorine if added before stocking, and caution after stocks in; L for others because of possible product contamination
CyanoHAB	Ozonation, cavitation, ultrasound	M–L	L due to power costs and low effectiveness of commercial units, unknown effective doses, impacts on product productivity

A.B. Issue	Strategy	Effectiveness (H,M,L)	Implementation practicality (H,M,L)
HAB and cyanotoxins	Depuration, detoxification procedures	H–L	M–L, as detoxification is HAB- and toxin-specific, with highly variable detoxification periods
Cyanotoxins	Use of U.S. EPA, WHO, EU, and Australia TDI guidance; better communication to overcome ethnic/cultural customs; rigorous understanding of food-processing limitations; aerosol advisories	H–L	H–L because of variability for local-national staffing for product testing and public outreach
General unfamiliarity with toxin-induced illness	Educate medical community of toxin-induced symptoms; continue development of toxin-specific therapies	M–L	L but improving due to education/outreach efforts in U.S. CDC, WHO, EU, etc.

Note: Only effective strategies are listed for both followed by a ranking of likely implementation of the practice.

(Table 12.2B) identifies some in-pond or in-lake techniques that could be used to reduce cyanoHAB impacts, again with the same prioritization for effectiveness and practicality. Finally, Table 12.2A.B lists issues and remedies common to both natural and cultured operations.

Marine fish aquaculture systems are mostly open systems as the open ocean is a potential suitable venue for fish mariculture that holds vast potential, but will require a shift from small growing operations to very large production to justify the added expense and difficulty of operating in open-ocean habitats. HAB events may also be fewer in some of these systems. Many of the HAB mitigation procedures used in freshwater systems would likely be ineffective in the open ocean due to diffusion of substances or conditions that could be generated up-current of mariculture facilities. Coastal ocean or estuarine aquaculture is also increasing, and HAB threats are nearly always possible in these locations. Hence, Table 12.3 focuses on approaches to prevent any HAB exposure (i.e., activities to implement to minimize HAB for shellfish or fish) as well as those in reaction to a bloom (i.e., those procedures that address specific HAB events when fish or shellfish are imminently threatened or already damaged). The recent success of very low concentrations of modified clays in Chinese waters with accompanying minimal nutrient, DO, or benthic fauna impacts (Yu *et al*., 2017) offers promise for expansion of this technique into appropriate regions where bathymetry and currents can assist in flocculated bloom dispersal or deposition. Many of the methodologies previously listed would be effective in identifying sustainable natural harvest areas or sites for locating commercial fish or shellfish mariculture (e.g., examining local land use, history of HAB in an area, and local hydrodynamics); others are specific to minimizing co-occurrences of HAB and natural or culture operations (e.g., detecting high chlorophyll and HAB species and toxins, forecasting HAB delivery to an area, and moving product to HAB-free areas); and still others are potential ways to minimize human exposures to intoxicated fish or shellfish (cleansing intoxicated fish or shellfish, outreach to inform potential recipients of toxic product, and medical treatment).

Mariculture and eutrophication: Many coastal seas are increasingly affected by cultural eutrophication from terrestrial, riverine, and atmospheric nutrient sources, but all sources are important to address when attempting to improve present conditions. Intensive and high-density fish mariculture, and to a lesser degree shellfish mariculture, may contribute to estuarine or marine eutrophication if carrying capacity for nutrient assimilation is exceeded for all nutrient inputs. Fish mariculture has been addressing this issue through reduced waste feed losses, improved food conversion ratio, carrying capacity limitations in some regions of nutrient sensitivity, protective siting rules, and relocating facilities out of poorly flushed backwaters toward the open ocean where water circulation and biological assimilation capacity is increased. Intensive shellfish mariculture reduces nutrient content on an annual basis within restricted flushed areas, but some of these nutrient-sensitive areas may be perturbed by inappropriately large and intense shellfish rafting operations. Increased supply of reactive dissolved nutrients to the upper water column, in vertically stratified systems or shallow mixed systems, can probably increase the likelihood of ongong algal blooms in nutrient-limited systems. Prior assessments of this have often been technically insufficient or focused on small fish mariculture farms, while high farm density in some other regions has not been comprehensively assessed. The consensus of many HAB experts worldwide is that links between anthropogenic nutrient supply and occurrence of HAB events are not consistent, but depend on HAB species, location-specific physics (stratification/mixing, currents, meteorological controls of waves and turbulence, etc.) and habitat attributes (e.g., temperature, salinity, and light). Likely, HAB and cyanoHAB threats to the freshwater and marine industries shown in Table 12.3 provide some potential guidance for aquaculture operation locations and management of sustainable operations for these needed protein sources.

Future sustainable supplies of fish and shellfish for the rapidly increasing global population are reliant upon planning and forceful action to maximize protein production, while minimizing HAB threats to these valued commodities and aquatic habitats. Both wild stocks and aquaculture stocks require protection of water and sediment quality, but to date the trend in many countries has been to exploit these resources for short-term gain without a long-term view to protect and enhance the necessary habitat. Practical use of historical, current, and expected future conditions in a given region for setting catch limits and siting culture operations could ensure sustained high yield of fish and shellfish from natural stocks and long-term profitability for commercial culture operations, thereby protecting the globally overharvested stocks

Table 12.3 Strategies for potential control of HAB and toxins in open marine systems.

Issue	Strategy	Effectiveness (H,M,L)	Implementation practicality (H,M,L)
Elevated nutrient loads	Watershed-wide implementation of BMPs effective in nutrient and sedimentation control, wetlands, flow-ways for estuarine natural stocks, or culture facilities	H	L due to very high cost, land use rights, lack of public/political will
Long residence time	Limit natural harvest; or, for aquaculture farms, limit based on carrying capacity estimates, or site elsewhere	H	H if natural harvest limits are enforced and H if farm operators/planners critically review local hydrodynamics for identifying aquaculture farm locations
Vertical stratification	In shallow and eutrophic estuaries, restricted fjords, and inland seas, identify local hydrodynamics and seasonal subsurface to bottom hypoxia/anoxia for aquaculture farm siting; for small embayments, use mixers/circulators	M	M due to need for experts to determine variability of seasonal stratification intensity from poorly predictable year-to-year local meteorology; L as mixers/circulators are costly
HAB history	Examine records for HAB occurrences, species for life cycles (resting-stage producers or not), and toxin production, HAB biomass levels, and reported concentrations causing chronic or acute effects and mortality	M	M due to need for trained experts to examine and interpret data for limiting possible impact of recurring HAB, those derived from local cyst excystment, and those yielding local hypoxia/anoxia
HAB and toxins	Increase species identification expertise and use automated equipment for species enumeration and toxin detection capacities	H	H as most toxin producers are known; H if competent regional toxin analyses are available
HAB	Broaden use of remotely sensed chlorophyll, other pigments, suspended sediment, and submersed grasses for HAB and environmental conditions	H	M due to need for remote sensing expertise for use in assessing general estuarine or oceanic productivity, potential for multiple uses of the coastal ocean, storm-induced events
HAB	Develop, distribute, and use local-to-regional scale HAB-hydrodynamic (particle tracking) and cell growth models similar to NPZ models already widely employed	H	H–M if cross-discipline need identified for multiple users (HAB, WWTPs, permitting, BMP siting, etc.); would combine remotely sensed chlorophyll, cell identifications, and local meteorology to forecast HAB landfall locations
Shellfish and fish HAB exposure	Pump HA cell-free water from depth; move shellfish to depth, relocating shellfish or fish pens to HAB-free areas; early shellfish harvest	H–M	H–M, basic costs of "doing business" in HAB area; some expertise required to identify and enable access to historic HAB-free areas
HAB and toxins purging	Provide harvesters and aquaculture farm operators with region- and toxin-specific depuration and detoxification procedures	H–L	M–L, as detoxification is HAB- and toxin-specific, with highly variable detoxification periods
Toxin exposures	Implementation of U.S. NSSP guidance suggestions internationally, EU testing; communications to address ethnic/cultural customs and harvest and aerosol advisories; rigorous understanding of food-processing limitations	H–L	H–L because of variability for local-national staffing for product testing and public outreach
General unfamiliarity of toxin-induced illness	Educate medical community of toxin-induced symptoms; continue development of toxin-specific therapies	M	L but improving due to education/outreach efforts in U.S. CDC, WHO, EU, APEC, etc.

Note: Only effective strategies are listed, followed by a ranking of likely implementation of the practice (high, medium, or low).

of fish and shellfish (e.g., Jackson *et al.*, 2001; Worm *et al.*, 2005; Beck *et al.*, 2011). Additionally, these efforts could reduce culture operation failures that occur due to ignoring likely HAB events for an area that could be attributable to the HA species and toxins present, cysts or akinetes possibly accumulating in a region, local nutrient sources and hydrodynamics, and future habitats likely from changing climate and land use. Planning and execution of strategies as described above are important for the next few years as aquaculture is recognized and implemented as the next major source of animal protein. Governments and industries must work together in the next decades for sustained sustenance for the world's increasing numbers of consumers.

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13

Harmful Algae Introductions: Vectors of Transfer, Mitigation, and Management

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13.1 Summary

- Sophisticated molecular tools are now available to identify new introductions and range expansions, or the new blooming of previously unnoticed cryptic species of harmful algae.
- Anthropogenic (ship ballast water, shellfish translocations, fishing waders, and sediment dredging) and natural vectors (climate change and storm events) behind such potential harmful algal bloom (HAB) introductions are described.
- Effective responses by resource managers depend on knowing whether a species' dominance is caused by its physiological or functional characteristics, changes in local environmental conditions, or a combination of both.
- A precautionary approach to management is recommended: (1) not assuming that a harmful species is not present and therefore does not need to be monitored for, (2) controlling all possible sources of unwanted translocations, and (3) being mindful of the risk of climate-driven range extensions of HAB species.
- Extensively researched ballast water treatment technologies (e.g., mechanical separation, heat treatment, UV irradiation, and biocides) serve as a guide toward suitable emergency treatment technologies to curb unwanted HAB translocations.

13.2 The Biogeographic Ranges of Harmful Algal Bloom Species

For many decades, it was thought that phytoplankton had a ubiquitous distribution in similar environmental conditions in oceans around the world. Oceans were viewed to lack barriers to gene flow, and microscopic planktonic organisms were considered to be present in every area supporting their ecological preferences (Finlay, 2002; Taylor *et al.*, 2007). Their ubiquity was attributed to large population sizes and a vast capacity for dispersal, making local extinction almost impossible (Fenchel and Finlay, 2004). As microalgae are passively transported and distributed by currents, physical dispersal barriers that exist on land such as mountain ranges were difficult to identify, and therefore speciation events were thought to be rare, and intraspecific diversity was thought to be low.

In the past decades, molecular research has begun to reveal a high degree of genetic diversity within microbial eukaryotic and prokaryotic species, showing that populations can be geographically structured in open ocean and benthic environments (de Vargas *et al.*, 1999; Martiny *et al.*, 2006; McCauley *et al.*, 2009; Casteleyn *et al.*, 2010; Penna *et al.*, 2010; Casabianca *et al.*, 2012). It is now accepted that phytoplankton populations can exhibit genetic structure over areas as small as 100 km and over time frames of months (Alpermann *et al.*, 2009; Härnström *et al.*, 2011; Richlen *et al.*, 2012). While some species appear to have a global distribution, others show narrow endemism (Vanormelingen *et al.*, 2008). In view of major advances in molecular technologies, it is now possible using population genetic markers to conclusively identify species that have been newly introduced to another region or are subject to recent range expansion.

Harmful Algal Blooms: A Compendium Desk Reference, First Edition. Edited by Sandra E. Shumway, JoAnn M. Burkholder, and Steve L. Morton.

13.3 Vectors of Transfer

13.3.1 Natural Factors

The geographic range of microalgal species can expand depending on natural factors (e.g., climate change, catastrophic storms, ocean currents, and transport of spores via wind or bird feet; Proctor, 1966; Schlichting, 1969) or human-mediated vectors. While prediction of the impact of global climate change on marine HAB is fraught with difficulties, range expansion of warm-water species at the expense of cold-water species that are driven poleward is expected. On top of range extensions driven by gradual climate change, ship ballast water and aquaculture product translocations continue to alter species distributions. The two mechanisms interact since ecosystems disturbed by pollution or climate change are more prone to invasions (Stachowicz *et al.*, 2002). Resource managers should fully understand and plan for the likelihood of significant range expansions or increases in biotoxin problems, which may be particularly unexpected in poorly monitored areas. Whereas the present chapter focuses on anthropogenic transport via ship ballast water and associated with the translocation of aquaculture products such as shellfish, other potential vectors such as fouling on ship hulls as well as transport via dredging or aquaculture equipment have not yet been adequately investigated to date with respect to microalgae.

13.3.2 Ballast Water

The global transport of microalgal species via ship ballast water, which commenced in the 1870s (Carlton, 1985) and is now transported annually on a worldwide basis at an estimated volume of 2–3 billion tonnes, has received considerable attention in the past two decades with nearly 400 papers published in the last 30 years (reviewed by Bailey, 2015). The potential for transport of non-indigenous marine microalgae via ship ballast water has been amply demonstrated, and nearly all known HAB species have been documented in viable form from ship ballast water. In extensive Australian ballast water surveys, 80% of ships contained up to 30 culturable diatom species, including several *Pseudo-nitzschia*, potential producers of amnesic shellfish toxins (Forbes and Hallegraeff, 2001). More seriously, viable cultures of the paralytic shellfish toxin–producing dinoflagellates *Alexandrium catenella* and *Alexandrium tamarense* were produced from ballast water entering Australia from Japan and Korea (in 5% of ships; Hallegraeff and Bolch, 1992), from ships entering British ports (*Alexandrium minutum* and *A. catenella/tamarense*; 17% of ships; Hamer *et al.*, 2001) as well as ships entering Canadian ports (*A. tamarense*: 19–32% of ships; *Pyrodinium bahamense*: 8–32%; Roy *et al.*, 2014). Both commercial ships and recreational boats were implicated in the dispersal of the brown tide *Aureococcus anophagefferens* into the North American Great Lakes (Doblin *et al.*, 2004b), and the potentially fish-killing dinoflagellate *Pfiesteria piscicida* was confirmed by molecular probes and cultured from ballast water entering U.S. and Australian ports, respectively (Doblin *et al.*, 2004a; Park *et al.*, 2007). Abundant *Dinophysis* dinoflagellate cells, causative organisms of diarrheic shellfish poisoning, were found in ships discharging near French aquaculture areas (Masson *et al.*, 2013). The recent application of increasingly sophisticated genetic tools (e.g., pyrosequencing; Burkholder *et al.*, 2007; J. Shaw *et al.*, University of Adelaide, unpublished) continues to add to this evidence, and the soft walled raphidophytes *Chattonella* and *Heterosigma*, and dinoflagellates *Karlodinium* and *Noctiluca* are also now confirmed from ballast water.

13.3.3 Translocation of Aquaculture Products

Viable transport of dinoflagellate cells and cysts of *P. piscicida, Pfiesteria shumwayae, Karenia brevis, Karenia mikimotoi, Alexandrium monilatum, Alexandrium tamarense*, and *Prorocentrum minimum*, after passage through the digestive tract of shellfish, has also been demonstrated (Scarratt *et al.*, 1993; Shumway *et al.*, 2004). This latter vector can introduce unwanted harmful microalgae directly into sensitive aquaculture areas. Biofouling ascidians attached to aquaculture gear can similarly contain viable harmful microalgae in their digestive tracts (Rosa *et al.*, 2013). The contribution of shellfish translocation to the spreading in western Japan of the dinoflagellate *Heterocapsa circularisquama* has been discussed by Imada *et al.* (2001). The fine-scale genetic structure of *Alexandrium pacificum* populations in Japanese coastal waters suggests a role for human-assisted dispersal associated with the expansion of the aquaculture industry and translocation of shellfish stocks into new areas (Nagai *et al.*, 2007). Finally, the potential impact from escape of aquaculture microalgal feedstocks (i.e., the globally used microalgal strains of [tropical] *Isochrysis galbana*, *Thalassiosira pseudonana*, etc.) remains to be assessed.

13.4 Molecular Evidence for Introductions of New Species to a Region

Determining whether a phytoplankton species has invasive characteristics – and, if so, is non-native to a region and a likely recent introduction – is

important for developing policy, management, and research responses to species with harmful impacts. This is because effective responses depend on knowing whether a species' ecological dominance is caused by its physiological or functional characteristics, changes in local environmental conditions, or a combination of both (Taylor and Bothwell, 2014).

Several potential new introductions of species to a region have been identified for HAB-forming organisms. The strength of evidence for these new introductions varies, and is based on features such as cyst records over decades, population genetic evidence linking geographically widely dispersed populations of a species, and long-term detailed monitoring records that have documented the arrival and expansion of a species not previously identified in a region. Not all instances of a HAB species forming blooms in a new area indicate that the species has been recently introduced to that area. Changing ecological conditions can stimulate blooms of species that have long been present in low abundances and may have previously gone unnoticed. This possibility has been supported by recent environmental genetic sequencing results, which have shown a "rare biosphere" of species that are present in extremely low abundances (Logares *et al.*, 2014) and would likely be overlooked using standard sampling techniques. Studies of plankton diversity using molecular genetic "meta-barcoding" techniques have shown the existence of ~10 times the number of operational taxonomic units (OTUs) compared to known described phytoplankton species that are documented in taxonomic or monitoring studies (de Vargas *et al.*, 2015), indicating substantial cryptic diversity is present. This would suggest that common phytoplankton monitoring using light microscopy is likely to undersample rare or cryptic species that may be present in a region.

Given these difficulties in identifying the complete phytoplankton community present in a region, it is understandable that in some cases, a species that was originally thought to be a new introduction to a region has been later found likely to have long been present in that region, and likely not closely related to populations from elsewhere in the world (Masseret *et al.*, 2009). There are several explanations for an apparent increase in the abundance of HAB in a region, even if species may not have been recently introduced from elsewhere: (1) improved scientific awareness and analytical capabilities; (2) increased use of coastal waters for aquaculture; (3) improved detection, preservation, and quantification methods; and (4) changing environmental conditions in a region, stimulating growth of previously rare species. For this reason, evidence must be examined carefully to determine whether a species has been recently introduced to a region via a human vector, such as a shellfish translocation or ballast water discharge. The careful collection of long-term data sets is necessary to determine baseline information, so that new introductions can be assessed.

In this section, examples of both new introductions and newly blooming species (that have been present for considerable periods of time in a location) are discussed.

13.4.1 The Stalk-Forming Freshwater Fouling Diatom *Didymosphenia geminata*

The species *Didymosphenia geminata* (often referred to as *didymo*) is a stalked diatom inhabiting nutrient-poor freshwater streams and rivers worldwide. It grows semi-benthically, attached to surfaces by way of polysaccharide stalks that form into thick gelatinous colonies, with an appearance like woolly mats (Kilroy and Unwin, 2011). While it does not produce toxins, it has adverse ecological and economic impacts on ecosystems. *D. geminata* may impact plant, invertebrate, and fish communities (Kilroy *et al.*, 2009; James *et al.*, 2010). It is aesthetically problematic, as the thick masses can degrade swimming areas and have the appearance of toilet paper, generating concerns regarding sanitation (Kilroy *et al.*, 2009). This diatom, considered to be native to North America and northern Europe (Bothwell *et al.*, 2014), has been reported with increasing frequency worldwide. One of the most closely documented case studies is from New Zealand. It was first reported on the South Island of New Zealand in 2004, and has now spread to over 140 rivers, several of which had been extensively sampled previously, confirming its prior absence (Kilroy *et al.*, 2009). In North America, reports of *D. geminata* began to appear in Vancouver in the early 1990s. In 2007, blooms were reported for the first time in New York, Vermont, and New Hampshire, and increasing numbers of streams have reported this species since then (Root and O'Reilly, 2012); it has been predicted to spread to many more areas of the United States (Kumar *et al.*, 2008). In South America, blooms have recently been reported for the first time in Chile (Jaramillo *et al.*, 2015).

New reports of this species have often corresponded with patterns of recreational use of streams and rivers, for example for fishing. It has been widely discussed that fishing waders and other fishing equipment may be important vectors for human translocations, as *D. geminata* may be retained as viable cells for considerable

periods in the felt sole of waders (Root and O'Reilly, 2012; Bothwell *et al.*, 2014). Local diffusion (e.g., within rivers) also appears to be important for its expansion. This species also appears to be a good example of a harmful algal species whose spread has been facilitated by changing environmental conditions, as low dissolved phosphorus conditions are necessary for bloom formation (Bothwell *et al.*, 2014). Other authors have challenged that *D. geminata* has increased its distribution (Taylor and Bothwell, 2014), suggesting that instead changing environmental conditions may be the major reason for increasing blooms, rather than translocations or spreading. This has been disputed (Bergey and Spaulding, 2015), indicating that more evidence, in the form of more detailed population genetic data, will be critical in resolving this issue.

13.4.2 *Alexandrium pacificum* and *A. minutum* in European and Japanese Waters

The species *A. pacificum* (as *A. catenella*, Group IV genotype) was documented from France, apparently for the first time, in 1995, based on a phytoplankton-monitoring program that had been in operation for 10 years prior to that (Lilly *et al.*, 2002). Following this, blooms of this species resulting in paralytic shellfish toxins in oysters were first detected in 1998, and have continued to occur since then (Masseret *et al.*, 2009). Based on phylogenetic evidence of the similarity of the ribosomal DNA gene regions, toxin profile, and restriction fragment length polymorphism (RFLP) data of two French and one Japanese strains, it was suggested that *A. pacificum* may have been transported to the Mediterranean, possibly via ballast water introduction, from Japan or other east Asian locations, likely prior to 1990 (Lilly *et al.*, 2002). More recently, these conclusions have been overturned. A population genetics study based on 61 strains of *A. pacificum* from the Mediterranean and 23 Japanese strains (Masseret *et al.*, 2009) has now shown that the European *A. pacificum* strains are not closely related to those from east Asia, but have likely long been present in the region. This example shows that highly specific molecular techniques, such as population genetics studies using methods such as microsatellite analyses, are necessary to verify translocation claims.

Such molecular techniques were also used to examine populations of *A. pacificum* (as *A. tamarense*, Group IV genotype) in Japanese and Korean coastal waters, and to try to detect the signal of human-assisted dispersals of this species causing shellfish toxicity in the region (Nagai *et al.*, 2007). In Japanese coastal waters, until the 1980s, paralytic shellfish toxins were only found in the northern part of the Pacific coast, but for the past 20 years, areas of western and eastern Japan have been impacted by blooms. While in most cases, genetic distance correlated with geographic distance between populations of *A. pacificum*, as would be expected of locally indigenous populations, some cases were also found of populations that were unusually similar, despite being separated by large distances (Nagai *et al.*, 2007). It was suggested that populations from Hiroshima and Sendai Bay, separated by about 1000 km, were unusually similar. These authors suggested that translocations of oyster spat, which occur between these two regions, may be a possible vector for human-assisted translocation. They concluded that further molecular genetic studies would be necessary to substantiate these hypotheses.

The case of the possible introduction or spreading of *A. minutum* in European waters has similarly been complex to decipher. In the Mediterranean, populations of *A. minutum* have been shown to be highly genetically diverse and structured, with apparently relatively low levels of gene flow between them (Casabianca *et al.*, 2012). These authors analyzed connectivity of the *A. minutum* populations in the Mediterranean and compared them to ocean circulation patterns. The combined evidence suggested that dispersal of this species broadly followed the main oceanic circulation patterns of this region, suggesting an indigenous population. *A. minutum* was thought to have been introduced to the North Sea in the mid-1980s, likely from the Mediterranean. This analysis was based on decadal data sets of phytoplankton monitoring using light microscopy, which first documented *A. minutum* as being present in the mid-1980s at the North Sea monitoring sites (Nehring, 1998). Genetic evidence based on microsatellites of *A. minutum* from regions all over the world showed that populations of *A. minutum* from England, Ireland, and France, representing the Irish Sea and English Channel regions with connectivity to the North Sea, formed a highly distinct cluster, not closely related to the Mediterranean populations (McCauley *et al.*, 2009). This would suggest that the appearance of populations in the German Bight regions of the North Sea may represent a range expansion of this species from other regions of the North Sea, which may have been related to factors like current patterns and increasing water temperatures (Nehring, 1998), rather than a human-assisted translocation of the species.

13.4.3 *Gymnodinium catenatum* in Australia and Europe

The species *Gymnodinium catenatum*, and its proposed introduction to several regions in the world, is one of the better known examples of a new phytoplankton species introduction, and it has been discussed extensively in the literature (Hallegraeff and Bolch, 1992). While some lines of evidence support the recent introduction of *G. catenatum* to some regions, for others, evidence has been equivocal. In Australia, *G. catenatum* was first recognized as a problem species in 1986, when it caused cases of paralytic shellfish toxins in shellfish, leading to the closure of shellfish farms for several months. A survey conducted of 11 Tasmanian estuaries, looking for cysts in sediments, found that *G. catenatum* cysts were present in the Huon and Derwent estuaries, as well as Spring Bay and Georges Bay, sites on the east and south coasts; but they were absent from five sites on the north and west coasts (McMinn *et al.*, 1997). A study of four cyst cores from Deep Bay, in southern Tasmania, found that cysts were present in the most recent sediments, and absent from those deeper sediments, dated at earlier than ~1972 (McMinn *et al.*, 1997). At the time, *G. catenatum* was thought to be absent from mainland Australia, and it has more recently been found present in South Australian and New South Wales waters (Bolch and de Salas, 2007). This cyst evidence in particular indicated that this species had been recently introduced to Tasmanian waters, possibly by means of ballast water translocation, as shipping to Tasmania from four Japanese ports, in regions where *G. catenatum* can be abundant, began in the 1970s. Studies to determine the viability of *G. catenatum* cysts in ballast water, and whether or not they were present, confirmed that they were present and viable in the ballast water tanks of some ships from Japan (Hallegraeff, 1998).

A population genetic study on 24 strains of *G. catenatum* from Australia, four Japanese strains, and seven strains from Spain and Portugal, based on randomly amplified polymorphic DNA (RAPD), showed that Australian strains were separate from those of both Spain and Japan (Bolch *et al.*, 1999). Geographic and temporal clustering of *G. catenatum* from Tasmania showed that genetic exchange between subpopulations appeared to be limited, and that blooms were composed of localized, estuary-bound populations. This evidence could neither confirm nor refute the hypothesis of a new introduction, or suggest an origin for the Tasmanian populations. Later work (Bolch and de Salas, 2007) detected a single nucleotide polymorphism among 63 rDNA-ITS sequences examined, separating *G. catenatum* strains into two rDNA-ITS ribotypes. Australian and New Zealand strains clustered together with a strain isolated from the Seto Inland Sea, Japan, separate to strains from other global populations. A re-examination of population genetics using a larger number of strains and broader and more variable marker regions such as mSATs, restriction site–associated DNA markers (RADseq) could help to resolve this question.

In European waters, *G. catenatum* was thought to have been first noticed along the west coast of the Iberian Peninsula (Portugal and Spain) in the mid-1970s (Ribeiro *et al.*, 2012). It was thought to have been introduced to the North Sea region around 1983, possibly from Spain (Nehring, 1998). This was based on long-term phytoplankton monitoring data, which did not document *G. catenatum* as being present in that region prior to the early 1980s. A survey of cysts from sediment cores from Portugal suggested that *G. catenatum* was first present ~100 years previously, in the first decade of the twentieth century (Amorim and Dale, 2006). As the cysts of several other cryptic but similar species of *Gymnodinium* can appear highly similar, it is possible that these records represented a similar species. A similar study of cyst cores from three areas along the west Iberian shelf showed that the earliest confirmed presence was ~1890–1950 (Ribeiro *et al.*, 2012). This would suggest that it is an invasive species in the region that has expanded its range northward, possibly from northern Africa, where it appears to have long been present, in recent decades.

In conclusion, a critical review of the evidence shows that many phytoplankton species may have been overlooked in certain regions if they are part of the "rare biosphere" in that region, and they likely have a wider distribution than has been previously thought. Therefore, baseline phytoplankton monitoring data are absolutely necessary, and this type of monitoring needs to be as detailed as practicable, preferably occasionally including new techniques such as environmental sequencing approaches where possible. Given the possibility that species may be present in a region even when they have not been reported before in routine monitoring, a precautionary approach to management is necessary. This would include: (1) not assuming that a harmful species is not present in a region, and therefore it is not necessary to monitor for it, even if it has not been detected in several years of previous data; and (2) controlling

all possible sources of translocations, and being mindful of the risk of range spreading of HAB species.

The option of not doing anything to curb harmful algal and aquatic pathogen translocations is no longer acceptable.

13.5 Prevention and Risk Reduction

13.5.1 Code of Practice on Translocation with Aquaculture Products

The 2005 *ICES Code of Practice on the Introductions and Transfer of Marine Organisms* primarily seeks to warn about the inadvertent consequences of introduction of new (or genetically modified) aquaculture species, but also warns against the impacts of translocation of unwanted contaminants such as pathogens. In Maine, United States, and Tasmania, Australia, shellfish relays can only be used to reduce bacterial contamination but not biotoxin contamination of shellfish, and require permits. These policies apply to all movements of bivalves from restricted, closed, or unclassified areas, but not to hatchery-reared spat or shellfish translocated from open harvest areas. In many countries (e.g., Australia), regional agreements are in place dictating that biotoxin-contaminated shellfish stocks cannot be translocated to non-affected areas, because of the risks of translocation of cysts and viable dinoflagellate cells.

13.5.2 Warning for HAB in Ballast Water-Uptake Zones and When Translocating Aquaculture Products

Prevention is better than to cure. The most effective measure to prevent ship uptake of HAB would be to avoid taking on ballast water during known harmful bloom events in the world's ports (International Maritime Organization [IMO] resolution A.868[20]). This has been applied, for example during the 1988 *Prymnesium* (*Chrysochromulina) polylepis* bloom in Norwegian coastal waters (K. Tangen, personal communication) and the 1993 *K. mikimotoi* blooms in New Zealand. Precautionary procedures also need to be developed when taking up ballast water in shallow areas with known sediment cyst beds of harmful species. For this purpose, Australia instigated an extensive monitoring program for toxic dinoflagellate cysts in major shipping ports. Any sediment-dredging or even jetty-building activities in Australia often involve a risk assessment that includes surveys for sediment toxicants (e.g., PCBs and heavy metals) but also dinoflagellate cyst beds.

13.5.3 Ballast Water Management

With the growing awareness of the problem of introduced marine pests in the past 30 years, a number of national and international regulations have been developed to reduce the risk of transfer of nonindigenous organisms. A critically important agreement to date has been the adoption in 2004 of the IMO's *International Convention for the Control and Management of Ship's Ballast Water and Sediments* (see www.imo.org). Comparable *Guidelines for the Control and Management of Ships' Biofouling to Minimize the Transfer of Invasive Aquatic Species (Biofouling Guidelines)* (Resolution MEPC.207[62]) are less advanced. The IMO's 2004 Ballast Water Convention prescribes strict performance standards for ballast water exchange (BWE; ≥95% volumetric exchange) to be exercised as an interim measure, as well as standards for ballast water treatment to be implemented in future. The IMO Convention only entered into force, however, after over 30 member states representing more than 35% of global shipping tonnage ratified. In September 2017, that is, over 10 years after its creation, this milestone was finally achieved with ratification from Finland. Of note is that the United States decided not to sign the IMO Convention, arguing that the U.S. Coast Guard regulations (https://homeport.uscg.mil) are even stricter. In essence, ships have the following options: (1) not to discharge ballast water; (2) discharge to another vessel or an onshore treatment facility; (3) use only water from a U.S. public water system; or (4) install a U.S. Coast Guard–approved ballast water management system, which upon discharge will not exceed 10 viable organisms per m^3 >50 µm minimum dimension (mostly zooplankton), not exceed 10 viable organisms per mL <50 µm and >10 µm minimum dimension (mostly phytoplankton), and not exceed 1 colony forming unit (dfu) per 100 mL of toxicogenic *Vibrio cholera*, <250 cfu of *Escherichia coli*/100 mL, and <100 cfu intestinal enterococci/100 mL. While a deliberate decision was made not to refer to target species in the zooplankton and phytoplankton size categories, the microbial standard is highly prescriptive and unachievable in a cost-effective way with currently available technologies, and hence will not be discussed here. Ballast water treatment technologies have been extensively researched in the past two decades (reviewed by Gregg *et al.*, 2009; updated by California State Lands Commission, 2013), and also serve as a guide toward suitable treatment technologies for aquaculture products, sediment dredging, and so on.

Table 13.1 Removal efficiency of microalgae by seawater filtration.

Filter type	Filter screen/nominal pore size (μm)	Removal efficacy (%)	Net lost flow due to backwash (%)
	25		10.6–21.2
Screen filters		99 (dinoflagellates)	
		37.5 (total phytoplankton)	
		88 (particles 25 μm or greater)	
	40	88.7 (particles 40 μm or greater)	N/A
		91 (dinoflagellates) 8.3 (total phytoplankton) 50–70 (particles greater than 50 μm) 91.9 (particles 50 μm or greater) Ineffective against *Phaeocystis globosa* (4–6 μm cell diameter; 20–500 μm colony diameter) Ineffective (particles >2–<63 μm)	6.8–13.5
Disk filters	55	80 (organisms >50 μm)	N/A
Crumb rubber	>500 (media size)	86.8 (particles 10 μm or greater)	N/A
		93.6 (particles 15 μm or greater)	
		51.7 (particles 2 μm or greater)	
		70 (phytoplankton); 45 (zooplankton)	

Source: Adapted from Gregg *et al.* (2009).

Specific treatment technologies examined for microalgae include mechanical separation (Table 13.1), heat treatment (Table 13.2), UV irradiation (Table 13.3), cavitation, de-oxygenation, and active substances (Table 13.4). To date, no single treatment option has proved to be universally effective, and increasing attention has focused on multi-component treatment systems comprising multiple technologies. The high flow rates during deballasting (1000 and 10,000 m^3/h) and the volumes of ballast water (typically, 25,000 to 80,000 tonnes) that must be treated pose significant technological challenges, and the presence of sediment in ballast tanks reduces the efficacy of many treatment options as this provides a habitat for resting stages of phytoplankton. Mechanical separation devices would best be used as a primary stage of a treatment system comprising multiple technologies because free-living organisms and sediment below a certain size are likely to be largely unaffected (Table 13.1). UV treatment systems are unlikely to eliminate all ballast water organisms, as they are not able to deliver a stable lethal dose across a wide range of water quality conditions and many organisms are resistant to UV exposure or can recuperate after treatment (Table 13.3; Buma *et al.*, 1995). At the current stage of development, cavitation would not be considered appropriate for the shipboard treatment of ballast water due to high capital and operating costs and high power requirements. The heating of ballast water using waste heat from ships' engines has been demonstrated to be a practical and cost-effective treatment option for eliminating ballast water zooplankton and phytoplankton (including resting stages) (Table 13.2). For example, 30 to 90 s exposure to temperatures above 40 °C were effective in killing cysts of the dinoflagellates *G. catenatum* and *A. tamarense*, whereas temperatures as low as 35 to 38 °C were sufficient after 4 h of heating (Hallegraeff *et al.*, 1997). These laboratory findings were confirmed in full-scale shipboard trials, where the ship's pipework was modified to enable waste heat from the main engine cooling circuit to heat the water in one of the ballast tanks by flushing with the heated water, which reached 37–38 °C (Rigby and Hallegraeff, 1994; Rigby *et al.*, 2004). Concerns have been expressed that attainable temperatures (40–45 °C) may not eliminate bacterial pathogens, and that this approach does not apply to ships traversing colder seas and may affect the integrity of vessel structures. Promising research has been conducted on several

Table 13.2 Effectiveness of heat treatment against microalgae.

Organisms	Species	Treatment
Diatoms	*Detonula pumila*	35 °C, 1 h
	Pseudo-nitzschia cuspidata	35 °C, 1 h
	Skeletonema costatum	35 °C, 1 h
	Thalassiosira rotula	35 °C, 1 h
	Amphora sp.	35 °C, 5 h
	Navicula sp.	35 °C, 5 h
	Navicula jeffreyi	35 °C, 5 h
Chlorophytes	*Dunaliella tertiolecta*	42.5 °C, 24 h
Raphidophytes	*Chattonella* sp. cysts	45 °C, 3 min
Picoplankton	*Nannochloropsis oculata*	53 °C, 100 sec
	Nannochloropsis oculata	42.5 °C, 24 h
Dinoflagellates	*Gymnodinium catenatum*	35 °C, 30 min
	Gymnodinium catenatum cysts	38–40 °C, 2 min
		44.5–46.3 °C, 30 sec
		37.5 °C, 1 h
	Alexandrium catenella cysts	42 °C, 30 min
		40 °C, 75 min
		38 °C, 4.5 h
	Alexandrium sp. cysts	45 °C, 3 min
	Scrippsiella sp. cysts	45 °C, 3 min
	Gymnodinium sp. cysts	45 °C, 3 min
	Protoperidinium sp. cysts	45 °C, 3 min
	Gyrodinium sp. cysts	45 °C, 3 min

Source: Adapted from Gregg *et al.* (2009).

systems that are able to achieve temperatures capable of eliminating bacteria (55 °C), but these technologies are still under development (Quilez-Badia *et al.*, 2008).

Biocide dosing systems (Table 13.4) have low capital costs and power requirements, but the costs of using active substances are significant (Gregg and Hallegraeff, 2007). Chemical treatment costs and space requirements can be significantly reduced by using onboard chemical generators, but the capital cost of these systems is significant,

Table 13.3 Effectiveness of UV irradiation against microalgae.

Organism	UV dosage	Efficacy (%)
Cyanobacteria	300–600 mWs/cm^2	90
Amphidinium sp., *Gymnodinium catenatum*	<50 mWs/cm^2	100
Skeletonema costatum	2.5 KW total output	100
Tetraselmis sp.	96–115 mWs/cm^2	87.6
Prorocentrum minimum	96–115 mWs/cm^2	84.7
Gymnodinium catenatum cysts	1600 mWs/cm^2	Ineffective
Chattonella cysts	30 lux	94
Scrippsiella cysts	30 lux	52
Phytoplankton	N/A	78

Source: Adapted from Gregg *et al.* (2009).

and all have biological efficacy, safety, operational, and environmental (poor biodegradation) concerns. Treatment systems that produce free hydroxyl radicals would be favorable over other chemical treatments as they are claimed to produce fewer or no toxic by-products at ballast discharge, but these technologies have high power requirements. Each treatment option requires further research on their biological and operational efficacy and safety under full-scale shipboard conditions and under a variety of environmental and source port water conditions. As of May 2015, 55 systems using active substances had received basic approval and 37 systems final approval from IMO, with 57 systems receiving type approval certification.

13.5.4 Other Precautionary Measures

Any commercial operation that seeks to pursue port dredging or translocation of aquaculture products needs to make the case that everything possible has been done to minimize risk (e.g., disposal of dredged sediments on landfills, and depuration of shellfish digestive tracts). Countries such as Australia and New Zealand maintain very strict quarantine conditions against the importation of microalgal culture strains from overseas collections. Each import application containing microalgae will be examined on a case-by-case

Table 13.4 Efficacy of chemical treatment against microalgae.

Treatment option	Organism	Treatment	Efficacy
Chlorine	Vegetative microalgae	1–100 ppm, 24–72 h	100% mortality
	Dinoflagellate cysts (*Gymnodinium catenatum*)	>500 ppm, 24 h	100% inactivation
Electrolytic chlorine	Phytoplankton	3–4 ppm	72% to >99% mortality
Chlorine dioxide	Vegetative microalgae	3–25 ppm, 30–120 min	100% mortality
	Dinoflagellate cysts	25 ppm, 2 weeks	100% inactivation
Chlorine dioxide generators	Vegetative microalgae	5 ppm, 24 h	99.6% mortality
Ozone	Dinoflagellates	>5 ppm TRO, 10 h	>99% reduction
	Microflagellates	>5 ppm TRO, 10 h	96–99% reduction
	Diatoms	>5 ppm TRO, 10 h	17–135% of initial concentrations
Hydrogen peroxide	Vegetative microalgae	3–100 ppm, 15 min–48 h	100% mortality
	Dinoflagellate cysts	100–10,000 ppm, 24–96 h	100% inactivation
Peraclean® Ocean	Vegetative microalgae	50–200 ppm, 48 h	100% mortality
	Dinoflagellate cysts	150–400 ppm, 2 weeks	100% inactivation
SeaKleen®	Vegetative microalgae	0.5–2 ppm, 24–48 h	100% mortality
	Dinoflagellate temporary cysts	2 ppm, 2 h	100% mortality
	Dinoflagellate resting cysts	6–10 ppm, 2 weeks (*Alex. catenella* no control at 10 ppm)	100% inactivation
Acrolein®	Phytoplankton	1 ppm	>99.999 reduction
Hydroxyl radicals	Unicellular algae, protozoans, bacteria	0.63 mg/L, 2.67–8 sec	100% mortality
Venturi Oxygen Stripping™	Phytoplankton	<1 ppm O_2, 120 h	100% mortality
pH adjustment	Dinoflagellate cysts	pH 2–10	No effect
Salinity	Dinoflagellate cysts	15–50 salinity	No effect
		100 salinity	No germination

Source: Adapted from Gregg *et al.* (2009).

basis. These regulations operate at the species level, but not at the genotype or strain level (e.g., highly toxic Chilean strains of *A. catenella* can be imported into Australia on the basis that this species already exists in Australia). Under 2015 Australian Quarantine regulations, any live water samples collected from >12 nautical miles offshore require a permit before they can be imported into a research laboratory. These restrictions do not apply to formalin or preserved microalgal samples or DNA extracts. In the United States, the importation of microorganisms (bacteria, fungi, viruses, algae, and protozoans) and infectious cell lines into the State of Hawaii requires an import permit from the Hawaii Department of Agriculture, but no such regulations appear to exist for imports into the U.S. mainland.

13.6 Emergency Treatment Options

Biocide dosing remains the only documented means for emergency treatment of newly arrived invaders. In March 1999, an infestation of the black striped mussel (*Mytilopsis sallei*) in Darwin, Australia, marinas was eradicated with chlorine/copper sulfate dosing at a cost of AU$2.2 million (Willan *et al.*, 2000). Similarly, Burson *et al.* (2014) killed the dinoflagellate *Alexandrium ostenfeldii* in

the enclosed Ouwerkerkse Kreek by dosing with 50 mg/L hydrogen peroxide. A successful application of heat treatment was undertaken to "clean" oyster spat from contaminating dinoflagellate cysts during the 2000 New Zealand *G. catenatum* bloom. To prevent the spreading of the New Zealand freshwater diatom pest *D. geminata*, strict quarantine protocols apply to researchers working in affected streams, and to fishermen who could carry viable microalgae on wading equipment. This includes soaking equipment for >1 min in 60 °C water, 2% household bleach, 5% salt solution, nappy cleaner, and antiseptic hand cleaner or dishwashing detergent (www.biosecurity.govt.nz/didymo).

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14

Culture and Culture Collections

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14.1 Introduction

Isolating and perpetuating cultures of harmful algal species is not fundamentally different than doing so for nonharmful taxa. Excellent references with robust protocols are available in print and on the internet (see the "Further Reading" section). Nevertheless, scientists and government agency personnel who are not familiar with standard microalgal isolation and culturing protocols may find themselves in the position of wanting to capture a harmful or putatively harmful organism for subsequent identification, chemical analysis, or biological study. This short chapter is for such individuals. This chapter will not serve as a substitute for specialized training in microalgal cultivation, but it is intended as a brief overview of considerations that may help a layperson to avoid common pitfalls on the way toward capturing and keeping alive a harmful alga until specialists can take over. It is divided into steps in the sequence of sampling, locating, perpetuating, and isolating an organism in a natural body of water.

14.2 Step 1: Sampling the Environment

The two most common reasons for sampling a body of water for harmful microalgal taxa are: (1) suspicion that a harmful organism is present, or (2) curiosity about the possibility that harmful taxa may be present. The difference may seem arcane, but essentially the first reason implies a forensic approach, whereas the second represents essentially a monitoring survey.

a) *Forensic approach.* Certain features of the environment, for example dead or moribund sea life or discolored water, suggest that a harmful microalga is present. The first pitfall in responding to environmental cues is that the responsible organism may be gone from the environment by the time the effects are apparent. The second pitfall is that the most abundant phytoplankton taxa may not include the causative organism. Finally, some environmental or biological phenomenon other than a harmful alga may be responsible for the observed ecological perturbations. Attributing response to a specific stimulus in aquatic ecosystems is not straightforward, and it is not uncommon for the public and even nonspecialist technical people to jump to the conclusion that a harmful algal bloom (HAB) is occurring when that is not the case. It is of fundamental importance to keep an open mind when following up on suspected HAB events and to understand that cause and effect may be impossible to establish. That said, what procedures and precautions can serve to increase the chance of capturing a harmful alga should one be present at an affected site?

Harmful Algal Blooms: A Compendium Desk Reference, First Edition. Edited by Sandra E. Shumway, JoAnn M. Burkholder, and Steve L. Morton.

i) *Whole-water sampling.* By far, the preferred method of sampling water to capture a harmful microalgal organism is to collect water rather than filtrate. Harmful taxa may be quite small or very delicate and easily damaged by plankton netting. Some known harmful taxa such as the brown tide organisms are smaller than commonly used plankton nets. A container to collect water may be as complex as a remote, automated sampler or a tethered, oceanographic sampling device (e.g., a Van Dorn or Go-Flo bottle), or as simple as a bucket or a bottle. Regardless, the collection container should be clean and free of detergents or other chemical residues that could harm collected organisms. As the microbiology of the water to be sampled is unknown, wearing water-proof gloves will protect hands from risk of skin exposure. The first step to use any container is to rinse it several times with the water to be collected so that any residues are rinsed away immediately before sampling. Automated or tethered samplers should be lowered to the depth at which the harmful species is suspected to be present and tripped to sample. Surface sampling – by far the most common approach when one is responding to an unexpected event – should be done without actually capturing the surface film of the water (i.e., submerge the bottle or bucket upside down, and then tip the container to allow it to fill with water from below the water surface). The reason for this precaution is that many airborne and surface-associated particles and organisms may be present at the water surface that could interfere with detection of truly planktonic microorganisms. As the size of most harmful microalgae is smaller than 150 µm, it is customary, and good practice, to pass the water through a 180–200 µm screen by pouring gently through a screen above another receiving container. This post-collection screening can remove grazing zooplankton that, if left in the water, may eat the relevant microalgae before they are isolated. Often, however, a screen is not available, and this step in the protocol is relevant to selecting conditions under which to transport the sample from the field to a laboratory or technical center for next steps. If the sample can be screened, generally it is better to maintain the sample at the temperature of the water when sampled. If, however, no screen is available, it is preferable to chill the sample in a cooler with ice or cold-packs to slow grazing, thereby protecting the organism of interest from being eliminated.

ii) *Plankton-net sampling.* Many protocols for harmful algal sampling (e.g., for toxic *Alexandrium* spp.) use a specialized device called a plankton net. A plankton net is pulled through the water, allowing water and small particles to pass through the net while capturing particles larger than the net mesh size, which generally is in the range of 5–30 µm. The advantage of using a plankton net is that relatively large volumes of water are sampled so that rare organisms are captured and concentrated. The disadvantages, as alluded to in this section, are that species smaller than the net size or those easily damaged may not be captured or may be collected too badly damaged for subsequent cultivation. If a plankton net is used as the first step in capturing and perpetuating a microalgal organism, similar guidelines concerning coarse screening to remove grazers (now also concentrated by the net) and transport temperature are recommended.

b) *Survey approach.* In most cases, monitoring or survey activities are designed to detect the presence of harmful algae in an environment, not to capture and cultivate living microorganisms. Environment sampling may emphasize spatial coverage and may focus on specific organisms. As with the forensic approach, whole-water or net sampling may be applied. If chemical preservation (e.g., iodine, formalin, or alcohol) of samples immediately after collection is part of the protocol, obviously this needs to be skipped if capture of live organisms is desired. Precautions mentioned in this section involving cleaning and rinsing in local water of sampling and transport containers – generally, not part of a survey protocol – should be implemented, as should predator screening and temperature choices if available. Overall, the objectives of detecting one or more species or capturing living individuals are different, so thought should be given to modifying sampling and transport protocols to maximize the chance of delivering living cells to the laboratory rather than achieving quantitative environmental sampling objectives.

14.3 Step 2: Processing a Field Sample in the Laboratory to Confirm Presence of the Target Organism

Whether collected by you or by someone else, a sample from which one wishes to isolate a living microalga should be processed immediately upon arrival. Processing may follow one or several of the options mentioned in this section; often, it is advantageous to try several approaches simultaneously, multiplying the potential for serendipity. Regardless, the first step should be microscopic examination of the sample and assessment of the 5–10 most abundant protistan and multicellular organisms (bacteria are likely to be more abundant than the protists, but it is impractical to assess them quantitatively with a simple, light microscope). Of course, the main objective is to recognize taxa that may be harmful, but note also should be made of potential grazers, including ciliates and other protozoans. If a putatively harmful taxon is recognized, take note also of microalgae of similar size. To proceed, one needs a target organism to perpetuate and an assessment of the co-occurring competitors and predators that will need to be removed or managed in the near term. Without a microscope, some of the subsequent steps still can be taken, but this effort may be futile if a target organism is not present in the water sample.

a) *Selective filtration.* As mentioned, screening out large and possibly predatory organisms is an important strategy to protect the target alga. Pouring the water through a screen with a mesh size in the range of 180–200 µm into another container is a good general precaution, but if the identity and size of the target alga are known, a mesh closer to, and larger than, the target alga will serve to remove both competitors and predators. In these examples, the intention is to allow the target organism to pass through the mesh while removing larger organisms.

   If, in contrast, the target alga is larger than most co-occurring microorganisms, concentrating the target organism by retaining it on a mesh may be a good strategy. To do this, rather than pouring the sample through a mesh, one can minimize physical stress on the target organism by suspending the mesh within a container and gently filling the mesh "liner" with the sample. Then the mesh is lifted gently within the container so that the volume of water within the mesh is a fraction of the original volume (the rest of the water volume having passed gradually through the mesh). One then pipets a smaller volume from inside the mesh to another container.

b) *Incubation.* Following screening, or without screening, the simplest way to try to perpetuate a target HAB organism is to incubate the sample under conditions conducive to its survival and possibly growth. A common mistake at this step is to assume that increased light and cooler temperatures will increase chances of survival. If no lighted incubator is available (and even if one is), the most conservative approach to lighting is to keep the sample on a north-facing windowsill that receives no direct sunlight. This exposes the target organism to a natural light regime and sufficient, but not excessive, light for photosynthesis. Direct sun on a small container with a relatively short light path often will overwhelm the photosynthetic capacity of most microalgal cells and lead to photoinhibition, reactive-oxygen generation, and mortality. Thus, if a lighted incubator for microalgal culture is available, it is best to incubate a field sample in a darker location of the incubator or with a mesh screen (black plastic window screen works well), so that the photosynthetically active radiation (PAR) level is in the range of 70–100 $\mu Em^{-2}/sec^{-1}$. Temperature near the range at which the target organism was collected is recommended to maximize its ability to compete with co-occurring microalgae.

   It should be understood that the most likely outcome of this initial incubation is that the target organism will disappear, either through predation by grazers or by being outcompeted by other, faster growing microalgae. Thus, an initial incubation should be considered a high-risk and temporary step in isolation for perpetuation.

c) *Enrichment.* A very intuitive approach to perpetuating a recently collected target microalga is to enrich the sample with nutrients at or near levels found in culture media, such as f/2. Unfortunately, this strategy works only when the target organism is the fastest growing component of the mixed microalgal community, which is the case very seldom. Nutrient enrichment is not recommended prior to effective removal of competing microalgae.

d) *Bulk separation.* This category is presented to include sorting methods other than sieving with mesh, specifically focusing on behavioral

responses of target HAB taxa to gravity and/or light. The simplest example is to allow non-motile organisms, such as diatoms, to sink, then collect them from the bottom of a container. A sometimes effective application of this approach is to settle a field sample in the dark in a cone-bottomed tube, pipetting the settled cells from the tip of the cone at successive time intervals, thereby enriching the "heaviest" cells first. If, in contrast, the target organism is less likely to sink than contaminating organisms, settling can be used to enrich the target organism in the overlying water. Settling can be used with the addition of light to further enrich the target organism. Some motile HAB taxa, especially dinoflagellates, are known to exhibit strong phototaxis, swimming toward a light source or collecting at the water surface (at the meniscus of a tube) in response to side or above lighting. As with sinking, sampling a lighted surface or the meniscus periodically following establishment of the light regime is recommended.

e) *Microscopic cell picking.* This is the most time-honored method for isolating clonal cultures of microalgae; it is described in detail in volumes listed in "Further Reading." Briefly, a light microscope and very fine pipets (handmade or manufactured) are used to capture a single cell for transfer into culture medium. Important considerations for picked cells relative to the receiving container are: the receiving container should contain only a small volume of medium, the medium should not be over-enriched with nutrients, incubation should be in dim light at moderate temperature, and containers should be observed frequently to monitor survival and any cell division. Flat-bottomed, 96-well or 48-well plates are well suited for receiving containers as many picked cells can be handled and managed together, and the flat bottom prevents trapping of cells in a conical bottom that inhibits any motility and access to nutrients. Generally speaking, half of single-cell picks inadvertently include other, contaminating microalgae that eventually overgrow the target species, and the other half end in mortality of the picked, target cell. A rule of thumb is that 1/100 (or 1%) of picked, single-cell isolations is successful in yielding a unialgal, viable, dividing population. This statistic underscores the need to invest in many replicate picks to increase the odds of success. Plates should be incubated with covers in place to minimize evaporation that can increase salinity rapidly when small volumes of water are involved.

Another oft-described step in microscope cell picking is referred to as *washing*. An individual, target cell is picked by pipet and placed in a small volume (200–500 µL) of sterile seawater, then picked from this container and placed in another well of sterile seawater, and this process is repeated until, theoretically, all contaminating microorganisms have been "diluted" out so that the final deposition of the target cell is free of contaminating organisms. Although often described, this method has yielded very limited success in our experience.

f) *Semisolid-medium isolation.* This approach also is described in great detail in the references in the "Further Reading" section. Of note is that spreading a mixed assemblage of microorganisms on a semisolid medium and picking a single colony for subsequent cultivation comprise a long-time standard method in bacteriology. This approach works best for microalgae that are meroplanktonic – having both benthic and planktonic association – such as pennate diatoms and some cyanobacteria. Dinoflagellates tend to perform poorly on agar, though, so application is limited for many prominent HAB taxa.

g) *Advanced cell sorting.* Analytical applications of flow cytometry to aquatic and marine microbiology have increased over the past 2–3 decades, leading to increased availability of instruments that may include physical sorting capabilities in environmental laboratories. A detailed description of flow-cytometric cell sorting is beyond the scope of this brief overview, and often only a specially trained individual is permitted to operate a cytometer in many institutions.

Instrument operator guidelines for successful single-cell isolations of microalgae are as follows: check that the target organism is being "gated" for sorting by depositing a few cells on a microscope slide prior to sorting into liquid medium; if possible, sort into 96-well plates containing 100–200 µL of sterile seawater or medium per well; adjust the sort option to place a single cell in each well; adjust cytometer settings to maximize selectivity, rather than ensuring that each sorted droplet contains a cell; sort at the lowest nozzle pressure possible; mix the source tube often to keep target cells evenly dispersed in the sample; and sort at least several plates (several hundred wells) to

improve the odds of obtaining a viable isolate. Incubation criteria and precautions detailed in this chapter should be implemented. For comparison, as many as 50% of wells in a successful sort may yield viable, growing cultures, so this is a case in which the technology provides a genuine benefit.

h) *Hatching cysts.* Many cultures of cyst-producing dinoflagellates have been isolated by incubating sediment samples under conditions conducive to excystment. Such conditions may be as simple as exposure to light at slightly elevated temperature. Alternately, a sediment sample can be sieved to retain cysts of known dinoflagellate taxa (e.g., with a 20-µm mesh for *Alexandrium* cysts), followed by excystment and isolation of single cells using the methods described here.

14.4 Step 3: From Spark to Flame

The description of pitfalls and low odds of success given here should present the strong impression that a dividing population of unialgal cells is a very precious miracle that needs careful attention to nurture. Common pitfalls beyond this point are under-inoculating scale-up cultures and over-lighting, as described. To avoid under-inoculation, a good rule of thumb is to double the volume of a growing culture, but certainly to not dilute the inoculum by more than 5×. For most HAB taxa, division times tend to be in the range of 2 days; therefore, doubling volume can be done every 2–3 days until sufficient volume is reached to move to a maintenance routine. A 5× dilution provides approximately a week of growth before cell division slows.

This brings to mind a note about expectations for final cell densities of HAB taxa before cell division ceases. Some HAB taxa are genuine "weeds" that may reach 10^5–10^6 cells mL^{-1}, as do aquaculture species, but many, especially dinoflagellates, may cease to grow beyond 10^3–10^4 cells mL^{-1}, regardless of nutrient availability, possibly because of autotoxicity. A culture that takes more than 5 days to divide can be considered to be in the wrong conditions (in terms of salinity, light, nutrients, etc.), to be stressed, and to be at risk of loss. As mentioned, matching the salinity, temperature, and light regimes of the environment from which it was isolated is good practice to perpetuate any algal isolate. For nutrients, f/2 generally is excessive, and further dilution to f/4 or even f/10 is used often with HAB cultures. Finding conditions supporting sustainable perpetuation of a new algal isolate is a challenge and is well served by placing many replicate cultures in different situations to increase the chance of happening upon an acceptable set of conditions. Certainly, experiments can be designed to define optimal conditions for a culture, but these require dependable inoculum cultures that must be produced in the absence of experimental results. In other words, subjecting newly isolated cultures to ranges of conditions and relying upon chance are realities with new isolates.

14.5 Step 4: Long-Term Perpetuation of HAB Cultures

Again, it is beyond the scope of this chapter to describe fully procedures and skills needed for long-term perpetuation of microalgal cultures; therefore, this section will highlight special precautions that should be observed with HAB taxa. In the United States, the National Science Foundation assists in the funding of two culture collections, the University of Texas Culture Collection (UTEX) and the Provasoli-Guillard National Center for Marine Algae and Microbiota (NCMA) at Bigelow Laboratory for Ocean Science. The UTEX collection contains over 3000 strains of algae representing 200 different genera with a focus on freshwater and soil algae. This is the main collection of harmful freshwater algae in the United States. The NCMA collection contains over 2700 strains, including over 280 known toxin-producing species. Both collections have strains either cryopreserved or actively growing. These collections may be willing to access new isolates of harmful algae; they should be contacted once a confirmed unialgal, actively growing culture is available for shipping by mail or commercial carrier (Table 14.1).

Perhaps the most difficult aspect of perpetuating cultures of some HAB microalgae involves sexual reproduction, especially in dinoflagellates and diatoms. Cyst-forming dinoflagellates, such as *Alexandrium* spp., spend most of the year in nature as relatively inactive, benthic cysts. In spring or summer, excystment occurs, and for a short period vegetative cell division occurs in the plankton. After some number of cell divisions, 2 N vegetative cells transform into gametes that mate and form planktonic zygotes that sink and transform into resting

Table 14.1 Institutions with Culture Collections.

Culture Collection	Address	Website
University of Texas Culture Collection (UTex)	University of Texas at Austin 205 W. 24th St. Biological Labs 218 Austin, TX 78712 USA	https://utex.org
National Center for Marine Algal and Microbiota (NCMA)	Bigelow Laboratory for Ocean Science 60 Bigelow Drive East Boothbay, ME 04544 USA	https://ncma.bigelow.org
American Type Culture Collection (ATCC)	10801 University Boulevard Manassas, VA 20110 USA	https://www.atcc.org
Australian National Algae Culture Collection (CSIRO)	Private Bag 10 Clayton South VIC 3169 Australia	http://www.csiro.au/en/Research/Collections/ANACC
Culture Collection of Algae and Protozoa (CCAP)	Scottish Marine Institute Argyll PA35 1QA Scotland, UK	http://www.ccap.ac.uk
Freshwater Algae Culture Collection (FACHB)	Institute of Hydrobiology No. 7 Doughu South Road Wuchang District, China	http://algae.ihb.ac.cn/English/
Norwegian Institute for Water Research (NIVA-CCA)	Gaustadalléen 21 Oslo 0349 Norway	https://niva-cca.no
Scandinavian Culture Collection of Algae and Protozoa (SCCAP)	University of Copenhagen Oster Farimagsgade 2D DK-1353 Copenhagen K	http://www.sccap.dk

cysts again. In some taxa, gametes are homothallic and dioecious, that is, morphologically indistinguishable, and compatible "plus" and "minus" strains are necessary for successful mating. In culture, inadvertent transformation of vegetative cultures to gametes, followed by unsuccessful mating or production of nonviable zygotes, can occur, leading to loss of isolates. Thus, for *Alexandrium* and related dinoflagellates, successful long-term perpetuation of populations in culture may require cultivation of multiple isolates and periodic mating to pass the organism through the cyst stage to re-establish vegetative populations. As there are no morphological cues related to mating compatibility, finding compatible strains is relegated to trial and error, and only after at least one successful mating can one have confidence in the long-term sustainability of a mating population.

Some diatoms, including toxic *Pseudo-nitzschia* spp., also have a sexual stage, necessitated by reduction in mean cell size during vegetative cell division. It is even more difficult to have compatible strains for mating in the same stage of sexual development for diatoms compared to dinoflagellates; therefore, it is not uncommon for harmful diatom strains to have limited longevity in culture.

Beyond challenges related to sexual life-history stages, establishing subculture regimes for HAB taxa can be more challenging than for aquaculture strains. HAB strains may grow slowly and to lower final cell densities than aquaculture algae, suggesting longer times between subcultures, but some strains are very intolerant of stationary-phase conditions and die very quickly once they stop dividing actively. There has been a suggestion that programmed cell death may play a role in culture "crashes," so a rule of thumb is to keep HAB cultures growing actively to maintain them in viable condition.

14.6 Epilogue

Despite low odds for successful isolation and particular challenges to long-term perpetuation, microalgal strains isolated from natural HAB events and perpetuated for decades have made enormous contributions to the growing scientific

understanding of the physiological ecology of HAB organisms. Because of recurring suspicions that there are significant differences in environmental tolerance, toxicity, and bioactivity between "strains" or local populations of HAB taxa, it can be worth the effort and an important contribution to science to capture, isolate, and cultivate HAB microalgae for experimental research. In this chapter, we provide guidance on early steps and identify issues to be considered for successful perpetuation of harmful algal isolates. We stress, however, that the knowledge, skills, and experience of a specialist should be mobilized as soon as possible in projects involving high-profile blooms that affect public and environmental health.

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15

Harmful Macroalgal Blooms in a Changing World: Causes, Impacts, and Management

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15.1 Introduction

Macroalgae are loosely defined as multicellular algae that are generally considered to have a simple plant-like body, or thallus, that is macroscopic (visible to the human eye) and ranges from simple to complex in structure (Graham *et al*., 2016). In freshwaters, where there are very few thalloid macroalgae, the term has been broadened to include filamentous, colonial, tuft-forming, crustose, tissue-like, coenocytic algae, or cyanobacteria that have forms recognizable to the naked eye (Sheath and Cole, 1992; also see, as examples, Burkholder, 2009; Fetscher *et al*., 2015). That definition is also used to include sheet-like, filamentous, coarsely branched, calcareous, and crustose algae in estuarine and marine environments as well. This review emphasizes macroalgae in U.S. inland, estuarine, and coastal marine waters, but examples from other nations are included to emphasize some points as appropriate. Macroalgae mainly include red, green, and brown taxa (Rhodophyta, Chlorophyta, and Streptophyta; and mostly photosynthetic stramenopiles [Heterokontophyta] – Class Phaeophyceae, respectively) (Graham *et al*., 2016 and references therein). An increasing number of freshwater, estuarine, and marine habitats also include certain harmful benthic mat-forming cyanobacteria (Cyanobacteria; or blue-green algae, Cyanophyta) as well.

In aquatic ecosystems, macroalgae are largely beneficial by providing important structural habitat for invertebrates and fishes (Dayton, 1985; Marx and Herrnkind, 1985; Holbrook *et al*., 1990; Pérez-Matus *et al*., 2008; Pérez-Matus and Shima, 2010; Lapointe *et al*., 2014), and a source of nutrition for herbivore and detrital food webs (Sammarco *et al*., 1974; Tenore, 1977; Lewis, 1986). Macroalgae are a natural, common feature of inland waters as well as estuaries, coastal waters, and (to a limited extent) oceanic waters (Bartsch *et al*., 2012), particularly the Gulf of Mexico, North Atlantic Ocean, and Caribbean Sea where pelagic *Sargassum* is distributed (Lapointe *et al*., 2014). When excessive growth and biomass accumulation occur from overstimulation by nutrient (primarily nitrogen [N] and phosphorus [P]) pollution and other human-related factors, however, algae are considered harmful because of the potentially severe environmental and economic impacts they can cause. Excessive biomass of macroalgae is commonly referred to as a *bloom*. Although many begin growth in a benthic habit, their mats often become dislodged and growth continues as floating mats, sometimes referred to as *metaphytic* (e.g., Hudon *et al*., 2014). As the causes and effects of macroalgal blooms are similar in many ways to those associated with harmful phytoplankton species, scientists use the term *harmful algal bloom* (HAB) (ECOHAB, 1995; HARRNESS, 2005) to describe this diverse array of bloom phenomena. The frequency and extent of macroalgal HAB have increased in estuaries and coastal waters throughout North America during the past five decades to now include all coastal states as well as Hawai'i (Figure 15.1); in addition, macroalgal HAB have become common in inland freshwater systems, including lakes, streams, rivers, springs, and reservoirs.

Harmful Algal Blooms: A Compendium Desk Reference, First Edition. Edited by Sandra E. Shumway, JoAnn M. Burkholder, and Steve L. Morton.

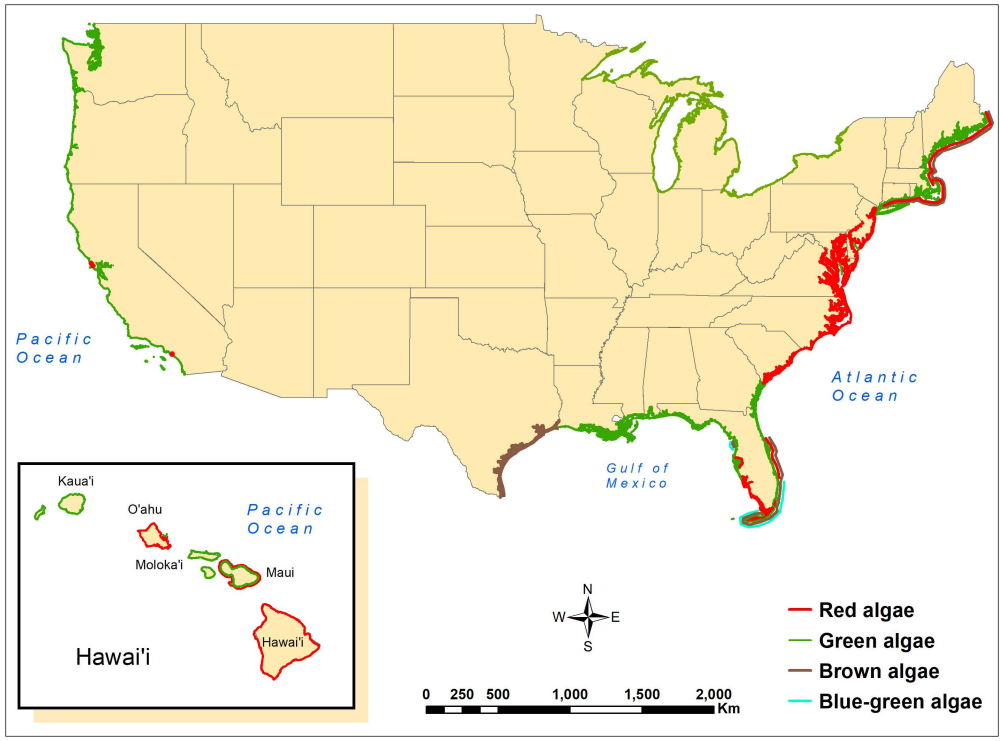

Figure 15.1 Map showing the distribution of estuarine and coastal marine harmful macroalgal blooms (by phylum) in North America and Hawai'i, also including freshwater taxa along the Laurentian Great Lakes.

Here, harmful macroalgal blooms are operationally defined in both ecological and socioeconomic contexts, as in Burkholder (2009). Macroalgal HAB cause undesirable ecological changes in habitats and food webs or, in some cases, produce potent bioactive substances that adversely affect beneficial aquatic life. From a socioeconomic perspective, the excessive biomass also causes undesirable effects for humans, such as decreased recreational uses of beaches and waterways (due to rotting biomass, offensive odors, reduced water clarity, fish kills, and reduced waterfront real estate values, and through provision of habitat for microbial pathogens, mosquitoes, snails as vectors for schistosomiasis, and other noxious species); increased fouling of pumps, filters, and intake pipes; taste and odor problems in drinking water supplies; increased costs of water treatment; increased costs of managing aquatic resources; and, less commonly, direct toxicity to humans and other animals (wild and cultured fish, larvae of commercially important shellfish, waterfowl, livestock, and domestic pets). Socioeconomic impacts also include losses of commercially important finfish and shellfish due to habitat loss and fouling of fishing gear.

15.2 Freshwater and Other Inland Macroalgae

The harmful macroalgae of freshwaters (wetlands, springs, streams and rivers, and lakes and reservoirs) and brackish to highly saline inland waters are low in diversity relative to estuarine and marine macroalgae, but most of the species are so widely distributed among the states that a map of their distribution is not included. Few species of red and brown algae inhabit freshwaters, and they tend to be restricted to nutrient-poor (oligotrophic) habitats (Graham *et al.*, 2016 and references therein). Thus, certain benthic cyanobacteria and green algae are the major macroalgal taxa in freshwaters that form noxious high-biomass blooms or outbreaks (Figure 15.2). In U.S. freshwaters, the noxious benthic bloom-forming filamentous taxa apparently are all native and mainly include the cyanobacteria *Lyngbya wollei*, *Oscillatoria* spp., and *Phormidium*/*Microcoleus* spp.; the green algae *Cladophora* spp. and *Pithophora oedogonia* (Chlorophyta, Ulvophyceae); and the higher green algae *Spirogyra* spp. (Streptophyta-Order Zygnematales).

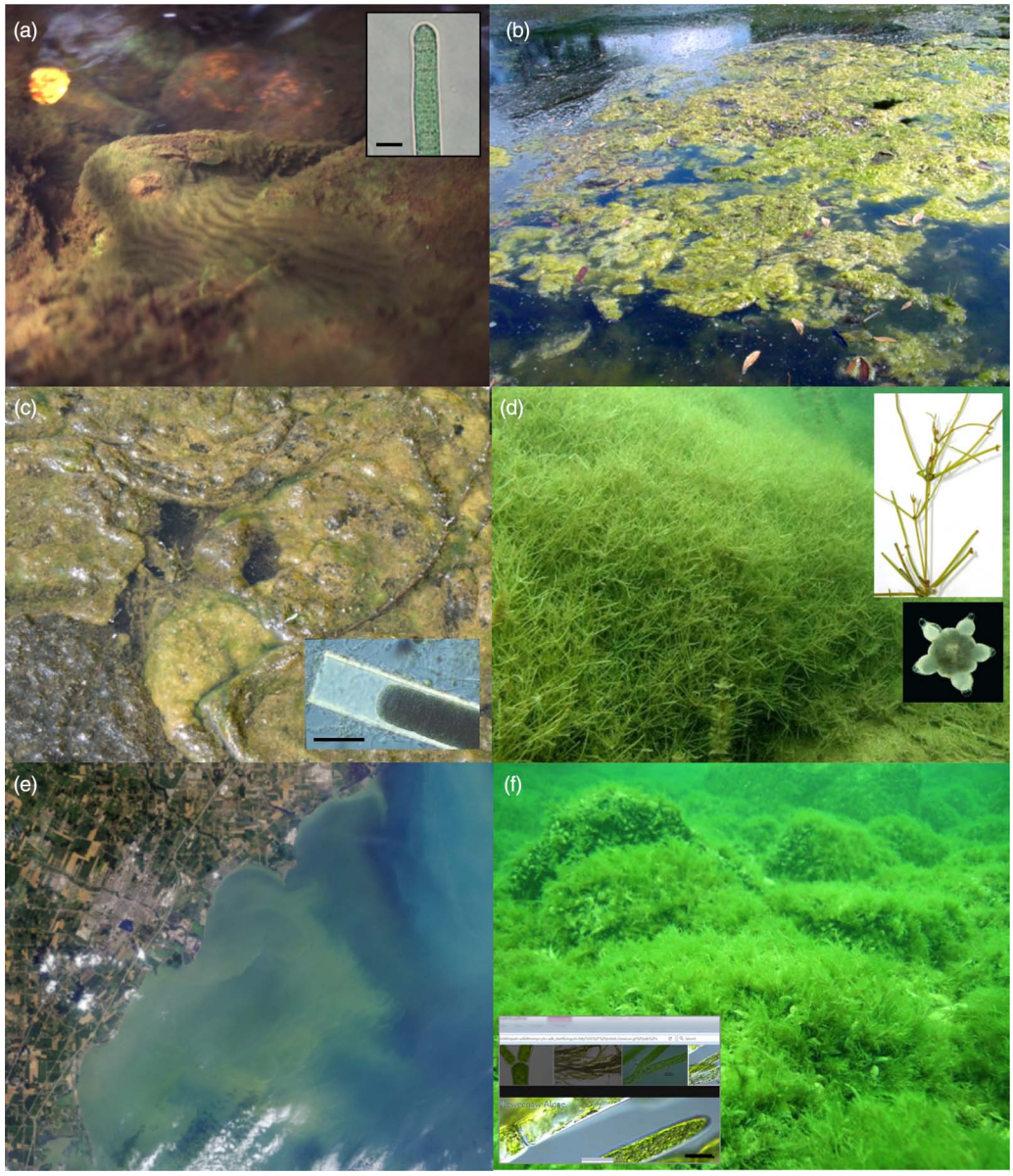

Figure 15.2 Examples of potentially harmful freshwater macroalgae: (a) The cyanobacterium *Phormidium* (both freshwater and marine) – benthic mat in a mildly acidic softwater stream (gold object in upper left is a floating leaf); insert is a light micrograph (scale bar: 20 μm). *Source*: Photos by J. Burkholder. (b,c) The cyanobacterium *Lyngbya wollei*: (b) Floating mats suspended from a benthic habit in a piedmont reservoir, North Carolina, USA. *Source*: Photo by E. Allen, NCSU Center for Applied Aquatic Ecology. (c) Closeup of floating mats mixed with some filamentous green algae (Chlorophyta). *Source*: Reprinted from Burkholder (2009, figure 9l), with permission from Elsevier. Insert is a light micrograph (scale bar: 50 μm). *Source*: Photo by B. Speziale, Clemson University, Clemson, South Carolina, United States, with permission. (d) Invasive starry stonewort (*Nitellopsis obtusa* – Streptophyta, Charales) in an inland lake in Michigan, United States. *Source*: Photo from E. Nat at the Robert B. Annis Water Resources Institute [AWRI], with permission. Courtesy of Ray Van Goethem, http://www.aquaticnuisanceplantcontrol.com/Algae-Album.html. Upper insert is a specimen from the Oneida Lake Education Initiative. *Source*: http://www.seagrant.sunysb.edu/oli/olei-stonewart.htm, with permission from E. Nat at the AWRI. Courtesy of Jeroen Huls, https://www.verspreidingsatlas.nl/2160. Lower insert is a star-shaped rhizoid (diagnostic, 1.5–2 mm in diameter). *Source*: http://deptsec.ku.edu/~ifaaku/jpg/Nat/Nat.html), with permission from the Nationaal Herbarium Nederland, the Netherlands. Courtesy of Emile Nat. (e,f) The filamentous chlorophyte *Cladophora*: (e) Satellite image of a major suspended and benthic bloom in the west basin of Great Lake Erie (Landsat natural color image). *Source*: Courtesy of the National Aeronautics and Space Administration, with permission. (f) Benthic overgrowth in Lake Michigan. *Source*: Photo courtesy of H.A. Bootsma. Insert is a light micrograph (scale bar, 45 μm – at http://www.keweenawalgae.mtu.edu/gallery_images/ulvophyceans/Cladophora_j74-1a_20125z.jpg. *Source*: Photo by J. Oyadomari, with permission.

The major thalloid green macroalgae in freshwaters are (1) the mostly beneficial charaleans (Streptophyta – Order Charales), which grow along shorelines of hardwater habitats (most species) or in mildly acidic softwaters; and (2) *Ulva* (Chlorophyta, Ulvophyceae – now including the former genus, *Enteromorpha*; Hayden *et al*., 2003), found in some brackish and highly saline inland waters, but much more prevalent in marine waters (below) (Burkholder, 2009 and references therein). Charaleans *Chara* and *Nitella* can sometimes become problematic in shallow waters when growth reaches the water surface and impedes human recreational uses (Lembi, 2003). Some *Ulva* spp. can grow in habitats spanning from freshwaters to salt springs and brackish lakes, to the Great Salt Lake, Utah, United States (salinity > 50) (as *Enteromorpha*; Flowers, 1934). A recent bloom of *Ulva flexuosa* (formerly *Enteromorpha flexuosa*) in Muskegon Lake, Michigan, United States, was described as invasive, and covered up to 80% of the littoral zone in some areas, mostly as epiphytic overgrowth (Lougheed and Stevenson, 2004). The affected lake had low grazing pressure, increased salinity from industrial discharge of chlorinated compounds, and a history of nutrient over-enrichment.

Benthic filamentous cyanobacteria, mostly the genera *Lyngbya*, *Oscillatoria*, and *Phormidium/Microcoleus*, commonly form nuisance or potentially toxic growth in inland waters worldwide (e.g., Figure 15.2). They can be both high-biomass and toxic bloom formers. The toxins can include microcystins, anatoxin-a, homo-anatoxin-a, aplysiatoxins, cylindrospermopsin, deoxy-cylindrospermopsin, dihydroanatoxin-a, dihydrohomoanatoxin-a, lyngbyatoxin, and saxitoxin analogs (Quiblier *et al*., 2013; McAllister *et al*., 2016). Five benthic *Phormidium* species and one recently renamed species (*Microcoleus autumnale*, formerly *Phormidium autumnale*; Strunecký *et al*., 2013) have been reported to be toxigenic thus far, including at least four of the six in North America (*M. autumnale*, *Phormidium corium*, *P. favosum*, and *P. tenue*; Tilden, 1910) (Quiblier *et al*., 2013 and references therein). Toxigenic benthic *Oscillatoria* species (e.g., *O. formosa* and *O. limosa*) are poorly identified and easily confused with *Phormidium* spp. (Quiblier *et al*., 2013 and references therein). Similarly, benthic *Lyngbya* spp. (with the exception of *Lyngbya wollei* – see Chapter 16 of this volume) can easily be confused with *Phormidium* and *Oscillatoria* spp., and are usually not identified to species.

Benthic filamentous cyanobacteria thrive in a wide array of habitats ranging from oligotrophic to eutrophic, including wetlands, lake littoral zones, wastewater ponds, hypersaline and geothermal ponds, streams and rivers, deep springs, saltmarshes, and seagrass meadows (Quiblier *et al*., 2013; authors' personal observation). These form cohesive mats that typically consist of a mixture of toxic and nontoxic cyanobacterial strains along with various other microbes (McAllister *et al*., 2016). The mats are often 1 cm or more in thickness (up to 70 cm thick; Dasey *et al*., 2005), so that the environment within the mat biofilm or "microscale ecosystem" (Quiblier *et al*., 2013) becomes distinct and somewhat isolated from that of the overlying water (Stevenson *et al*., 2007; Wood *et al*., 2015a). Although water-column nutrient concentrations (both N and P) during initial substratum colonization can strongly influence establishment and mat formation (Cowell and Botts, 1994; Stevenson *et al*., 2007; Wood *et al*., 2014, 2015b), the interior of the developed mat is characterized by steep chemical gradients that control nutrient uptake and recycling, which are largely independent from nutrient fluxes in the overlying water (Stal, 2012; Wood *et al*., 2015a).

Only sparse information is available about the environmental factors that control toxic, mat-forming benthic cyanobacteria. Sites with high *Phormidium* coverage in some rivers have been linked to high total N to total P (TN:TP) ratios, usually exceeding 20:1 (Wood and Young, 2012; but see Sabater *et al*., 2003; Vilalta *et al*., 2003). These sites also were characterized by elevated dissolved inorganic nitrogen (DIN = ammonium + nitrate + nitrite) concentrations (> 100 µg/L) and low water-column phosphate (< 10 µg soluble reactive phosphorus [SRP]/L), but had higher loads of fine sediments enriched with biologically available P (McAllister *et al*., 2016).

Harmful filamentous green algae generally thrive in shallow littoral areas of nutrient-rich (eutrophic) habitats (Burkholder, 2009 and references therein), and may be free-floating (e.g., *Pithophora oedogonia* and *Spirogyra* spp.) and/or attached to substrata (e.g., *Cladophora*). The most widely known of these are within *Cladophora* (Figure 15.2), which is the most widely distributed macroalgal genus throughout the world's freshwater ecosystems (Dodds and Gudder, 1992; Higgins *et al*., 2008). These species occur in many alkaline freshwater and brackish lakes and rivers, and in estuarine and marine waters. Some may be grazed when small or dislodged by some fauna, but they generally are considered a poor, nonpreferred food source (Zulkifly *et al*., 2013). These green algae have a relatively high light

optimum for photosynthesis and can rapidly acclimate to low or high light (Graham *et al*., 1982, 1996; Zulkifly *et al*., 2013). Maximum biomass or coverage of *Cladophora* has exceeded 900 g dry wt m^{-2}, and its filaments can be 0.5 m or more in length (Burkholder, 2009 and references therein). Worldwide, freshwater *Cladophora* thrives in P-enriched waters with dependable substrata (e.g., large boulders) for attachment (Pitcairn and Hawkes, 1973; Dodds and Gudder, 1992; Zulkifly *et al*., 2013 and references therein).

The noxious filamentous chlorophyte *Pithophora oedogonia* is restricted to freshwaters and thrives in shallow littoral areas of eutrophic habitats (Lembi *et al*., 1980, 1988). This species has low affinity for DIN (as nitrate) and SRP in comparison to *Cladophora glomerata*, but generally higher temperature tolerance and wider pH tolerance extending from alkaline to mildly acidic habitats. Spring growth begins in a benthic habit, and then oxygen bubbles trapped within the filaments carry them to the water surface where mats develop. Photosynthetic rates are positively correlated with external concentrations of both N and P (Spencer *et al*., 1985). This organism is well adapted to low light conditions and even extended periods of darkness, and can survive severe self-shading within the mats where about 95% of the available light is absorbed within the first 5 mm of mat thickness (O'Neal *et al*., 1985; Spencer *et al*., 1985). A computer model simulating growth dynamics of *P. oedogonia* in a north temperate lake predicted more biomass reduction from 50% lower nitrate concentrations than from a similar decrease in TP (Spencer *et al*., 1987).

Filamentous algae within the order Zygnematales are common bloom formers in alkaline and mildly to moderately acidic eutrophic freshwaters throughout the United States (Berry and Lembi, 2000; McKernan and Juliano, 2001 and references therein). Rapid overgrowth by *Spirogyra* spp. sometimes occurs in lakes, ponds, slowly flowing streams, and ditches affected by anthropogenic nutrient sources such as agricultural runoff or sewage effluent (McKernan and Juliano, 2001). Species within this genus can also be abundant in benthic habitats of large lakes; for example, Askari (1992) described a mat of *Spirogyra* that was more than 0.6 m thick washed up on the shores of Lake Huron. *Spirogyra* forms benthic and floating mats, and some species have responded rapidly to N and P enrichment (O'Neal and Lembi, 1988). This alga is less tolerant of low light than *Pithophora oedogonia*, and the mats tend to disintegrate under high temperature and high light, or in darkness (O'Neal and Lembi, 1988; Adrian, 1994; Graham *et al*., 1995 and references therein). The species *Spirogyra fluviatilis* can withstand current velocities up to 30 cm/sec, if phosphate is available to offset the apparent increase in cellular P demand (Borchardt *et al*., 1994). It can adjust short-term SRP uptake to compensate for the suboptimal conditions imposed by rapid flow. In Lake Baikal, a World Heritage Site where 3700 species live in the world's oldest, deepest, and most voluminous lake, extensive mats of *Spirogyra* have developed around urban areas as a result of inadequate sewage treatment (Nuwer, 2016). Various other filamentous and colonial chlorophytes can overgrow inland waters and adversely affect other aquatic life and local economies (Burkholder, 2009), exemplified by the taxa discussed above.

15.3 Estuarine and Coastal Marine Macroalgae

Estuarine and coastal marine macroalgae (seaweeds) are a rich, diverse group of macroscopic, multicellular organisms that grow along temperate, subtropical, and tropical coastlines (Taylor, 1960, 1972; Abbott and Hollenberg, 1976; Schneider and Searles, 1991; Littler and Littler, 2000; Gabrielson *et al*., 2006; Dawes and Mathiesen, 2008). Blooms of macroalgae can occur naturally, but have become increasingly common features of urbanized bays, harbors, and coastal waters where thick submersed mats are formed that can overgrow economically important coastal habitats such as seagrasses and coral reefs. Excessive biomass of these blooms can strand on beaches and along shorelines, where they decompose and become a public nuisance. These blooms transform coastal habitats and have myriad social and economic consequences. The occurrence of estuarine and coastal macroalgal blooms is increasing globally, and now affects virtually all coastal states within the United States, including Hawai'i (Smith *et al*., 2002; Anderson *et al*., 2008; Bricker *et al*., 2008; Table 15.1 and Figure 15.3). Estuarine and coastal macroalgae are eukaryotes within three phyla: the Chlorophyta (green algae), Rhodophyta (red algae), and Phaeophyta (brown algae). The Cyanobacteria (Cyanophyta, or blue-green algae), which are prokaryotic, unlike the other phototrophs included here, are also included because they include filamentous macroalgae, act similarly as other macroalgae from an

Table 15.1 Coastal macroalgal blooms reported for various locations within the continental United States and Hawai'i.

Location	Species	Impacts	Reference(s)
New England, mid-Atlantic U.S.	*Gracilaria vermiculophilla*	Non-native, high biomass, overgrowth of benthos	Freshwater *et al.* (2006), Saunders (2009), Nettleton *et al.* (2013)
New England	*Ulva compressa*, *Ulva lactuca*, *Ulva rigida; Codium fragile* subsp. *tomentosoides, Colpomenia peregrina, Grateloupia turuturu*	High biomass, toxin uptake, non-native, loss of shellfish habitat	Sawyer (1965), Thornber *et al.* (2008), Guidone and Thornber (2013), Cheney *et al.* (2014), Ramus (1971), Malinowski and Ramus (1973), Hanisak (1979), Mathieson *et al.* (2003), Green *et al.* (2012), Villalard Bohnsack and Harlin (1997), Mathieson *et al.* (2008)
Massachusetts	*Dasysiphonia japonica*	High biomass on beaches, non-native	Schneider (2010)
Nahant Bay, Massachusetts	*Cladophora* sp., *Pylailella littoralis*	High biomass on beaches, surf zone	Auer (1982), Wilce *et al.* (1982)
Waquoit Bay, Cape Cod, Massachusetts	*Gracilaria tikvahiae, Cladophra vagabunda*	High biomass, overgrowth of seagrasses, loss of shellfish habitat	Valiela *et al.* (1992, 1997), Peckol *et al.* (1994), Hauxwell *et al.* (1998, 2001a)
Jamaica Bay, New York	*Ulva rigida*	High biomass, loss of shellfish	Wallace and Gobler (2015)
Delaware and Maryland Coastal Bays	*Ulva lactuca*	High biomass, odors	Cole (2002), McGinty *et al.* (2002)
Indian River Lagoon, Florida	*Gracilaria* spp., *Ulva* spp., *Acanthophora, Caulerpa*	High biomass, overgrowth of seagrasses, shellfish and wildlife mortality	Benz *et al.* (1979), Virnstein and Carbonara (1985), White and Snodgrass (1990), Riegl *et al.* (2006), Lapointe *et al.* (2015)
Southeast Florida	*Codium isthmocladum, Caulerpa verticillat, Caulerpa racemosa, Caulerpa brachypus, Lyngbya* spp.	High biomass, overgrowth of coral reefs	Lapointe (1997), Paul *et al.* (2005), Lapointe *et al.* (2005a, 2005b), Lapointe and Bedford (2010)
Biscayne Bay, Florida	*Anadyoneme*, *Dictyota*, *Halimeda*, *Laurencia* spp.,	High biomass, overgrowth of seagrasses and corals	Lirman *et al.* (2008), Collado-Vides *et al.* (2011, 2013)
Florida Keys and Florida Bay	*Cladophora vagabunda, Dictyota* spp., *Halimeda* spp., *Laurencia* spp., *Spyridia filamentosa*	High biomass, overgrowth of corals and seagrasses, hypoxia	Lapointe *et al.* (1994), Lapointe *et al.* (2004, 2007), Smith *et al.* (2007), Green *et al.* (2015)
Bermuda	*Cladophora prolifera*	High biomass in Harrington Sound	Lapointe and O'Connell (1989)
Southwest Florida	*Gracilaria* spp., *Hypnea* spp.	High biomass, mass strandings on beaches	Lapointe and Bedford (2007)
Tampa Bay, Florida	*Chaetomorpha*, *Gracilaria,* Ulva *spp.*	High biomass, odors, overgrowth of seagrasses	Hagan (1969), Mangrove Systems (1985), Kelley (1995), Johansson (2003)
Texas and Florida	*Sargassum* spp. (drift macroalgae)	High biomass on beaches and coastal waters	Lapointe (1995), Gower *et al.* (2006), Kopecky and Dunton (2006)

San Juan Island, Washington	*Ulva lactuca* (reported as *Ulva fenestrata*), *Ulvaria obscura* (reported as *Monostroma fuscum*)	High percent cover, changes in infaunal abundances	Price and Hylleberg (1982), Bulthuis (1995)
Ship Harbor, Anacortes, Washington	*Ulvaria obscura*	Allelopathy (dopamine) – lab experiments	Van Alstyne *et al.* (2014)
Blakely Island, Washington	*Ulvaria obscura*, *Ulva lactuca* (reported as *U. fenestrata*), *Ulva linza* (reported as *Enteromorpha linza*)	Reduction in eelgrass (*Zostera marina*) shoot density, high biomass	Nelson and Lee (2001), Nelson *et al.* (2003)
Penn Cove, Coupeville, Washington	*Ulva lactuca*, *Ulva* spp.	Alterations of seawater pH and oxygen levels, high percent cover	Van Alstyne (2015), Van Alstyne *et al.* (2015)
Puget Sound, Washington	Ulvoid algae	High percent cover, noxious odor, hypoxia	Frankenstein (2000), Nelson *et al.* (2009)
Seahurst Bight Seattle, Washington	*Monostroma grevillei*, *Ulva lactuca* (reported as *U. fenestrata*), *Ulva linza* (reported as *Enteromorpha linza*)	High percent cover (in summer)	Thom and Albright (1990)
Grays Harbor, Washington	*Blidingia minima var. subsalsa/Ulva intestinalis* complex	High percent cover	Thom (1984)
Netarts Bay and Yaquina Bay, Oregon	*Ulva linza*, *Ulva lactuca*, *Ulva flexuosa*, *Ulva intestinalis*, *Ulva* spp.	High biomass	Davis (1981), Kentula and Dewitt (2003), Brown *et al.* (2007), Boese and Robbins (2008)
Yaquina Bay and Coos Bay, Oregon	Unspecified macroalgal species	Negative correlation between algal and eelgrass (*Zostera marina*) percent covers	Hessing-Lewis and Hacker (2013)
Coos Bay, Oregon	*Ulva* spp., *Ulva* spp. (reported as *Enteromorpha* spp.)	Negative effects on eelgrass (*Zostera marina*) in riverine, but not marine, sites; high biomass in summer months	Pregnall and Rudy (1985), Hessing-Lewis *et al.* (2011)
Bodega Harbor, California	*Ulva expansa*	Reductions in abundances of phoronids (*Phoronopsis)* and clams (*Macoma*)	Everett (1991)
Tomales Bay, California	*Gracilariopsis* sp.	Reduction of eelgrass (*Zostera marina*) shoot densities and growth rates	Huntington and Boyer (2008)
Elkhorn Slough, California	*Ulva* spp.	High biomass and percent cover	Schaadt (2005)
Huntington Harbor, California	*Caulerpa taxifolia*	Invasive, high biomass	Williams and Grosholz (2002)
Upper Newport Bay, California	*Ulva intestinalis* (reported as Enteromorpha intestinalis), *Ulva expansa, Ceramium* spp.	High percent cover	Kamer *et al.* (2001)
Magu Lagoon, Ventura County, California	*Ulva* spp.	Alter macrofaunal distributions and shorebird foraging behavior	Green (2011)
Mugu Lagoon, Tijuana River Estuary, and Upper Newport Bay, California	*Ulva intestinalis, Ulva expansa*	High percent cover	Kennison and Fong (2014)

(*continued*)

Table 15.1 (*Continued*)

Location	Species	Impacts	Reference(s)
Southern California Bight, California	*Ulva* spp.	High percent cover, high biomass	McLaughlin *et al.* (2013)
Hawai'i	*Gracilaria salicornia*	High percent cover	Smith *et al.* (2002)
SW Maui, Hawai'i	*Cladophora sericea*	High percent cover, overgrowth of coral and other macroalgae	Smith *et al.* (2005)
Maui, Hawai'i	*Ulva lactuca, Hypnea musciformis, Acanthophora spicifera, Hypnea musciformis*	High percent cover	Smith *et al.* (2002), Dailer *et al.* (2012)
Kaneohe Bay, Oahu, Hawai'i	*Dictyosphearia cavernosa*, *Kappaphycus* spp., *Gracilaria salicornia*	High percent cover, overgrowth of coral reefs	Stimson *et al.* (2001), Conklin and Smith (2005)
Oahu, Hawai'i	*Acanthophora spicifera*, *Gracilaria salicornia*, *Kappaphycus* sp.	High percent cover, high biomass	Smith *et al.* (2002, 2004)

ecological standpoint, and are increasingly important macroalgal bloom formers in estuarine and marine systems.

Opportunistic green macroalgae, including the genera *Ulva*, *Chaetomorpha*, *Cladophora*, *Codium*, and *Caulerpa*, are perhaps best known for widespread blooms in urbanized bays and harbors in temperate and subtropical waters (Table 15.1 and Figure 15.3a, 15.3d, 15.3e, 15.3f, 15.3g, 15.3h). For example, extensive blooms of *Ulva* have developed throughout the United States in Boston Harbor (Sawyer, 1965), Narragansett

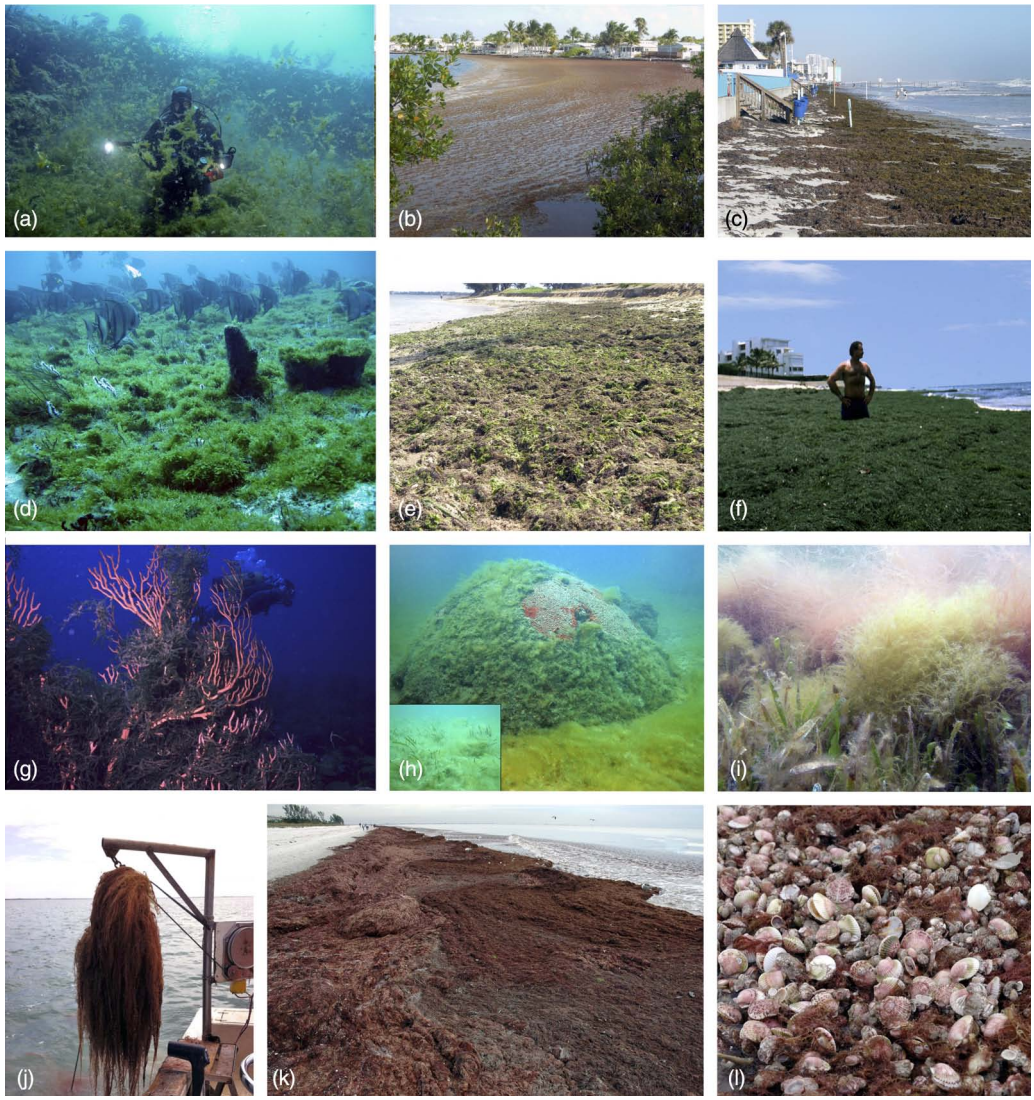

Figure 15.3 Examples of coastal marine harmful macroalgal blooms. (a) The green macroalga *Cladophora* sp. on coastal reefs off Boca Raton, Florida. *Source:* photo by R. Baumberger. (b,c) The brown macroalga pelagic *Sargassum* in the lower Florida Keys and Daytona Beach, Florida. *Source:* photos by B. Lapointe. (d) The non-native green macroalga *Caulerpa brachypus* forma *parvifolia* forming benthic mats on reefs off Riviera Beach, Florida. *Source:* photo by B. Bedford. (e) Mixed bloom of the non-native red macroalga *Hypnea musciformis* and the green *Ulva lactuca* on Maui, Hawai'i. *Source:* photo by B. Lapointe. (f,g) Blooms of the green macroalga *Codium isthmocladum* impacting beaches and coastal reefs off Palm Beach County, Florida. *Source:* photos by B. Lapointe. (h) Blooms of the green macroalga *Cladophora vagabunda* overgrowing a dead brain coral near the Content Keys in the lower Florida Keys. *Source:* photo by B. Lapointe. (i) Drift blooms of the brown macroalga *Dictyota* spp. and the red *Wrightiella blodgettii* overgrowing turtle grass meadows in the lower Florida Keys. *Source:* photo by B. Lapointe. (j) Fouling of stone crab traps by the red macroalga *Wrightiella blodgettii* near Cudjoe Key, Florida. *Source:* photo courtesy of M. Laudicina. (k) Blooms of red drift macroalgae (*Gracilaria* spp., *Hypnea* spp., and *Acanthophora spicifera*) on Sanibel Island beaches in Lee County, southwest Florida. *Source:* photo by B. Lapointe. (l) mortality of scallops by red drift macroalgae blooms at Sanibel Island. *Source:* photo by B. Lapointe.

Bay (Thornber *et al*., 2008), coastal bays in Delaware and Maryland (McGinty *et al*., 2002; Cole, 2002), the Indian River Lagoon in east-central Florida (Whitehouse and Lapointe, 2015), Tampa Bay (Hagan, 1969), embayments in southern California (McLaughlin *et al*., 2014), and the Salish Sea in Washington State (Frankenstein and Redman, 2000; Nelson *et al*., 2009). In these environments, *Ulva* forms thick mats that overgrow the benthos, resulting in hypoxia and anoxia, offensive odors, and loss of biodiversity. *Chaetomorpha* blooms have also developed in shallow, urbanized estuaries and coastal lagoons where benthic mats form, and they can eventually displace seagrasses as the dominant benthic primary producer (McGlathery, 2001). Blooms of *Cladophora* spp. in estuarine/marine waters have increasingly developed in urbanized bays and coastal waters, including Harrington Sound, Bermuda (Lapointe and O'Connell, 1989); Waquoit Bay, Cape Cod (Valiela *et al*., 1992; Peckol *et al*., 1994); as well as coastal waters in southeast Florida (Figure 15.3a), Florida Bay, and the Florida Keys (Dawes *et al*., 1999; Lapointe *et al*., 2004; Smith *et al*., 2007; Figure 15.3d). On coral reefs in southeast Florida, a succession of blooms since 1990 has included the green algae *Codium isthmocladum*, *Caulerpa verticillata*, *Caulerpa racemosa*, and the non-native *Caulerpa brachypus* var. *parvifolia* (Lapointe *et al*., 2005a; Figure 15.3d). All of these green macroalgal blooms are commonly referred to as "green tides" (Fletcher, 1996; Raffaelli *et al*., 1998).

Red macroalgal blooms have also been on the rise along developing coastlines for many decades. During the urbanization of the subtropical Tampa Bay area in the 1960s and 1970s, extensive blooms of the red macroalga *Gracilaria tikvahiae* developed (Hagan, 1969), where the excessive drift biomass accumulation overgrew seagrasses and decomposed on shores, causing offensive odors. Similar blooms of drift *Gracilaria tikvahiae* (and other genera of red macroalgae, including *Hypnea*, *Acanthophora*, and *Spyridia*) developed in estuaries and coastal waters of southwest Florida between 2003 and 2007, fouling beaches and shorelines (Lapointe and Bedford, 2007; Figure 15.3k and 15.3l). Similar blooms occurred in the Indian River Lagoon in east-central Florida since the 1970s, where macroalgae now account for greater than threefold biomass compared to seagrasses (Lapointe *et al*., 2015). Seasonal blooms of *Gracilaria* also occur in the temperate waters of Waquoit Bay, Cape Cod, where thick algal canopies cause light limitation and loss of the seagrass *Zostera marina* (Valiela *et al*., 1997; Hauxwell *et al*., 2001a; Hauxwell and Valiela, 2004). In the subtropical Florida Bay/Florida Keys region, the red drift macroalgae *Laurencia* spp., *Spyridia filamentosa*, and *Wrightiella blodgettii* have formed increasing blooms with increasing urbanization in the Keys and N loading from the Everglades; these blooms are increasingly impacting both seagrasses and coral reefs (Lapointe *et al*., 1994; Collado-Villes *et al*., 2007; Green *et al*., 2015) as well as commercial fishing gear such as lobster and stone crab traps (Figure 15.3i and 15.3j).

Brown macroalgal blooms have also increasingly developed in temperate and subtropical waters. Blooms of a drift form of the brown macroalga *Pilayella littoralis* have fouled the shallow waters and beaches of Nahant Bay since 1903, where they form sludge-like masses that strand on beaches in accumulations up to 0.5 m thick (Wilce *et al*., 1982). Increasing biomass strandings of the floating, pelagic brown macroalga *Sargassum* have fouled tourist beaches in Texas and Florida for decades (Lapointe, 1995; Gower *et al*., 2006). In the Florida Keys, blooms of *Dictyota* spp. and *Cladosiphon* have increased since 1990 with increased freshwater flows and nitrogen loading from the Everglades, overgrowing seagrasses (Figure 15.3i) and corals as a consequence of nutrient enrichment and eutrophication (Lapointe *et al*., 2004).

Over the last decade, estuarine and marine macroalgal blooms have expanded globally to unprecedented spatial scales and biomass levels. In 2007, blooms of *Ulva prolifera* developed in the Yellow Sea, China; by the following summer of 2008, the Yellow Sea region experienced the largest green tides ever, comprising over 20 million metric tons of floating biomass and an area of 13,000–30,000 km^2 (Liu *et al*., 2009; Gao *et al*., 2010; Ye *et al*., 2011). These massive green tides severely impacted the Qingdao area, the site of the summer 2008 Olympic sailing competition, and over 1 million tonnes of *Ulva* biomass were removed by hand (Leliaert *et al*., 2008). Following the BP *Deepwater Horizon* oil spill in 2010, unprecedented high-biomass strandings of pelagic *Sargassum* (Figure 15.3b and 15.3c) impacted coastal communities beyond the Gulf of Mexico, including the east coast of Florida, Sierra Leone, and the entire Caribbean basin. These blooms were particularly severe throughout the Caribbean region in 2015 (Hu *et al*., 2016), where widespread and detrimental environmental and economic impacts occurred (Kirkpatrick, 2015; Stasi, 2015; Figure 15.5e). It is uncertain whether these recent *Sargassum* blooms are a short-term response to variable environmental conditions or reflect a

long-term trend of nutrient enrichment and eutrophication in offshore, oceanic waters on the North Atlantic Ocean.

15.4 Influences on Bloom Development

The ability of a particular alga to become a successful bloom former depends on its physiological responses to local environmental growth-limiting factors. Unlike toxic phytoplankton blooms, macroalgal blooms usually lack direct chemical toxicity, but typically have a broader range of distribution and ecological impacts. These blooms can result in the displacement of indigenous species, oxygen depletion (hypoxia/anoxia), noxious and toxic odors (hydrogen sulfide, dimethyl sulfide, and dimethyl disulfide), habitat loss, alterations of biogeochemical cycles and food webs, alterations in grazing, and die-offs of seagrasses and coral reefs (Jørgensen and Okholm-Hansen, 1985; Lapointe *et al*., 1994; ECOHAB, 1995; Valiela *et al*., 1997; National Research Council [NRC], 2000; McGlathery, 2001; HARRNESS, 2005; United Nations Environment Program [UNEP], 2005). Increasingly, macroalgal blooms foul beaches and shorelines important to local tourist economies, impact commercial and sport fisheries, and require ever more expensive biomass removal programs (Harris, 2005; Higgins *et al*., 2005; Morand and Briand, 1996; Lapointe and Bedford, 2007).

Macroalgal productivity and growth are controlled by interactions of physical, chemical, and biological factors including light, temperature, nutrient availability, salinity, grazing, water motion, water residence time, depth, and desiccation (e.g., Raven, 1992; Lobban and Harrison, 1994; Dawes, 1998; Vis *et al*., 2008). The physiographic setting, including geomorphology and hydrography, can also be important, as it determines connectivity of macroalgal recruitment, as well as the type of primary production base and biological communities present (e.g., freshwater marshes, submersed freshwater meadows, rocky shorelines, and soft substrata; and, in brackish and marine systems, mangroves, salt marshes, seagrasses, coral reefs, and rocky intertidal, soft-bottom, and planktonic systems). Physical factors such as winds, currents, and tides also influence the transport and accumulation of drift macroalgal blooms.

Of all these factors, however, *the increasing trend of macroalgal HAB results primarily from increased nutrient loading and eutrophication* in many lakes, reservoirs, streams, rivers, shallow bays, estuaries, and coastal waters (Hagan, 1969; Smith *et al*., 1981; Lapointe *et al*., 1994; Morand and Briand, 1996; Valiela *et al*., 1997; NRC, 2000; Higgins *et al*., 2005; Stevenson *et al*., 2007; Vis *et al*., 2008; Teichberg *et al*., 2010; Armenio *et al*., 2016). Thus, many studies have addressed the importance of nutrient loading to the development of macroalgal HAB (below). Compared to the effects of nutrients, other factors associated with global change (e.g., alterations to seawater and air temperatures and seawater pH and carbonate chemistry) on seaweed growth and distribution are poorly known (Harley *et al*., 2012). These interacting factors could be significant to HAB formation, especially in coming decades.

15.5 Nutrient Pollution

15.5.1 Sources

Although point-source sewage pollution has long been recognized as a cause of HAB (Sawyer, 1965; Hagan, 1969; Smith *et al*., 1981; Lapointe *et al*., 2005a; Teichberg *et al*., 2010), non-point-source inputs of sewage, such as from septic tanks, shallow injection wells, fertilizers, and nutrient-enriched submarine groundwater discharges, can also increase nutrient loading, eutrophication, and the development of macroalgal blooms (Johannes and Hearn, 1985; Lapointe and O'Connell, 1989; Lapointe *et al*., 1990; Valiela *et al*., 1990, 1997; Lapointe, 1997; Teichberg *et al*., 2010). In addition, siliciclastic environments tend toward stronger N-limitation of macroalgal blooms (Hanisak, 1979; Nixon and Pilson, 1983; Lapointe *et al*., 1992), compared to carbonate-rich waters that tend more toward stronger P-limitation due to adsorption of P in sediments (Lapointe *et al*., 1992; McGlathery *et al*., 1994; Lapointe, 1997). In general, human activities are increasing N loading to the biosphere at a greater rate than P loading (NRC, 2000), which will tend toward increasing N:P ratios and P limitation of macroalgal blooms. There is already evidence for this in pelagic *Sargassum* in the Gulf of Mexico, where plants have higher tissue N and N:P ratios than historical baseline values (Lapointe, 1995). This change likely reflects the increasing N and N:P ratios in the Mississippi and Atchafalaya rivers that account for an estimated 90% of the total N load and 87% of the total P load discharged annually to the Gulf of Mexico (Dunn, 1996). Deviations from this general pattern have been reported, however (Larned, 1998; Fong *et al*., 2001), and underscore

the complexities of eutrophication processes in coastal environments (NRC, 2000).

15.5.2 Indicators of Nutrient Pollution and Nutrient Sources

Among thousands of macroalgal species (Graham *et al.*, 2016 and references therein), relatively few have responded to nutrient pollution (below) by forming high-biomass blooms. Nevertheless, during the past century, macroalgal blooms have increased in frequency and extent in many inland waters and along North America's coastlines (Figures 15.1, 15.2, and 15.3, and Table 15.1) and are now considered a major element of global change (HARRNESS, 2005; UNEP, 2005). As a few of many examples, blue-green *Lyngbya wollei* mats are considered to be an indicator of freshwater ecosystem degradation (Hudon *et al.*, 2014 and references therein). Similarly, in coral reef ecosystems, the presence of massive occurrences of benthic cyanobacteria such as *Lyngbya majuscula* have been suggested to serve as indicators of coral reef health (Golubic *et al.*, 2010). Filamentous chlorophytes (*Cladophora* and others) commonly proliferate in response to nutrients in sewage and animal waste sources that contaminate freshwater streams and rivers (e.g., Stevenson *et al.*, 2012 and references therein). In estuaries and coastal marine systems, the chlorophytes *Ulva*, *Chaetomorpha*, and *Cladophora* spp. are common responders to sewage (Lapointe and O'Connell, 1989; Lapointe *et al.*, 2015 and references therein). The frequency and magnitude of blooms of certain "ephemeral" macroalgae such as *Ulva lactuca* have been considered as an indicator of high nutrient overenrichment, and seagrass success or failure (Fox *et al.*, 2010; Whitehouse and Lapointe, 2015).

Macroalgal blooms are key ecological indicators of nutrient pollution and coastal eutrophication, and can provide an ideal tool for nutrient monitoring programs. Macroalgae are often attached to benthic substrata (although many blooms can form drift populations) and therefore integrate nutrient availability at a given site over time scales of days to weeks. Opportunistic, fast-growing macroalgae can have rapid nutrient uptake rates, such as the red alga *Gracilaria tikvahiae* (D'Elia and DeBoer, 1978) and the green alga *Ulva lactuca* (Whitehouse and Lapointe, 2015). Accordingly, some macroalgae can be sampled to assess not only relative status of enrichment (nutrient quantity, and the internal tissue percentages of C, N, and P), but also the nutrient source(s) through stable isotope analysis (N [$\delta^{15}N$] and C [$\delta^{13}C$]) of tissues. Macroalgae can be used to discriminate specific nutrient sources in marine ecosystems because there is no fractionation of $\delta^{15}N$ values of N sources in N-limited systems (France *et al.*, 1998; Waser *et al.*, 1999; Cole *et al.*, 2004; Savage and Elmgren, 2004; Lapointe *et al.*, 2005b; Deutsch and Voss, 2006; Thornber *et al.*, 2008). Where fractionation has been documented between the N source (groundwater NO_3^-) and macroalgal tissue, enrichment in tissue $\delta^{15}N$ was slight (0.2–1.4%; see Umezawa *et al.*, 2002). These measurements allow use of some macroalgae to identify land-based N sources, which can assist policymakers in efforts to reduce nutrient loads through total maximum daily loads and/or basin management action plans (Lapointe *et al.*, 2005a, 2015). For example, studies using macroalgae in Florida have demonstrated utility in discriminating between agricultural and sewage nitrogen sources in estuarine (Lapointe and Bedford, 2007; Lapointe *et al.*, 2015) and coastal environments (Barile, 2004; Lapointe *et al.*, 2004, 2005a). The technique has been used successfully in an array of geographic areas throughout the United States, including Boston Harbor and Waquoit Bay, Massachusetts (France *et al.*, 1998; McClelland and Valiela, 1998); Narragansett Bay, Rhode Island (Thornber *et al.*, 2008); the Florida Keys (Lapointe *et al.*, 2004); southwest Florida (Lapointe and Bedford, 2007); the Indian River Lagoon, Florida (Lapointe *et al.*, 2015); and Maui, Hawai'i (Dailer *et al.*, 2010).

15.6 Uptake/Adsorption of Other Contaminants

Many seaweed species, including those that form blooms, take up inorganic and organic pollutants from the surrounding water or the sediments (Carafa *et al.*, 2007; Cheney *et al.*, 2014; He and Chen, 2014). Brown algae, such as *Sargassum* spp., are known to take up and sequester heavy metals such as lead, copper, cadmium, zinc, nickel, and chromium (reviewed by He and Chen, 2014). These higher uptake rates relative to those of green and red algae are due to the presence of alginate and other cell wall components that have strong affinity for cationic (positively charged) metals (Fourest and Volesky, 1995, 1997). Cations typically are absorbed more quickly by brown algae than anions (negatively charged molecules), and more cationic metals are absorbed in higher pH

waters (Davis *et al*., 2000). Red and green algae also absorb heavy metals (Rice and Lapointe, 1981; Sfriso *et al*., 1992; Chakraborty *et al*., 2014), but generally not as readily as brown algae.

Seaweeds can take up toxic organic compounds from the environment. For example, ulvoid green algae can accumulate polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and many types of chlorinated pesticides, including dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethane (DDD), dichlorodiphenyldichloroethylene (DDE), hexachlorocyclohexane (HCH), and hexachlorocyclobenzene (HCB) (Maroli *et al*., 1993; Pavoni *et al*., 2003; Cheney *et al*., 2014). Sfriso *et al*. (1992) documented seasonal cycles of several persistent organic pollutants in sediments under mats of *Ulva rigida*, which take up and store the compounds. Concentrations of PCBs and nonylphenol polethoxylates in surface sediments markedly increased starting in midsummer as mats of ulvoid algae were decomposing and the organic pollutants in the algae were transferred into the sediments. Thus, the movement of algal mats from a contaminated to an uncontaminated area could also result in the transfer of pollutants that are being absorbed and stored in the algae. Pollutants may also be transferred up the food chain by consumers of the algae that are concentrating the toxins (Cheney *et al*., 2014). Bloom-forming seaweeds are useful as bioindicators of pollution as they can rapidly absorb heavy metals and organic pollutants (Eide *et al*., 1980; Chakraborty *et al*., 2014) and as a means for decontaminating polluted sites (Vieira and Volesky, 2010).

15.7 Impacts on Human Health: Macroalgae as Substrata for Pathogens

Recent research has established that freshwater harmful macroalgae such as *Cladophora* spp. and *Lyngbya wollei* on beaches can concentrate pathogenic fecal bacteria such as *Escherichia coli*, enterococci, and *Clostridium fringens* (Whitman *et al*., 2003; Paul *et al*., 2004; Vijayavel *et al*., 2013). High concentrations of these fecal bacteria were found in *L. wollei* that was growing abundantly in nearshore waters adjacent to a popular recreational beach in Lake St. Clair, Michigan, whereas much lower densities of fecal bacteria were measured in the surrounding water (Vijayavel *et al*., 2013). A decade earlier, stranded *Cladophora* mats in Lake Erie were found to sustain and nourish the growth of fecal bacteria (Whitman *et al*., 2003). Samples of *Cladophora* mats along ten beaches in four states bordering Great Lake Michigan were collected in summer 2002, and both *E. coli* and enterococci were ubiquitous (up to 97% occurrence) in the *Cladophora*. The fecal bacteria survived for more than six months in sun-dried mats stored at 4 °C and readily grew upon rehydration at warmer temperature.

Stranded *Cladophora* mats can harbor other human pathogens as well. During a two-year study of the Little Calumet area (Chicago–Lake Michigan and an adjacent ditch), Shiga toxin–producing *E. coli* (STEC) and *Shigella* were detected in 100% and 25% of *Cladophora* samples, respectively, during one year but not the other (Ishii *et al*., 2006). In addition, the human pathogen *Salmonella* was found in 40% and 80% of ditch and lakeside samples, respectively, with densities as high as 1.6×10^3 cells per gram of *Cladophora*. In addition, there were up to 5.4×10^2 cells of *Campylobacter* per gram of *Cladophora* in 60% and 100% of lake and ditch samples, respectively (Ishii *et al*., 2006). Such macroalgal repositories for pathogenic microorganisms represent a present-day, potential human health threat along beaches of several Great Lakes where *Cladophora* has again proliferated.

Massive growth of this macroalga, up to 940 g dry mass m^{-2} (median: ~170 g m^{-2}), can extend more than 6 m from shore out into the lake along its northern shoreline, covering up to 100% of the substrata in many locations (Higgins *et al*., 2005; Stauffer, 2005). Die-off begins in mid-summer and extends into fall each year, wherein *Cladophora* senesces, sloughs from its substrata, and washes ashore. Harris (2005) provided a compelling description:

> The beached algae often accumulate in mats [more than a meter thick] mixed with decaying zebra mussels [*Dreissena polymorpha*], other invertebrates, and fish. The combination results in unsightly, malodorous conditions that drive visitors away from popular beaches and force homeowners to keep their windows shut. . . . Stranded *Cladophora* mats can sustain and nourish the growth of fecal bacteria from gull droppings, sewage overflows, and/or runoff from urban and agricultural areas (Whitman *et al*., 2003; Paul *et al*., 2004). Because of its septic odor, the organic mess has been mistaken for manure or sewage from failing septic systems or municipal sewer overflows. In the swash zone, the algae may turn into a

brown-black organic soup with an oily sheen, prompting some people to suspect an industrial waste or oil spill.

Although a number of illnesses occur in recreational users of marine waters (Henrickson *et al*., 2001), to our knowledge, there are fewer associations between marine macroalgal blooms and human diseases in the United States; however, in the United Kingdom, *Vibrio cholerae* has been found to be associated with macroalgae known to form blooms (*Ulva* spp. and *Polysiphonia lanosa*) (Islam *et al*., 1994).

15.8 Non-native Invasions

Macroalgae represent about 20% of marine introduced species globally (Andreakis and Schaffelke, 2012), and non-native macroalgal invasions are a major driver of coastal ecosystem change worldwide (UNEP, 2005; Williams and Smith, 2007). Alien macroalgae are increasingly invading coastal waters globally (Grosholz and Ruiz, 1996; Wonham and Carlton, 2005; Miller *et al*., 2011), and there are more than 120 known introduced species globally (Mathieson *et al*., 2008). These non-native macroalgae can outcompete native species, reducing biodiversity and leading to alterations of ecosystem structure and function (Vitousek *et al*., 1997).

To our knowledge, the only freshwater macroalga that has invaded inland U.S. waters is starry stonewort (*Nitellopsis obtusa*) (Figure 15.2d). Thought to be native to Europe, this invader has occurred in various lakes of the upper Midwest (mostly Michigan) and New York since 1978 (United States Geological Survey [USGS], 2016). This charalean species can grow up to 2 m in height at 9-m depths (Pullman and Crawford, 2010). It forms dense mats of vegetation that can completely cover the bottom of shallow lakes and ponds. As its biomass and coverage increase, it covers nearly all of the sediment in a given area and forms irregular, undulating "pillows" of biomass (Pullman and Crawford, 2010). When in decline or dormant, holes open in the starry stonewort mats that resemble the hole pattern of Swiss cheese. Starry stonewort grows well in a wide range of lake types such as clear water or dark water systems, and it is easily transported from lake to lake among aquatic plant debris entangled on boat trailers and anchors. It has shown no apparent preference for shade or full sun (Pullman and Crawford, 2010). Starry stonewort is considered aggressive and has outcompeted invasive macrophyte species (aquatic vascular plants) such as Eurasian watermilfoil (*Myriophyllum spicatum*), fanwort (*Cabomba caroliniana*), and curly leaf pondweed (*Potamogeton crispus*). Its maximum biomass has been reported at $\sim$260 g m^{-2} (Schloesser *et al*., 1986; Nichols *et al*., 1988). This invasive macroalga adversely affects fish spawning habitat by forming a thick mat that impedes access to substrata for nest creation. Its thick growth can completely eliminate spawning activity in the area of infestation (Pullman and Crawford, 2010). On the other hand, there is a strong association between starry stoneworts and invasive zebra mussels, suggesting a mutualistic relationship: the zebra mussels provide nutrients via pseudofeces and feces, and the macroalga provides suitable attachment substrata (Pullman and Crawford, 2010).

Macroalgae that have made transoceanic invasions into coastal waters of North America include the green algae *Codium fragile* var. *tomentosoides* (Fralick and Mathieson, 1973; Malinowski and Ramus, 1973; Carlton and Scanlon, 1985), *Caulerpa taxifolia* (Williams and Smith, 2007), and *Caulerpa brachypus* var. *parvifolia* (Lapointe *et al*., 2005b); the red algae *Gracilaria tikvahiae* (introduced to Hawai'i; University of Hawai'i, 2001), *Gracilaria vermiculophilla* (Freshwater *et al*., 2006), *Dasysiphonia japonica* (Schneider, 2010), *Caulocanthus ustulatus* (Miller, 2004), and *Grateloupia turuturu* (Mathieson *et al*., 2008); and the brown algae *Sargassum muticum* (Scagel, 1956; Aguilar-Rosas and Galindo, 1990), *Sargassum horneri* (Miller *et al*., 2007), *Colpomenia peregrina* (Green *et al*., 2012), *Undaria pinnatifida* (Silva *et al*., 2002), *Lomentaria hakodatensis* (Curiel *et al*., 2006), and *Cutleria cylindrical* (Hollenberg, 1978). Furthermore, many species that are now considered to be cosmopolitan may owe their broad distributions to transport on the hulls of wooden ships that were transiting the oceans long before the baselines that are used to determine whether a species is native or invasive were established (Carlton, 1996).

Non-native macroalgae are considered harmful when excessive biomass blooms form under certain conditions and environments. Several exotic/invasive macroalgae have invaded coastal waters along the U.S. East Coast. As mentioned above, *Codium fragile* subsp. *tomentosoides* has spread from its native Japan to various parts of the world, including both coasts on the U.S. mainland (Provan *et al*., 2005 and references therein). Native to Japan, this macroalga is thought to have been carried to New England from Europe on ship hulls

and then spread north to New England in the late 1950s, coincident with the launch of the world's first satellites, the Soviet Union's *Sputniks*. Thus, suspicious New England fishermen called it "sputnik weed," and then "oyster thief," because attachment of its sporelings to oyster (and scallop) shells caused overgrowth and smothering of shellfish aquaculture operations (Trowbridge, 1998 and references therein).

As other examples, *Caulerpa brachypus* f. *parvifolia* was discovered overgrowing deep (25–43 m) coral reef communities off southeast Florida in May 2001, forming dense mats (5–15 cm) covering up to 90% of the reef surface (Figure 15.3d; Lapointe *et al*., 2005b). Fortunately, this invasive bloom was largely removed by the "twin hurricanes" in summer 2004 (Lapointe *et al*., 2006), although some re-emergence has since occurred. Along the mid-Atlantic coasts of North America, *Gracilaria vermiculophilla* was first detected in North Carolina in 2000, and in the following years extensive blooms fouled fishing gear and the intake screens at the Brunswick Nuclear Plant (Freshwater *et al*., 2006).

More than a dozen species of non-native seaweeds have been documented to occur on the U.S. West Coast, but to date none have formed harmful blooms in that region (Miller *et al*., 2011). These species include algae known to form large, deleterious blooms in other locations, such as *Caulerpa taxifolia* (Williams and Grosholz, 2002), *Codium fragile* spp. *tomentosoides* (Provan *et al*., 2008), *Undaria pinnatifidia* (Thornber *et al*., 2004), and *Sargassum muticum* (Britton-Simmons, 2004). The lack of spread of the invasive alga *C. taxifolia* in southern California was due to early detection of the introduction and rapid, coordinated eradication efforts by a variety of local, state, and federal agencies in combination with private groups and nongovernmental organizations (Anderson, 2005).

Numerous invasive species have formed harmful blooms in the Hawaiian Islands (Smith *et al*., 2002; Figure 15.3e). Problematic species have included the rhodophytes *Acanthophora spicifera*, *Gracilaria salicornia*, *G. tikvahiae*, *Hypnea musciformis*, and *Kappaphycus* spp., and the chlorophytes *Avrainvillea amadelpha* and *Dictyosphaeria cavernosa* (Stimson *et al*., 2001; Smith *et al*., 2002). Some of these introductions occurred accidentally via transport by boats (e.g., *A. spicifera*), whereas others, such as *G. salicornia*, *G. tikvahiae*, and *Kappaphycus* spp., were deliberately introduced for aquaculture operations (University of Hawai'i, 2001; Smith *et al*., 2004). The ability of many of these species to spread by vegetative propagation (Smith *et al*., 2002, 2004), coupled with rapid growth in response to N inputs (Stimson *et al*., 2001), enable populations of these invasives to rapidly expand, especially in areas where N inputs from sewage and stormwater runoff occur (Lapointe and Bedford, 2011).

15.9 Ecological and Ecosystem-Level Impacts

The ecological impacts of macroalgal blooms depend on many factors, including the level of biomass, duration of bloom, morphology of the species involved, habitat (e.g., seagrass meadow, coral reef, rocky intertidal, or soft-bottom community), and a variety of local physical, chemical, and biological factors. Recent studies have attempted to quantify at what biomass level(s) environmental effects on natural benthic communities occur. Bona (2006) reported effects on benthic habitats at biomass levels of ~90 g dry wt. m^2 and > 70% cover; while Scanlan *et al*. (2007) suggested an "effects" threshold of 70–120 g dry wt m^{-2}, and Green *et al*. (2014) reported significant impacts on benthic invertebrates at 110–120 g dry wt m^{-2} and 100% cover following a month of biomass cover. Additionally, a range of 3–15 g dry wt. m^{-2} was recently suggested as a transition zone from reference conditions in eight California estuaries (Sutula *et al*., 2014). Given this range of estimates, it is likely that effects vary by geographic location and conditions.

Macroalgal response to nutrient enrichment often translates to rapidly developing canopies ranging from 0.75 m to more than 2 m in thickness (Sfriso *et al*., 1992; Hauxwell *et al*., 2001a) with high biomass – for example, 650 g dry wt m^{-2} of *Gracilaria tikvahiae* mixed with other macroalgae known to thrive in nutrient overenriched conditions, such as the chlorophytes *Ulva* and *Cladophora* (Havens *et al*., 2001). These high-biomass macroalgal outbreaks typically form thick layers or "blankets" on the bottom or over the water surface that impede or outright block oxygen diffusion into the water from the overlying air (also see Section 15.10.1). Thick macroalgal drift assemblages can promote increased hypoxia/anoxia and hydrogen sulfide stress in the water column and sediments below, exacerbated by high macroalgal respiration during the night (Hauxwell and Valiela, 2004; Van Alstyne *et al*., 2015b). Due to high respiration rates, the macroalgal canopies

themselves commonly become hypoxic or anoxic at night, and sometimes even during the day (Hauxwell and Valiela, 2004). In shallow coastal ecosystems, the respiration of all of that biomass can also deplete the water column of dissolved oxygen, leading to suffocation and death of beneficial aquatic life (Harlin, 1995; Burkholder and Glibert, 2013 and references therein).

Macroalgal-dominated systems sustain inputs of large amounts of labile organic matter when the algae die and decompose periodically due to self-shading, other stressors, and seasonal growth patterns (Havens *et al*., 2001 and references within). Macroalgae generally release labile (readily biologically available) nutrients rapidly during decomposition, which fuel additional outbreaks when conditions become favorable (Buchsbaum *et al*., 1991; Havens *et al*., 2001). Major quantities of DIN (directly used by most algae) and dissolved organic N and P (some forms used by algae, various forms used by bacterial decomposers) are released to the water (up to 850 μmol/m^2) when the algae die and decompose (Havens *et al*., 2001; Gao *et al*., 2013 and references therein). Macroalgal die-offs commonly cause a sudden increase in oxygen demand as well (Valiela *et al*., 1992; Duarte, 1995). A major ecosystem impact that repeatedly has been noted is that harmful macroalgal blooms depress biodiversity (NRC, 2000; Lyons *et al*., 2014 and references therein). The loss of biodiversity can, in turn, alter bioturbation, nutrient generation, invasion resistance, secondary production, and resource use (Solan *et al*., 2004; Stachowicz *et al*., 2007).

Following decades of macroalgal blooms, unprecedented phytoplankton blooms (including a brown tide) developed in 2011 and 2012 in the Indian River Lagoon in east-central Florida (Lapointe *et al*., 2015). These blooms caused severe light limitation and extensive seagrass loss (> 60%) that coincided with unusual mortality events (UMEs) involving endangered manatees, dolphins, and pelicans. Necropsies of dead manatees revealed stomachs full of macroalgae, especially *Gracilaria* spp., along with severe intestinal irritation and bleeding, suggesting an apparent "toxic shock syndrome." This raised concerns about possible intoxication of the manatees following the phytoplankton HAB events. During the period of unusually high manatee mortalities in the northern Indian River Lagoon in spring and summer 2013, *Gracilaria tikvahiae* and associated drift macroalgal communities, which became a primary food source for manatees following seagrass die-off, were sampled at a manatee mortality "hot spot" at Shorty's Pocket, an embayment along the Banana River, in May and July of 2013. The mixed macroalgal communities were dominated by red drift algal species including *G. tikvahiae*, *Acanthophora spicifera*, *Hypnea musciformis*, and *Hydropuntia secunda*, but also included conspicuous blooms of the green alga *Chaetomorpha linum*, as well as cyanobacterial mats. The macroalgae in this section of the northern Indian River Lagoon have unusually high N:P ratios, which has been known to cause increased toxicity in some species (Lapointe *et al*., 2015). As such, toxin dose–response assays of *G. tikvahiae* extracts showed high toxicity activity to mammalian cells (Neuro 2A cells, MCF7 cells) from abundant cyanogenic glycosides (Lapointe and Herren, 2015). Although the drift macroalga *G. tikvahiae* is not generally considered toxic to humans, human intoxication due to consumption of *Gracilaria edulis* (now *Polycavernosa tsudai*) did affect 13 people who ingested the raw seaweed in Guam, three of whom died (Halstead and Haddock, 1992). Similarly, a novel glycosidic macrolide, polycavernoside A, was isolated from the *G. edulis* and was considered responsible for the poisoning (Yotsu-Yamashita *et al*., 1993). A sewage outfall at 18 m depth, to the north of the reef flat where the *G. edulis* was collected, was considered a possible factor by the Guam Environmental Protection Agency. These seemingly unrelated events suggest that nutrient enrichment and alterations of N:P ratios may increase toxicity of macroalgae to mammals.

15.9.1 Regime Shifts

Many of the ecological and ecosystem impacts caused by macroalgal blooms are relatively short-lived effects from which systems recover as blooms die out or are removed. In some cases, however, changes to ecosystems persist even after the blooms are gone. These regime shifts (also referred to as *phase shifts* or *changes to alternate stable states*) are abrupt, persistent changes in ecosystem structure that typically involve multiple interacting abiotic and biotic drivers (Scheffer *et al*., 2001; Scheffer and Carpenter, 2003; Rocha *et al*., 2015).

The following traits are characteristic of regime shifts involving blooms of macroalgae: (1) one of the most common causes of regime shifts in estuaries is excessive nutrient input (Troell *et al*., 2005; Petersen *et al*., 2008; Rocha *et al*., 2015). (2) Multiple causes often interact, such as an invasive species in combination with eutrophication, which can lead to total reorganization of the

food web (Kraberg *et al*., 2011; Lapointe and Bedford, 2011). (3) Community composition, species dominance, and peak abundance of species can be affected by a regime shift. (4) The new dominant species in the affected ecosystem remains long-term (years), as documented for various macroalgal taxa in coastal lagoons that have undergone a regime shift in response to eutrophication (e.g., Curley *et al*., 1971; Lee and Olsen, 1985; Valiela *et al*., 1997). While benthic macroalgae often replace rooted macrophytes (Burkholder *et al*., 2007; Hastings, 2013 and references therein), shallow macrophyte-free systems have also been shown to undergo regime shifts to undesirable benthic or benthic and floating macroalgae (Genkai-Kato *et al*., 2012).

Two notable types of regime shifts caused by macroalgal blooms in U.S. waters are ecosystem regime shifts on coral reefs, which have been increasingly impacted by expansion of macroalgae and filamentous algal turfs and loss of hermatypic (reef-forming) corals, and shifts between rooted macrophytes and undesirable benthic or benthic and floating macroalgae in estuaries (Burkholder *et al*., 2007; Genkai-Kato *et al*., 2012; Hastings, 2013 and references therein).

Case studies have linked macroalgal blooms to regime shifts and degradation of seagrass habitats in a broad range of environmental settings (NRC, 2000). Early studies in an urbanized estuary (Tampa Bay, Florida) showed that excessive nutrient loading from sewage, especially N, supported extensive blooms of *Gracilaria*, *Ulva*, and *Chaetomorpha* spp. (Hagan, 1969; Guist and Humm, 1976) that averaged up to 195 g dry wt m^{-2} in bay-wide surveys (Mangrove Systems, 1985). These drift blooms not only were harmful to seagrasses and overall biodiversity in Tampa Bay, but also caused noxious odors along shorelines that became unacceptable to the public (Hagan, 1969). Odor was the driving issue that forced improved sewage treatment, including nitrogen removal. Following more than 90% reduction of N loading to Tampa Bay since 1979, macroalgal and phytoplankton blooms have diminished over the subsequent decades and seagrass cover has recently returned to its 1950s level (Greening *et al*., 2014). Similar excessive macroalgal blooms have developed in other nutrient-enriched seagrass systems, including the Florida Keys, Florida (Lapointe *et al*., 1994; Green *et al*., 2015); Waquoit Bay, Cape Cod, Massachusetts (Valiela *et al*., 1992, 1997; Peckol *et al*., 1994; Hauxwell *et al*., 2001b); Narragansett Bay, Rhode Island (Thornber *et al*., 2008); and the Indian River Lagoon, Florida (Lapointe *et al*., 2015).

Regime shifts on coral reefs are now happening globally; however, the first example of this came from Kane'ohe Bay, Hawai'i, where the green "bubble alga" *Dictyosphaeria cavernosa* overgrew and killed corals as a result of nutrient enrichment from sewage (Banner, 1974; Smith *et al*., 1981). Dramatic macroalgal blooms and loss of corals occurred on fringing reefs in Jamaica during the 1980s, and the cause of this regime shift was suggested to be solely due to reduced grazing following the die-off of the long-spined sea urchin *Diadema antillarum* and overfishing of herbivorous fishes; the various fringing reefs that were monitored in the study were all assumed to be unpolluted sites (Hughes, 1994). Other studies, however, had documented elevated N (as NO_3^-) availability from groundwater and river discharges on many of the affected reefs, as well as NH_4^+ at some sites impacted directly by sewage associated with urbanization and tourist resorts (Lapointe, 1997; Lapointe *et al.*, 2011). Following years of scientific debate on this issue, it is now generally accepted that bottom-up (nutrients) and top-down (grazing) operate simultaneously to influence the outcome of regime shifts on a particular coral reef. For example, increasing nutrients combined with overfishing on reefs in Negril, Jamaica, have led to the combination of low cover of corals (5–10%) and turfs (15%) and high macroalgae (65%) (Figure 15.4a); in comparison, increasing nutrients in combination with intense grazing by large, mobile herbivorous fishes (parrotfishes and tangs) at Looe Key Sanctuary Preservation Area (SPA) in the Florida Keys have resulted in equally low coral cover (5–10%), moderate levels of macroalgae (20–25%), and high levels of algal turfs (50%; Figure 15.4b; Lapointe and Thacker, 2002). The escalating nutrients in both Jamaica and the Florida Keys are driving the cover of coral lower and overall benthic algae higher, but the intense grazing at Looe Key SPA maintains relatively lower cover of macroalgae and higher cover of turfs. Additionally, physical disturbance (e.g., turbulence, storm events) can act to keep macroalgal cover at low levels, allowing turf algae to dominate. Recognizing that corals are adapted to oligotrophic conditions, the regime shifts toward less coral and more macroalgae and/or algal turfs that has been occurring throughout the Caribbean since the 1970s appear to be driven by increasing nutrient subsidies from upland watersheds and not solely related to changes in herbivorous fishes (Suchley *et al*., 2016).

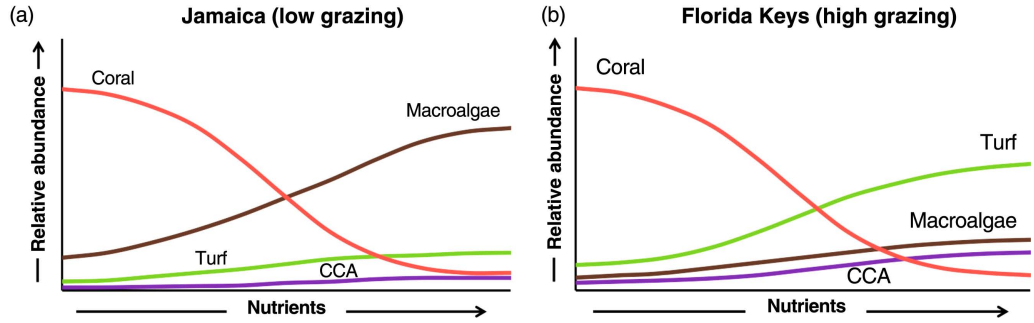

Figure 15.4 Effects of increasing nutrient availability on relative abundance of macroalgae, turf algae, and crustose coralline algae (CCA) on coral reefs. Under conditions of increasing nutrients and low grazing such as the north coast of Jamaica (a), macroalgae become dominant relative to corals, turf algae, or CCA. Under conditions of increasing nutrients and high grazing (or high levels of physical disturbance such as water turbulence) such as the Florida Keys (b), turf algae become dominant relative to corals, macroalgae, or CCA.

15.9.2 Freshwater Macroalgal HAB

15.9.2.1 Filamentous Cyanobacteria

Among the best-known noxious benthic cyanobacteria bloom formers is *Lyngbya wollei*, which is closely related to *Lyngbya majuscula*, a noxious cyanobacterial mat former in marine waters (Speziale and Dyck, 1992). This freshwater species occurs in temperate to tropical, alkaline, or mildly acidic freshwaters in lakes and rivers across North America (Hudon *et al*., 2014). Blooms of *L. wollei* have been documented for more than 100 years (e.g., Wolle, 1887), but reports of increased abundance are becoming more common in the eastern and southeastern United States (Hudon *et al*., 2014 and references therein). Thus, this alga is commonly considered as a native species that acts as an opportunistic invader (e.g., PBS&J, 2004; Hudon *et al*., 2014), or as "an initial aggressive colonizer," for example following natural disturbances (Cowell and Botts, 1994). In situations when other primary producers are limited by unfavorable environmental conditions or are physically removed, *L. wollei* has proliferated (Evans *et al*., 2007; Hudon *et al*., 2014). This species is diazotrophic (i.e., it can "fix" nitrogen gas into ammonia), and it can produce an array of aplysiatoxins: cylindrospermopsin, deoxy-cylindrospermopsin, lyngbyatoxin, and saxitoxin analogs (e.g., decarbamoylsaxitoxin and decarbamoylgonyautoxin) (Camacho and Thacker, 2006; Seifert, 2007; Foss *et al*., 2012).

Initially, *L. wollei* grows attached in a benthic habit, later forming dense free-floating mats in lakes, reservoirs, streams, and springs. Maximal biomass is attained in summer–early fall at a high optimum temperature for growth. Nevertheless, in warm temperate climates such as much of the southeastern United States, *L. wollei* has overwintered while maintaining high biomass (~120–440 g dry wt m^{-2}) (Hudon *et al*., 2014 and references therein). Other characteristics additionally enable *L. wollei* to proliferate: it is well adapted to low light, and viable populations occur down to 0.05% (15 μEinst m^{-2} s^{-1}) to 1% of incident photosynthetically active radiation (e.g., Speziale *et al*., 1991; Panek, 2012 and references therein). Photobleaching of external filaments protects the rest of the mat from damage by ultraviolet light. Very low dissolved inorganic carbon (C_i) concentrations can saturate its photosynthesis and growth (Speziale *et al*., 1991), which would enhance survival in C-limited situations such as the interior of its thick mats. Conditions that enhance photorespiration (e.g., high midday temperatures and high oxygen concentrations) characterize floating mat environments, but photorespiration is minimal in *L. wollei*, probably because it has highly efficient C-concentrating mechanisms and can use bicarbonate as a C source (Beer *et al*., 1986, 1992 – note that in the latter publication, *L. wollei* was referred to as *L. birgei*).

This benthic cyanobacterium is strongly stimulated by nutrient over-enrichment. Harmful effects have included clogged water intakes, offensive odors, the production of potent toxins, and compromised recreational and potable water use (Hudon *et al*., 2014 and references therein). Massive mats of *L. wollei* have been reported, such as a mat that was estimated to be 10 m long, 1 m wide, and up to 0.5 m thick, with biomass as high as 1508 g dry wt m^{-2} (Hudon *et al*., 2014 and references therein). This species has caused major, adverse habitat alteration and aesthetic impairment in various reservoirs of the southeast

(Speziale and Dyck, 1992 and references therein), and in formerly clear, deep Florida springs (Stevenson *et al*., 2007). Increases have been observed in tributaries that drain agricultural lands in the St. Lawrence River (Hudon *et al*., 2014); and there has been recent proliferation in western Lake Erie (Bridgeman and Penamon, 2010). A shift in composition of benthic macroalgae in a fluvial lake, from chlorophyceans *Cladophora* and *Hydrodictyon reticulatum* to cyanobacteria *Lyngbya wollei* and *Gloeotrichia*, was related to elevated nitrate from agricultural drainage (Vis *et al*., 2008).

Wetlands dominated by *L. wollei* have been found to support lower biomass of invertebrates and large fish, lower species richness, and more slowly growing juvenile fish (perch, *Perca flavescens*) than macrophyte (vascular plant)–dominated wetlands (Hudon *et al*., 2012). Some strains of this cyanobacterium apparently are toxic to certain amphipod species (Camacho and Thacker, 2006; Gélinas *et al*., 2013). Grazer biomass was significantly lower in areas with abundant *L. wollei*, which apparently was related to reduced food and habitat availability through declines in beneficial macrophytes and associated epiphytes (Lévesque *et al*., 2012). Replacement of macrophytes by *L. wollei* mats shifted trophic structure, decreased carrying capacity for fish, and significantly altered ecosystem dynamics (Hudon *et al*., 2014). While mammal deaths have not been linked to *L. wollei*, dog deaths have occurred following ingestion of benthic *Phormidium* mats containing anatoxin-producing strains (Puschner *et al*., 2008; McAllister *et al*., 2016). Dogs also reportedly were killed by ingesting toxic benthic *Oscillatoria* mats (Gunn *et al*., 1992).

15.9.2.2 Filamentous Green Algae

These are common responders to nutrient pollution, often forming massive, slimy growths at or near the water surface in areas affected by sewage and other nutrient over-enriched conditions (Perrin *et al*., 1988; Mackay, 2006; Benke and Cushing, 2011), and the excessive growth can cause major diel DO fluctuations and "sags" (Pitcairn and Hawkes, 1973; Kirk, 1994). In the western United States, for example, filamentous green algae such as *Cladophora* and *Pithophora oedogonia*, as well as the streptophyte *Chara*, have been listed as among the most consistently problematic aquatic weed species (Anderson, 1990). In the Great Lakes, early post-invasion of zebra mussels was characterized by increased available light and a shift in dominance of benthic algae from diatoms to *Spirogyra* sp., later co-occurring with *Cladophora* (Pillsbury *et al*., 2002 and references therein). The best-known harmful macroalgae in freshwaters, *Cladophora* spp. (*C. glomerata* and others), are major responders to P enrichment (Higgins *et al*., 2008 and references therein; Auer *et al*., 2010). Rocky substrata in alkaline lakes and streams of the upper Midwest and the western United States provide habitat for *Cladophora* blooms (Lembi, 2003; Sandgren *et al*., 2005; DiTomaso *et al*., 2013). In the Illinois River basin, for example, attached *Cladophora* was found to grow optimally under high P enrichment (600 μg TP/L; Leland and Porter, 2000).

Among the most renowned habitats for *Cladophora* blooms are the Laurentian Great Lakes. In the 1960s–1970s, *Cladophora* (*C. glomerata* and others) proliferated in the west basin of Great Lake Erie in response to P pollution, then drifted into shore in rotting masses from major seasonal die-offs that were sometimes measured in tonnes of fresh weight (Higgins *et al*., 2005, 2008). Major reductions in point-source inputs of P through upgrades in wastewater treatment and detergent P bans led to a dramatic reduction of *Cladophora* during the 1970s and early 1980s, but the Great Lakes soon became a *Cladophora* story of "déjà vu" (Higgins *et al*., 2008 and references therein). Invasions of zebra mussels followed by quagga mussels (*Dreissena bugensis*) resulted in their domination of nearshore benthic environments by the late 1980s. The increased metabolic wastes from mussels were a major source of internal P loading, and their filtering activity cleared the water and increased light penetration (Hecky *et al*., 2004; Auer *et al*., 2010). By the 1990s, the mean peak biomass of *Cladophora* was similar to historic values in Lake Erie during the 1960s–1970s, and has remained so (Auer *et al*., 2010; Tomlinson *et al*., 2010); surface mats can extend more than 6.1 m out into the water (Stauffer, 2005). From 1995 to 2002, the northern shoreline of the eastern basin had maximum production of ~12,000 tonnes dry mass during the spring growth phase and removed an estimated 15 tonnes of P within 30 days (Higgins, 2005; Higgins *et al*., 2005); shorelines along portions of Lakes Ontario, Michigan, and Huron are also being fouled by rotting *Cladophora* growth (Garrison and Greb, 2005; Higgins *et al*., 2008).

The Canale and Auer model, later modified as the *Cladophora* Growth Model (CGM) and then as the Great Lakes *Cladophora* Model (GLCM), has been successfully validated on field populations in multiple locations of the Great Lakes (Tomlinson *et al*., 2010 and references therein). The models indicated that *Cladophora* growth

extended to deeper waters post-*Dreissena*, and that *Dreissena*-induced changes in water quality were responsible for the dramatic resurgence of *Cladophora* (Higgins *et al*., 2006). While dreissenid mussel abundance is a major factor controlling the magnitude of *Cladophora* production, catchment land cover and nearshore water quality (nutrient levels and suspended solids) are also important (Depew *et al*., 2011); P from dreissenid mussel wastes apparently is insufficient to produce severe blooms without localized P enrichment (Higgins *et al*., 2012); and P management from land-based sources "remains the appropriate mechanism for reducing nuisance levels of *Cladophora* growth" (Auer *et al*., 2010, p. 248).

Most ecological information about freshwater filamentous macroalgae is for *Cladophora*, which has overgrown and displaced beneficial aquatic plants, reduced invertebrate densities and fish spawning, and reduced species biodiversity (Neil, 1975; Ozimek *et al*., 1991; Zulkifly *et al*., 2013 and references therein). Decaying mats of *Cladophora* have caused or contributed to anoxia, resulting in kills of other aquatic life (Burkholder, 2009 and references therein), and the mats have retained pathogenic microbes that cause human disease as explained above. The substantial contribution of *Cladophora* (and, more recently, *Lyngbya wollei*) blooms to hypoxia/anoxia zones in Lake Erie has threatened the habitats and food resources needed by sport fish such as walleye (*Sander vitreus*) and yellow perch (*Perca flavescens*) (Arend *et al*., 2011; Hinderer *et al*., 2011). Recently, however, it has been increasingly recognized that *Cladophora* also can act as an ecosystem or ecological engineer (i.e., an organism that creates, modifies, and maintains habitat – Jones *et al*., 1994) by increasing benthic habitat complexity, providing spatial refugia, enhancing sedimentation, reducing current velocity, and shading substrata (Ward and Ricciardi, 2010; Zulkifly *et al*., 2013; note that similar findings have been reported for *Cladophora* in estuarine habitats – Kraufvelin and Salovius, 2004). This alga also transforms key resources such as P and C from one form to another, thereby controlling resource supplies and cycling on scales that may influence global littoral biogeochemistry (Zulkifly *et al*., 2013).

15.9.3 Estuarine and Coastal Marine HAB

Seagrass meadows are a common feature of many shallow estuaries and coastal waters in temperate, subtropical, and tropical regions. Seagrasses are highly productive and provide important ecological services, including biodiversity, fisheries habitat, and sediment stabilization, all of which are important to local and regional economies. Like all plants, they require nutrients to grow. In excess, however, nutrients cause decline of seagrass communities (Burkholder *et al*., 2007; Cabaço *et al*., 2013) as a result of several factors, including macroalgal blooms. Seagrasses grow relatively slowly in comparison to macroalgae, which can double their biomass in only a few days under nutrient-enriched conditions (Whitehouse and Lapointe, 2015). They also have higher light requirements than many macroalgae, so shading from increased epiphytic fouling, macroalgal overgrowth, and phytoplankton blooms reduces their productivity and growth. Macroalgae can initially grow as epiphytes on seagrasses and eventually break loose, forming thick drift mats that block light from reaching the underlying seagrasses, leading to the loss of seagrass meadows (Burkholder *et al*., 1992, 2007; Lapointe *et al*., 1994; Hauxwell *et al*., 2001b; McGlathery, 2001).

On the U.S. West Coast, nutrient enrichment from upwelling can contribute to the formation of macroalgal blooms that, in some locations, affect the native eelgrass (*Zostera marina*). On the Oregon coast, spatial and interannual variation in macroalgal abundances positively correlates with upwelling (Hessing-Lewis and Hacker, 2013). Although eelgrass abundances were negatively correlated with macroalgal abundances over large spatial scales, there was no temporal correlation. Field experiments conducted in the Coos Bay Estuary, Oregon, demonstrated that ulvoid macroalgae can cause declines in eelgrass abundance at riverine sites, but not at marine sites, suggesting that increased macroalgal growth due to upwelled nutrients along the Oregon coast impacts eelgrasses that experience other physiological stresses such as light limitation (Hessing-Lewis *et al*., 2011). Because upwelling in this region results in naturally high nutrient concentrations in marine and estuarine waters, it has been suggested that even small anthropogenic inputs of nutrients could cause nutrient levels to exceed a "tipping point," leading to increased algal growth in nearshore waters (Thom and Albright, 1990).

Coral reefs are among the most productive and biologically diverse ecosystems in the world. Coral reefs are distributed in nutrient-poor surface waters in the tropics and subtropics, and increases in nutrient loading can result in regime shifts away from coral to dominance by macroalgae and smaller filamentous algal turfs as noted previously

(Bell, 1992; Lapointe, 1997; NRC, 2000; Fabricius, 2005). For example, in Kane'Ohe Bay, Hawai'i, increased nutrient loading from sewage outfalls in the 1960s led to blooms of the green alga *Dictyosphaeria cavernosa* (Smith *et al.*, 1981). Since then, invasions of non-native macroalgae have led to harmful macroalgal blooms in coastal waters of the Hawaiian Islands, including urbanized areas in Maui where sewage pollution has led to blooms of *Hypnea musciformis* and *Ulva* spp. (Dailer *et al.*, 2012). In highly urbanized southeast Florida, sewage pollution was a primary factor causing a succession of macroalgal blooms and invasions on fringing coral reefs. Spectacular blooms of unattached *Codium isthmocladum* developed during summer months in 1989–1990 on deep reefs (24–43 m) off southern Palm Beach and northern Broward counties (Figure 15.3f and 15.3g); these were followed by blooms of the non-native *Caulerpa brachypus* in the late 1990s (Figure 15.3d). Studies measuring carbon: nitrogen: phosphorus ratios (C:N:P) and stable nitrogen isotopes ($\delta^{15}N$) in macroalgal tissue linked these blooms to nutrient enrichment from sewage outfalls and land-based runoff (Lapointe *et al.*, 2005a, 2005b; Lapointe and Bedford, 2010). Despite a well-funded Water Quality Protection Program since 1991, macroalgal blooms have also increased in frequency and extent throughout the Florida Keys National Marine Sanctuary (Figure 15.3h, 15.3i, and 15.3j). A distinct increase in the frequency and extent of these blooms followed the political decision to increase freshwater discharges and N-loading from the Everglades to Florida Bay between 1991 and 1995 (Lapointe *et al.*, 2004, 2007; Collado-Vides *et al.*, 2007). In response to this increased N-loading, coral reefs in southern Florida Bay were overgrown by thick mats of *Cladophora* spp. (Lapointe *et al.*, 2007), which respond to increasing N enrichment as inorganic N or urea by producing blooms (Zulkifly *et al.*, 2013). Research by Lapointe *et al.* (2004) in the lower Florida Keys indicated that both agricultural runoff and local sewage discharges from septic effluent leachate were significant N sources supporting blooms of the filamentous macroalgae *Cladophora catenata* (Chlorophyta, Ulvophyceae) and *Cladosiphon occidentalis* (Heterokontophyta, Phaeophyceae) in coral reef and seagrass ecosystems, respectively. Similar blooms of *Cladophora* spp. have developed in N-enriched environments in Bermuda (Lapointe and O'Connell, 1989) and Hawai'i (Smith *et al.*, 2005).

Benthic cyanobacterial mats are considered rare on healthy coral reefs, but proliferate on damaged colonies and coral rubble and form loose mats over sandy sediments (Golubic *et al.*, 2010). Highest abundance of these mats has been found on sheltered reefs close to urbanized areas, which has been related to anthropogenic nutrient pollution (Brocke *et al.*, 2015). Reefs with high abundance of benthic cyanobacterial mats have also been characterized by high benthic macroalgal cover and depressed cover of corals (Brocke *et al.*, 2015). Growth of the noxious inhabitant of coral reefs, *Lyngbya majuscula*, has been experimentally stimulated by P, N, and iron enrichment (Ahern *et al.*, 2008).

15.10 Effects of Blooms on the Chemistry of the Oceans and the Atmosphere

Macroalgal blooms consist of high biomass of algae that respire, photosynthesize, and take up and release inorganic and organic compounds from and into seawater, and thus can cause significant changes in seawater chemistry. Large blooms of intertidal and shallow subtidal seaweeds may also affect atmospheric chemistry by emitting volatile compounds either directly into the air or into shallow waters where they eventually cross the sea–air boundary and become airborne.

15.10.1 Changes to Carbonate Chemistry and pH

High macroalgal biomass can affect gas levels in the surrounding seawater through physiological processes (respiration and photosynthesis), by physically altering the air–sea interface, and by promoting bacterial growth. The specific patterns of dissolved gas changes will depend on the size and physiological state of the bloom as well as topographical features of the site and localized water flows.

CO_2 or bicarbonate (HCO_3^-) is removed from seawater during the day by photosynthesizing macroalgae, therefore daytime pH levels near macroalgal accumulations can be dramatically higher than nighttime levels (Middleboe and Hansen, 2007; Saderne *et al.*, 2013). In waters near a small ulvoid algal bloom in Washington State, the difference between daytime and nighttime pH levels exceeded 1.5 pH units during spring–early summer tides (Van Alstyne *et al.*, 2015a), well above the average pH changes expected due to ocean acidification. The high-pH and low-carbon

conditions generated by ulvoid algae in tide pools can reduce photosynthetic rates of other algae (Bjork *et al*., 2004). Similarly, photosynthetic rates of eelgrass (*Zostera marina*) growing beneath a single layer of *Ulva* were reduced in part because of the high pH caused by algal photosynthesis, but also because of the lower levels of light available to the eelgrass (Mvungi *et al*., 2012).

15.10.2 Release of Materials and Chemicals into Seawater

Macroalgal blooms are frequently ephemeral and can be a source of particulate matter and dissolved organic and inorganic compounds when chemicals leak through cell membranes of healthy algae or are released as algae senesce or decompose (e.g., Sieburth and Jensen, 1969; Hanson, 1977; Boyer and Fong, 2005). The production of this material can have ecological consequences, including providing a supply of nutrients and carbon for microbial and detritivore communities and moving nutrients from algae into nearby waters and sediments (Nielsen *et al*., 2004; Hardison *et al*., 2010).

Some bloom-forming seaweeds also produce and release toxic or allelopathic organic compounds that affect organisms in nearby pelagic or benthic communities. Many red macroalgae have gland cells with inclusions that contain halogenated (bromine, iodine) substances with antimicrobial, antiherbivore, or other allelopathic functions (Paul *et al*., 2006). Red algae produce the widest variety of toxic secondary metabolites among the algae, including many halogenated terpenoids and even domoic acid (Graham *et al*., 2016 and references therein).

Many species of ulvoid green algae produce allelochemicals whose effects include reducing densities of barnacles in tidepools (Magre, 1974); causing mortality in crab larvae (Johnson and Welsh, 1985), oyster larvae (Nelson *et al*., 2003a; Nelson and Gregg, 2013), and juvenile abalone (Wang *et al*., 2011); inhibiting the growth of planktonic microalgae (Jin and Dong, 2003; Wang *et al*., 2009; Tang and Gobler, 2011) and benthic macroalgae (Nelson *et al*., 2003a); and reducing fouling by epiphytic bacteria, algae, and invertebrates (Egan *et al*., 2000; Nelson *et al*., 2003b; Harder *et al*., 2004; Hellio *et al*., 2004).

The compounds mediating these interactions have been identified in some cases. For example, the bloom-forming ulvoid alga *Ulvaria obscura* releases dopamine into the surrounding seawater when it is stranded during low tide, becomes desiccated, and is rehydrated during an incoming tide (Van Alstyne *et al*., 2011, 2013). In seawater, dopamine oxidizes to form a variety of quinones. Dopamine or the quinones resulting from it reduce the growth and germination rates of other seaweeds and increase mortality rates of crab zoeae (Van Alstyne *et al*., 2014). All ulvoid algae examined to date produce dimethylsulfoniopropionate (DMSP), a small sulfonium compound (Van Alstyne, 2008) that has been shown to inhibit the growth of epiphytic bacteria on *Fucus vesiculosus* (Saha *et al*., 2014). Although the effects of DMSP from ulvoid algae have not been examined, DMSP may be responsible for mediating many of the allelopathic interactions involving these algae. The invasive bloom-forming macroalga *Caulerpa racemosa* produces several related sesquiterpenes that can have biological activity (Amade and Lemée, 1998). One of them, caulerpenyne, causes decreases in the photosynthetic efficiency of a native Mediterranean seagrass, *Cymodocea nodosa*, at concentrations of 10 ppm (Raniello *et al*., 2007), which may help the alga outcompete native macrophytes. Fucoid brown algae produce and release phlorotannins, phloroglucinol-based polyphenolic compounds (Amsler and Fairhead, 2005). Phlorotannins from *Sargassum natans* and *S. fluitans* have been shown to inhibit the growth of epiphytic bacteria and invertebrates (Sieburth and Conover, 1965).

Another group of harmful molecules that is released by macroalgae is reactive oxygen species (ROS). ROS are produced as a result of photosynthesis and respiration (Halliwell and Gutteridge, 2015; Lesser, 2006; Bischof and Rautenberger, 2012) and can damage lipids, proteins, and DNA (Fridovich, 1978; Asada and Takahashi, 1987; Halliwell and Gutteridge, 1989), but are also used as signaling molecules in stress responses to changes in temperature, salinity, and desiccation (Miller *et al*., 2008). Most ROS are scavenged by a variety of enzymatic and non-enzymatic antioxidants (Ledford and Niyogi, 2005); however, when algae are not able to scavenge ROS, which can occur when they are stressed, and the production of ROS increases or antioxidant production decreases (Collén and Davison, 1999a, 1999b, 2001), ROS can diffuse into the surrounding seawater where they can impact other organisms (Collén and Pedersén, 1994; Collén and Davison, 1997; Küpper *et al*., 2001; Abrahamsson *et al*., 2003; Choo *et al*., 2004; Barros *et al*., 2006; Van Alstyne *et al*., 2013). The generation and release of ROS during microalgal blooms can be toxic toward fish (see Chapter 7 of this volume). Seaweeds, including species that form large blooms, also release ROS

(van Hees and Van Alstyne, 2013); however, little is known about the effects on nearby organisms.

15.10.3 Release of Volatile Compounds

During low tide, marine macroalgae release into the atmosphere complex mixtures of small, volatile organic compounds (Moore, 1977; Paul and Pohnert, 2011), which can be the source of the characteristic odors of macroalgal blooms. These compounds include a variety of chemical types, such as halomethanes, halogenated hydrocarbons, halogenated and non-halogenated terpenes, aromatic compounds, oxylipins, and small sulfur compounds (Paul and Pohnert, 2011). Several of these compounds, especially isoprene and brominated organic compounds, can affect ozone depletion (Schauffler *et al*., 1999; Quack *et al*., 2004). The biogenic production of dimethyl sulfide (DMS) can contribute to acid precipitation and climate change, and has been hypothesized to impact cloud formation in remote environments (Charlson *et al*., 1987). Whether large growths of seaweeds are important sources of atmospherically significant volatile compounds is not well known. Bromoform and methyl bromide production by kelps (Manley *et al*., 1992) and the production of halogenated compounds by large seaweed farms (Leedham *et al*., 2013) have been suggested to be large enough to influence atmospheric chemistry. In contrast, with the exception of the release of volatile sulfur compounds by ulvoid algae, little is known about the potential significance of the release of volatiles by seaweed blooms.

In ulvoid algae, the biochemical cleavage of dimethylsulfoniopropionate (DMSP) to form DMS is triggered by a number of environmental factors, including physical damage from grazers (Van Alstyne and Houser, 2003; Van Alstyne *et al*., 2009), decreases in salinity, increases in seawater temperature, and especially desiccation (Van Alstyne *et al*., 2015a). Ulvoid algae respond in a species-specific manner to these stresses. For example, the low intertidal alga *Ulvaria obscura* increases DMS emissions strongly in response to hyposaline conditions, warm (35 °C) temperatures, and desiccation, whereas the high intertidal *Ulva intestinalis* dramatically increases DMS emissions when dried, but does not alter DMS emissions in response to changes in salinity or seawater temperatures (Van Alstyne *et al*., 2015a). The production of DMS and other volatile sulfur metabolites, including hydrogen sulfide (H_2S), occurs over *U. lactuca* mats in Danish estuaries at low tide and is in the range of 1–3 umol S m^{-2} h^{-1} (Jørgensen and Okholm-Hansen, 1985). At the end of the growing season, when ulvoid macroalgae senesce or decay, anaerobic bacteria that release H_2S often utilize these algae as substrate (Nedergaard *et al*., 2002), leading to ecological consequences. For example, H_2S trapped under decomposing mats of *Ulva* is the suspected cause of death for a horse and 30 wild boars on the coast of Brittany, France (Charlier *et al*., 2007). H_2S is also toxic to many other organisms, including humans as well as beneficial aquatic life (Bagarinao, 1992; Lamers *et al*., 2013). High sulfide concentrations in the water column have been implicated in mass mortalities of fish and other aquatic life (Bagarinao, 1992 and references therein).

15.11 Management Strategies

Because of the negative environmental and economic impacts that are often associated with harmful macroalgal blooms, government agencies and private stakeholders have increasingly sought various strategies for management and mitigation, although the main historic approaches, physical removal and herbicide treatment, remain common. The strategies used for a given bloom depend on the cause(s) and physical characteristics of the local setting, and can include multiple approaches. For example, herbicides such as copper sulfate have been used for decades to control algal blooms in freshwaters, and are effective for loose mat formers such as *Spirogyra* (Lembi, 2003 and references therein). However, dense mats of the cyanobacterium *Lyngbya wollei* or *Pithophora oedogonia* can block penetration of such chemicals (Lembi, 2003 and references therein), leaving managers with choices such as physical harvest or, if possible, draining ponds followed by application of bleach pellets (e.g., see Poovey and Netherland, 2006; Bishop *et al*., 2015; note that control efforts of this species have also been impeded by the presence of both surface and benthic mats). Triploid grass carp (*Ctenopharyngodon idella*), restocked as necessary, are effective in removing nuisance charaleans, but their effectiveness in controlling filamentous algal mats is mixed (Lembi, 2003 and references therein). Waterfowl (geese or swans) consume filamentous algae, and charaleans are favored food of herbivorous ducks, coots, and swans; however, waterfowl introduced for macroalgal control are usually flightless, require diet supplements for adequate nutrition, must be protected

from predators, are aggressive in breeding season, and excrete wastes that can accumulate along shorelines and stimulate phytoplankton blooms (Lembi, 2003 and references therein). Overall, Lembi (2003, p. 826) concluded that for freshwater macroalgae, "Methods for direct control . . . are available, but none of them ensure that problems will be solved other than in the short term."

In situations where nutrient pollution from a point source of sewage or other nutrient source has been identified as a controlling factor, *reduction of nutrient loading at the source* is effective at moderating or even terminating the bloom (NRC, 2000). Regarding freshwater *Cladophora* in the Great Lakes, for example, Harris (2005, p. 11) described P supply reduction as "the only feasible option" for biomass reduction, although noting that decreased external P loads may be insufficient to overcome the internal P loading from exotic/invasive dreissenid mussels on *Cladophora* abundance (Bootsma *et al*., 2004). In Tampa Bay, sewage nitrogen was recognized as a primary driver of eutrophication in the 1970s, including extensive blooms of macroalgae *Ulva*, *Gracilaria*, *Spyridia*, and *Chaetomorpha* in shallow waters of the portion of Tampa Bay known as Hillsboro Bay (Greening *et al*., 2014). The offensive odors of decomposing macroalgae along the shorelines provided the key impetus for government agencies and stakeholders to develop plans for N removal from the local wastewater treatment plant. In the following decades, a 90% reduction in N loading to Tampa Bay correlated with diminishing macroalgae and phytoplankton blooms and expansion of seagrasses throughout the bay. Today, this highly urbanized subtropical estuary provides a successful case study of how sound science can lead to successful management and termination of macroalgal blooms, and simultaneously leads to recovery of seagrasses (Greening *et al*., 2014).

Efforts to control harmful blooms in Hawai'i via nutrient reduction have produced mixed results. Diversion of sewage effluents from Kane'ohe Bay, which caused decreases in water-column DIN, was expected to reduce the abundance of *D. cavernosa*; indeed, a marked reduction in algal abundance occurred in the central bay where pre-diversion biomass levels on reef slopes were the highest in the entire bay (Smith *et al*., 1981). The lack of a response to water-column DIN reductions on reef flats may have been due to the lower biomass levels and the alga assimilating nutrients from the sediments and invertebrate excretions (Stimson *et al*., 2001). In this system, herbivores did not reduce the abundance of *D. cavernosa* on reef flats because algal species were available that were more preferred as food resources (Stimson *et al*., 2001).

Physical removal of *Cladophora* accumulations in freshwaters has been described as a short-term mitigation strategy (Harris, 2005). Smaller accumulations have been removed by frequent hand-raking followed by composting or landfilling. Mechanical removal of large accumulations, especially on public beaches, has been successful using front-end loaders, backhoes, or beach-grooming equipment, with the following caveat: the heavy equipment can grind the decaying algae down into the sand, which can stimulate growth of fecal bacteria such as *E. coli* (Harris, 2005). In addition, successful cleanup requires removal of the algal mats as soon as they wash ashore, because they can quickly decay into what has been described as an "organic soup that is extremely difficult to collect and remove" (Harris, 2005, p. 12).

Increasingly, popular tourist destinations have had to develop management strategies to harvest and remove excessive macroalgae from marine beaches. Beaches in Texas, United States, including Galveston, Padre Island, and Port Aransas, have sustained more frequent strandings of *Sargassum* in recent decades. Unlike the Caribbean region, where booms (Figure 15.5e) have been widely deployed to prevent landfall of *Sargassum* on beaches, management in Texas and other areas of the United States has been restricted by the federal Fishery Management Plan for *Sargassum*, which prevents any harvesting within about 161 km (100 miles) of shore (South Atlantic Fishery Management Council [SAFMC], 2002). On Galveston Island, the Galveston Island Board of Trustees rakes with the tractor-towed Barber Surf Rake (Figure 15.5a and 15.5b), which is available in three models. Recent studies comparing raked and unraked beaches at Galveston found no differences in beach height, although the study did not consider horizontal seaward expansion or change in slope of the beach (Williams *et al*., 2008). The Barber Surf Rake is widely used around the United States, and was used in summer of 2015 to clean *Sargassum* from beaches in Key West, Florida.

In May 2014, unusually large quantities of *Sargassum* washed ashore in Galveston. In situations like this when *Sargassum* mats on the beach become too high for the Barber Surf Rake or other beach-grooming equipment, front-end loaders are used to remove the seaweed to the dune line. In Key West, Florida, the height threshold is 25 cm. When *Sargassum* is higher than this, front-end

loaders are used in combination with dump trucks to transport the *Sargassum* to a desired location. In Port Aransas, this activity required a U.S. Army Corps of Engineers permit to move sand from the beach below the high-tide line. Similar issues are of concern in Florida, where the Florida Department of Environmental Protection does not allow collection of *Sargassum* below the water line. In addition to front-end loaders and dump trucks, graders are used to groom the beach following removal of *Sargassum*. Physical removal of macroalgae from coral reefs in Kaneohe Bay, Hawai'i, by use of the "super sucker" (Figure 15.5c and 15.5d) is labor-intensive and expensive, and tends to produce only temporary reductions in biomass (Smith *et al*., 2004; Weijerman *et al*., 2008).

There are few options available to effectively manage *Codium fragile* spp. *tomentosoides* (Global Invasive Species Database, 2016):

> Chemical herbicides are not a viable option of control and end up doing harm. Mechanical removal techniques such as trawling, cutting, and suctioning may reduce density temporarily, but they are expensive and the populations will quickly rebound. Manual removal will not work either. *C. fragile* spp. *tomentosoides* readily reproduces from fragments. There are a variety of naturally occurring organisms that feed on *C. fragile* spp. *tomentosoides*, but no one or combination of species can offer sufficient control [as] these species do not readily discriminate between the native and introduced *C. fragile* subspecies. Preventing the spread of *C. fragile* spp. *tomentosoides* through quarantine measures and public education are [among] the only ways to insure it does not spread.

These examples illustrate the present status, that is, the general difficulty in developing management strategies that are both economical and effective in reducing the biomass of macroalgal blooms, other than as short-term measures.

15.12 Economic Impacts

Harmful macroalgal blooms have been associated with various detrimental social and ecological impacts, such as inhibited recreation, diminished aesthetic enjoyment of the coastal zone, and interference with tourism, fishing, and mariculture (Lyons *et al*., 2014 and references therein), all of which lead to economic impacts. The toxic H_2S emitted from mats of rotting macroalgae can threaten human health (Chrisafis, 2009; Samuel, 2011). These noxious blooms also depress biodiversity and alter ecological processes as well (Fletcher, 1996; Raffaelli *et al*., 1998; Lyons *et al*., 2014), with potential adverse economic effects. Yet, the economic impacts of harmful macroalgal blooms are very poorly tracked, despite general acceptance that these blooms cause major aesthetic loss, adversely affect swimmers and other beachgoers, negatively affect tourism on beaches, and dramatically affect both aquatic ecosystems and recreational/commercial fisheries.

An example of economic impacts due to macroalgal HAB is the Great Lakes *Cladophora* problem. The presence of rotting *Cladophora* mats that sequester fecal bacteria at many beaches along the shores of Lakes Michigan and Erie is believed to have led to beach closures, and closing a Lake Michigan beach was estimated to cause economic losses as high as $37,000 U.S. per day in 2003 dollars (Rabinovici *et al*., 2004). Moreover, in 2006–2007, only 47% of U.S. beaches along Lake Erie were open for 95% or more of the beach season, and water quality continues to deteriorate (Environment Canada and the U.S. Environmental Protection Agency [EPA], 2009). Massive rotting *Cladophora* mats have led to property depreciation in the Milwaukee region, and financial burdens for industries with water intakes in Lakes Erie and Michigan, such as shutdown of a nuclear power plant because *Cladophora* clogged its emergency cooling pumps (Bootsma *et al*., 2004). Along the U.S. shores of Lake Ontario, nuisance filamentous algal impacts were noted by nearly half (19 of 42) of business respondents (Limburg *et al*., 2010). Most of those respondents (18 of 19) described decreased revenues; about half of them (9 of 19) altered some of their goods and services because of the nuisance filamentous algae, such as moving docks, washing boats more frequently, pumping out algal clumps from marinas, and selling beach rakes. Some respondents reported having spent at least $1,000 per season to remove algae, and one marina owner spent $11,200 (Limburg *et al*., 2010). The regional Great Lakes recreational fishery had an estimated value exceeding $7 billion U.S. annually as of a decade ago (Southwick Associates, 2007), and *Cladophora* blooms have threatened the habitats and food resources needed by sport fish such as walleye (*Sander vitreus*) and yellow perch (*Perca flavescens*) (Arend *et al*., 2011; Hinderer *et al*., 2011). The value of real estate on lakefront (Lake Erie) with *Cladophora* mats was reported to average only 80–85% of the value of clean frontage (Ormerod, 1970). Water intakes to

Figure 15.5 Some examples of mechanical methods for mitigating macroalgal blooms on beaches and in coastal waters. (a,b) The Barber surf rake used to remove macroalgae from beaches; *Source:* photos courtesy of H. Barber & Sons, Inc. (c,d) the "super sucker" used for removing invasive red macroalgae from coral reefs in Kane'ohe Bay, Hawai'i; *Source:* photos by B. Lapointe. (e) floating booms used to deflect pelagic *Sargassum* from coastal properties in the Caribbean. *Source:* photo courtesy of D. Jimenez.

power plants have been clogged by *Cladophora*, leading to power outages, and human health and safety on beaches has been threatened by *Cladophora* mats laden with pathogenic microbes as explained above. Unfortunately, despite many years of major impacts, an overall, quantitative economic analysis of *Cladophora*-related economic costs in the Great Lakes is not yet available.

Species of *Cladophora* are also well known in the extensive irrigation systems and aqueducts of the western United States, where they reduce flow rate and canal capacity (Lembi *et al*., 1988; Lembi, 2003; Ross, 2006 and references therein). Detached mats float downstream and clog pump inlets, irrigation siphons, trash racks, and sprinkler heads (Hansen *et al*., 1984 in Lembi, 2003). More generally, clogging of rivers, canals, and drainage ditches by *Cladophora* and other filamentous algae can cause flooding events (Lembi, 2003). Yet, only a few dated descriptions of associated economic costs in specific locales are available (Lembi *et al*., 1988; Lembi, 2003 and references therein).

Macroalgal blooms are having increasing impacts on coastal economies through loss of real estate values and tourism as well as escalating beach cleanup costs. Along Maui's Kihei coast in Hawai'i, over $20 million U.S. per year in tourism revenues and property values have been lost as a result of problems associated with blooms of the rhodophyte *Hypnea musciformis* (Cesar and Van Beukering, 2004; http://www.hawaii.edu/ssri/hcri/ev/kihei_coast.htm). In Maui County, some $250,000 U.S. is spent annually by condominium owners to remove excessive seaweed biomass from the beaches. In the Peel Inlet, Australia, removal of seaweeds cost $160,000 U.S. annually for 13,000 m^3 of macroalgae (Atkins *et al*., 1993). In France, the cost exceeded 3.6 million francs for 90,000 m^3 of green tides removed from the Brittany coastline in 1992 (Centre d'Etude et de Valorisation des Algues [CEVA], 1993). In Lee County, Florida, costs of beach seaweed removal programs were historically nominal but increased dramatically to $260,503 in fiscal year 2003/2004 with the onset of drift rhodophyte blooms (Lapointe and Bedford, 2007).

Expensive management actions followed the massive *Sargassum* landings on Caribbean beaches between 2011 and 2015. Manual labor activities involving *Sargassum* collection and removal from resort beaches were widespread, and beach management costs up to $33,000 per week were reported to maintain a typical resort with ~1000 ft. of beach. In Cancun and the Riviera Maya, the Mexican government spent up to $10 million U.S. in 2015 on the problem. Funds were used to hire 5000 temporary workers and equipment for *Sargassum* removal, as well as for field tests of methods to prevent *Sargassum* from coming ashore; this involved the Mexican Navy (Alexander, 2015). Booms have been deployed in a variety of locations around the Caribbean, including Mexico, to deflect *Sargassum* from beaches, bays, and coastlines (Figure 15.5e).

15.13 Recycling Macroalgae Biomass

There has been relatively little exploration of freshwater macroalgae for beneficial use in, as examples, fertilizers or biofuels. Focus has mostly been directed toward *Cladophora* spp., which have been described as relatively "easy" to harvest using meshes or mechanical scraping (Zulkifly *et al*., 2013). The harvested material has been suggested for use in extracting P to supply agricultural fertilizers (Zulkifly *et al*., 2013). Cellulose extracted from *Cladophora glomerata* has been hydrolyzed to provide glucose for the cultivation of bacteria that have been genetically engineered to produce desirable fatty acid precursors to biodiesel fuel (Hoover *et al*., 2011). The epiphytic diatoms on *Cladophora* may also be a promising source of fatty acids; it was estimated that *Cladophora*-dominated diatom-rich periphyton grown in wastewater effluent at U.S. wastewater treatment plants could generate about 7.6 billion L (2 billion gallons) of biofuel annually (Graham *et al*., 2012). Biomass of *Cladophora* spp. may also be a potential source of cyclic tetrapyrrolic photosensitizers for photodynamic therapy (Tang *et al*., 2012).

Many seaweeds are harvested commercially for use in the food, nutraceutical, cosmetic, agricultural, and biofuel industries (Zemke-White and Ohno, 1999; Dominguez, 2013; Wei *et al*., 2013; Balboa *et al*., 2015), and there is much potential for beneficial uses of species that form blooms. Many seaweeds, including *Ulva* spp., are edible (Chapman and Chapman, 1980) and could be used for human consumption, as well as livestock and aquaculture feeds (Bolton *et al*., 2009; Michalak and Chojnacka, 2009). In general, seaweeds have a number of qualities needed to be good sources of compost, although concentrations of accumulated metals or phytotoxins can be a concern (Han *et al*., 2014). Due to high C:N ratios, macroalgae are often mixed with other compostable materials to prevent ammonia volatilization (Han *et al*., 2014). Additionally, macroalgae can be used as feedstocks for the production of biochar, defined as a solid material obtained from the carbonization of biomass used to improve soil conditions (Milledge and Harvey, 2016). Macroalgae can also be used for the production of biofuels, such as ethanol (Huesemann *et al*., 2010) and biomethane (Langlois *et al*., 2012); however, most seaweeds are not good candidates for biodiesel production because of low lipid contents. A recent review of energy extraction from macroalgae concluded that it is

too early at the current stage of biofuel development to select definitively what method or combinations of methods for exploiting energy from macroalgae will be commercially feasible (Milledge *et al.*, 2014).

Some bloom-forming species produce specific products that are commercially desirable. For example, brown algae, such as *Sargassum* spp., are typically high in polyphenolic compounds (Amsler and Fairhead, 2005), which are antioxidants (Shibata *et al.*, 2008). Fucoidans, sulfated polysaccharides, are also produced by brown algae and have been reported to have antioxidant, antiviral, and anti-inflammatory activity (Vo and Kim, 2013). Macroalgae are often grown in order to harvest commercially important cell wall components, such as alginate, agar, and carrageenan (McHugh, 1991), and blooms could also be a source of these products.

15.14 Forecast

Given the projected trajectories for increased cultural eutrophication and climate change in the United States and worldwide (Intergovernmental Panel on Climate Change, 2015; World Resources Institute, n.d.), harmful macroalgal blooms ranging from freshwaters to brackish and coastal marine waters are predicted to increase in the coming decades. Considering benthic filamentous cyanobacteria, Quiblier *et al.* (2013) wrote, "As climatic conditions change and anthropogenic pressures on waterways increase, it seems likely that the prevalence of blooms of benthic cyanobacteria will increase." Blooms of benthic filamentous cyanobacteria and filamentous green algae tend to be promoted by higher temperatures and nutrient pollution (Burkholder, 2009 and references therein; O'Neil *et al.*, 2012). While more storms and rainfall would accelerate freshwater delivery of nutrients and flushing, longer drought periods would favor freshwater HAB by decreasing flushing and increase internal nutrient cycling. In coastal areas, predicted sea-level rise would increase the extent of continental shelf areas, providing shallow, stable coastal habitats that could favor macroalgal growth and/or expand suitable habitats inland (Harley *et al.*, 2012; Teichberg *et al.*, 2012 and references therein).

Increasing ocean acidification may also promote the growth of macroalgae and lead to an increase in blooms. As seawater pH decreases, the percentage of dissolved inorganic carbon (DIC) that occurs as HCO_3^- increases. The majority of marine macroalgae studied to date (> 85%) use a C3 photosynthetic process, including use of HCO_3^- as a carbon source (Koch *et al.*, 2013). In nutrient-replete environments, increasing ocean acidification could lead to more rapid growth rates and more frequent or larger blooms of algae that are not DIC-saturated.

Considering the dramatic rise in macroalgal blooms in response to eutrophication in recent years, more research is critically needed on effective methods for harvesting the biomass and recycling for beneficial purposes in mitigating climate change. In the 1970s, fast-growing red seaweeds such as *Gracilaria tikvahiae* were shown to be effective for recycling nutrients from municipal sewage into marine biomass with beneficial purposes, such as hydrocolloids, biofuels, or fertilizers (Ryther *et al.*, 1978). Given the rising energy cost of chemical fertilizers and the increasing opposition to usage because of environmental impacts, research is needed on the potential for conversion of macroalgal biomass into natural, low-cost, sustainable soil additives (N'Yeurt and Iese, 2014). Similar research efforts are needed on production of biofuels and carbon sequestration. For example, the high C:N ratio (50:1) of pelagic *Sargassum* in the Sargasso Sea (Lapointe, 1995), combined with the tendency of this macroalga to sink to the deep-sea floor, makes it an efficient target species to sequester carbon in the oceans compared to phytoplankton, which typically have C:N ratios < 10:1 (Smetacek and Zingone, 2013). The future of harmful macroalgal blooms could be much different if they become regarded as potential crops with beneficial uses rather than excessive biomass that adversely affects the health of freshwater and marine ecosystems.

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16

Harmful Algal Species Fact Sheets

Harmful Algal Blooms: A Compendium Desk Reference, First Edition. Edited by Sandra E. Shumway, JoAnn M. Burkholder, and Steve L. Morton.

Alexandrium

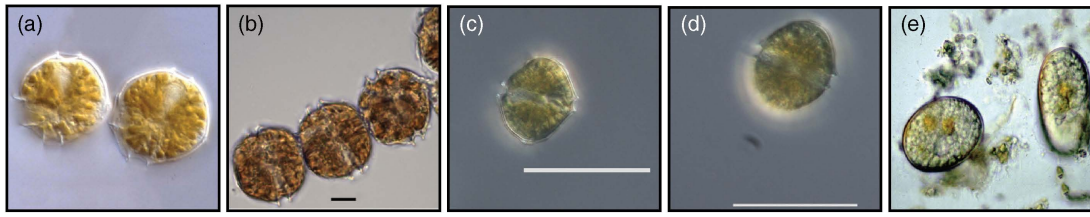

Figure 1 Optical micrographs of major toxigenic *Alexandrium* corresponding with original morphometric descriptions of vegetative cells. (a) *Alexandrium tamarense* (Lebour) Balech (featured specimen would belong to emended *A. catenella*, Prud'homme van Reine, 2017); (b) *Alexandrium catenella* (Whedon & Kofoid) Balech; (c) *Alexandrium minutum* Halim; (d) *Alexandrium ostenfeldii* (Paulsen) Balech et Tangen; (e) Live resting cysts (hypnozygotes) of *A. catenella* (formerly *A. fundyense*). Scale bar = 20 µm (b); 50 µm (c,d). *Photo credits*: N. Lewis, IMB, NRC, Halifax, Canada (a); A. Perez, FICOLAB, Universidad de Concepción, Chile (b); A. Kraberg, BAH, AWI, Germany (c,d); D. Anderson, WHOI, MA, USA (e).

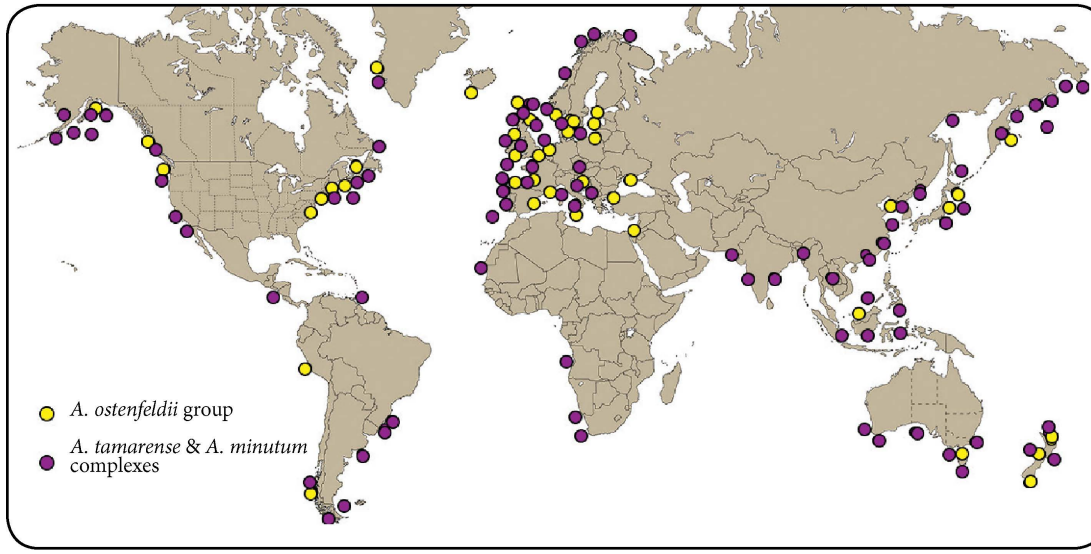

Figure 2 Known global distribution of the combined *A. tamarense* and *A. minutum* species complexes (purple–filled circles), coinciding with almost all PSP toxicity events attributed to *Alexandrium*; and the *A. ostenfeldii* species group (including *A. peruvianum*) (yellow–filled circles) and corresponding to the presence of spirolides (SPX), gymnodimines (GYM), and/or paralytic shellfish toxins (PST) linked to these species in the plankton or accumulated in shellfish. Compiled by L. Durán-Riveroll, CONACYT-UNAM, Mexico.

General: The genus *Alexandrium* (Halim) is perhaps the most intensively studied among toxic marine dinoflagellates. This is largely attributable to the devastating consequences of toxigenic blooms of this genus, with human poisonings from contaminated seafood, primarily from shellfish and more rarely from finfish; socio–economic losses to the aquaculture and fisheries industries; marine faunal mortalities; and food web disruptions common in coastal waters throughout the world. Members of this genus are globally distributed from the Arctic to the tropics, and in both hemispheres from sub–polar through temperate to sub–tropical to tropical waters. At least four distinct groups of marine phycotoxins are associated with various *Alexandrium* species, along with poorly characterized bioactive compounds (allelochemicals) that may affect species interactions among the plankton. According to the most recent iteration of the IOC–UNESCO reference list of toxic microalgae (Moestrup *et al.*, 2017), there are now more than 30 recognized morphological species of *Alexandrium* (Table 1), posing a daunting challenge for risk assessment and accurate identification in toxic phytoplankton monitoring programs.

Morphology: The first species assignable to *Alexandrium* was described as *Gonyaulax tamarensis* (Lebour) from the Tamar estuary in southern England, but was not associated with shellfish toxicity in the region. The genus *Alexandrium* was formally established with

Alexandrium

the description of its type species *A. minutum* (Halim, 1960) from a 'red tide' in the harbor of Alexandria, Egypt. This genus now includes several species formerly classified within a number of existing distinct or lapsed genera, such as *Gonyaulax*, *Protogonyaulax*, *Gessnerium*, *Goniodoma*, and *Pyrodinium*.

Until the 1980s, species identifications had been based exclusively on the analysis of morphological features, primarily the number, position, and form of cellulose plates of the internal cell wall ('theca') as defined in Kofoidean notation. Members of the genus *Alexandrium* are rather similar and undistinguished in surface morphological features. Detailed analysis of several key morphological characteristics is required for species identification, including the size and shape of the apical pore complex [APC], first apical (1′), sixth precingular (6″), and several sulcal plates (e.g., Sp and Sa). In addition, general morphological features such as cell size, shape, cell chain formation, ornamentation of the theca, cingular and sulcal excavation, the presence and orientation of sulcal lists, anterior and posterior attachment pores, and/or a ventral pore at the margin of the 1′ plate are diagnostic albeit rather variable species descriptors.

These diagnostic features are well described in the classic monograph on *Alexandrium* by Balech (1995), in which he also divided *Alexandrium* into two subgenera, *Alexandrium* and *Gessnerium*, a more heterogeneous group of species, based on the connection or lack of connection between the first apical (1′) and apical pore (Po) thecal plates.

With the advent of advanced molecular techniques in the past decade, the morphological classification of species within the genus, including species designations, has been frequently revised. These revisions have often led to confusing, albeit valid, species assignments, with alternative schemes for promotion of morphological varieties or molecular clades to species rank and assignment of newly defined taxa to occupy former species names. Molecular phylogenetic analysis has discriminated well-supported clades within the genus, although most phylogenies have not supported the taxonomic division of the genus into the two mentioned subgenera (Penna *et al*., 2008; Anderson *et al*., 2012). In any case, phylogenetic analyses based upon rDNA sequences confirm the existence of three well-supported complexes of species: the *A. ostenfeldii* species complex; the *A. minutum* species complex, containing two phylogenetic clades; and the *A. tamarense* species complex, which consists of five rDNA groups (or ribotypes) (John *et al*., 2003; Lilly *et al*., 2005; Anderson *et al*., 2012). On a global scale, these three species groups comprise most of the *Alexandrium* taxa associated with shellfish toxicity, particularly paralytic shellfish poisoning (PSP).

Molecular phylogenetic analyses have consistently revealed clusters of ribotypes for the *A. tamarense* species complex that are rather distinct (Lilly *et al*., 2005). The large genetic distance between the five ribotypes, plus evidence from interbreeding affinities, has led to their re-definition as separate species (Anderson *et al*., 2012; John *et al*., 2014). Detailed examination of the morphological criteria for taxonomic identification (e.g., presence/absence of a ventral pore, formation of long cell chains, etc.) has shown that many characteristics are shared among the five respective ribosomal groups, or that they are inconsistent within a morphospecies, and hence are inadequate for species definition and discrimination. In light of recent molecular evidence, in particular, the ribotype Group I of the *A. tamarense* species complex was renamed and emended as *A. fundyense* (John *et al*., 2014), which includes toxigenic members formerly assigned to the morphospecies *A. tamarense* (Lebour) Balech, and many chain-forming populations previously designated as *A. catenella* (Whedon & Kofoid) Balech. This proposal to subsume A. *tamarense* Group I within an emended A. *fundyense* was disputed by Fraga *et al*., 2015, who argued that only Group I cells were included in the original description (as *Gonyaulax catenella*) from the San Francisco region of California. This controversy was recently adjudicated by the ICN Nomenclature Committee for Algae, which recommended that the name *A. catenella* (formerly *G. catenella*) should not be rejected for Group I and that *A. fundyense* and *A. catenella* are conspecific, with nomenclatural priority being given to *A. catenella* (Prud'homme van Reine, 2017). Accordingly, although most taxonomists have accepted the proper designation of Group I cells to *A. catenella*, the nomenclatural reassignments have created confusion in comparing species identifications in the previous literature and in applying classic morphological criteria for

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routine monitoring by microscopic analysis.

Within the *A. minutum* group *sensu* Balech (1995), there are several defined morphospecies (*A. minutum*, *A. ibericum*, *A. angustitabulatum*, *A. lusitanicum*) that present similar morphological characteristics and express similar or identical PSP toxin profiles. These *Alexandrium* taxa may be synonymous or conspecific. The relationship of *A. minutum* to other members of the *A. minutum* group was also explored by molecular analysis, but members of this group are not readily distinguished via rDNA gene sequencing. Strains previously designated as *A. lusitanicum* or *A. angustitabulatum* were merged with *A. minutum* based on rDNA gene sequencing (Lilly *et al*., 2005). The species *A. tamutum*, *A. insuetum*, and *A. andersonii* were confirmed to be valid, but the latter species did not branch with any member of the "*A. minutum* group" as defined by Balech (1995).

Within the *A. ostenfeldii group*, cultured strains conforming to the descriptions of *A. ostenfeldii* and *A. peruvianum* have been recently examined from widely distributed geographical populations from Europe (Finland, Great Britain), North America, Peru, China, Japan, and New Zealand (Kremp *et al*., 2013). The diagnostic species descriptors were more variable than previously assumed, and exhibited extensive intra- and inter-strain variability. Phylogenetic analysis of rDNA sequences revealed that the strains formed a monophyletic clade, with a complex genetic structure consisting of six closely related groups with only slight genetic divergence. Kremp *et al*. (2013) therefore concluded that *A. ostenfeldii* and *A. peruvianum* belong to a single species, dubbed *A. ostenfeldii* because of nomenclatural priority.

General morphology and cell characteristics: The genus *Alexandrium* belongs to the gonyaulacoid dinoflagellates (Order: Gonyaulacales; family: Gonyaulacaceae), based upon the thecal plate tabulation, and morphological and ultrastructural features. Cells of *Alexandrium* species vary widely in size (20–80 µm transdiameter), and are roughly spherical to subspherical and without horns or spines (Figure 1a, b, c, d). The cell surface is rather featureless, but small pores and light reticulations are typically present. The descending median cingulum (girdle) is displaced 1–1.5 girdle widths and has poorly developed lists (margin extensions). The sulcal groove may be deeply excavated, but is also without prominent lists. Among *Alexandrium* species, the apical pore plate (Po) may be either directly connected to, or indirectly linked to, the first apical (l′) plate via a threadlike suture (e.g., in *A. ostenfeldii*), or the plates may be completely disjunct. The Po bears a relatively large comma-shaped cavity (or is fishhook-shaped in *A. catenella* and *A. tamarense*) as a diagnostic feature of the apical pore complex (APC). A ventral pore (vp) at the margin of the 1′ plate may be present or absent; if present it varies from small circular (e.g., in *A. tamarense*) to large kidney shaped (e.g., for *A. ostenfeldii*). All *Alexandrium* species share a common Kofoidean plate tabulation comprising an APC and 4′, 6″, 5‴, 2⁗, 6C, 9-10S plates.

Alexandrium cells are rather weakly armored with a relatively thin and delicate theca, e.g., for *A. ostenfeldii*, but which may be occasionally rugose. The theca is easily shed in sample handling and this complicates species identification. Species discrimination among close morphological relatives is rather difficult by light microscopy, and often requires thecal staining with iodine or a fluorescent marker, or examination by scanning electron microscopy. Under the conventional optical microscope, *Alexandrium* cells are typically dark- to reddish-brown in color with several radially distributed chloroplasts and a large equatorially located U-shaped nucleus. Many *Alexandrium* populations are bioluminescent, but this is highly variable even within a species.

Some species, including members of the *A. minutum*, *A. tamarense*, and *A. ostenfeldii* groups, are usually found in nature as individual cells, although short chains of two to four cells are common in rapidly growing populations. In contrast, some *Alexandrium* species, such as *A. catenella* and *A. fraterculus* among others, more often occur in chains of >8 cells and this feature can (with caution) aid in species identification of field material.

Known Distribution: The *Alexandrium tamarense/fundyense/catenella* species group is almost globally distributed from the Arctic and sub-Arctic waters of Canada, USA and Scandinavia (including Greenland) to the tropics (Figure 2). In North America, blooms are most prominent along the Pacific coast of North America from Alaska and British Columbia to California, and from the estuary and Gulf of

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St. Lawrence southward along the coast of Nova Scotia and Bay of Fundy to the southern Gulf of Maine. In South America, associated toxic blooms range from the fjord regions of Chile to Tierra del Fuego and from the northern Argentine Sea to the Magellan Strait. The *A. catenella* chain-forming morphotype may form blooms in the Benguela current region off Namibia and South Africa. In east Asia, the *A. tamarense* species group is found from Kamchatka southward to tropical waters, and with frequent appearance in Japanese coastal embayments. The *A. tamarense* group is common in plankton samples from Northern Europe in early summer, especially around the British Isles and along the Norwegian and Swedish west coasts. This biogeographical information is not exhaustive, and because it is largely dependent on morphological species identification, it is subject to revision and historical reassignments given the recent inconsistent reclassification of species within this group (John *et al*., 2014; Prud'homme van Reine, 2017).

The *A. minutum* group is widely distributed, with members of this group reported from the Atlantic coast of North America, Argentine Sea, and west European coasts (France, Iberia), and from the North Sea to the west coast of Sweden (Skagerrak). Populations are also found throughout the Mediterranean, in Southeast Asia (Taiwan), along the west coast of India, in New Zealand, and in southern Australia.

Alexandrium ostenfeldii (Paulsen) Balech & *Tangen* was originally described (as *Goniodoma ostenfeldii*) (Paulsen, 1904) from Iceland and is frequently found in north temperate and subarctic coastal waters of Scandinavia and Canada – giving rise to the view that it is a cold-water species (Figure 2). In any case, current reports of the occurrence of the *A. ostenfeldii* group (now including *A. peruvianum* [Balech & Mendiola] Balech & Tangen) has now expanded to include locations around the British Isles, the Atlantic coast of France and northern Iberia, low-salinity environments in the Netherlands and Baltic Sea, the Atlantic coast of Canada, and the Gulf of Maine, USA. In South America, the morphotype has been identified in Peru, southern Chile, and the Argentine Sea. In Asia, the species occurs in various locations in Japan, New Zealand, and southern Australia. Particularly striking is the frequent and widespread occurrence in the Mediterranean Sea – hardly a cold-water environment.

Cysts: Benthic resting (sexual) cysts (or hypnozygotes) and the life history of many *Alexandrium* species have been fully described from mating experiments with cultured isolates. Naturally occurring *Alexandrium* resting cysts are usually smooth and ovoid to round in shape (Matsuoka and Fukuyo, 2003) (Figure 1e), and except for *A. pseudogonyaulax*, which forms cysts with a distinct paratabulation (Montresor, 1995), cysts lack surface features. Mature cysts often have a mucilaginous layer with evident lipid globules and an orange-red carotenoid pigment spot, particularly prior to germination. *Alexandrium* cysts are robust, but do not fossilize well; therefore, cyst records are usually limited to about the last century within recent sediments. The existence of "seed beds" of benthic *Alexandrium* cysts has led to attempts to map their location and temporal abundance for risk evaluation of incipient regional toxic blooms and even for modeling bloom dynamics. The efficacy and accuracy of such surveys are complicated by lateral advection and high hydrodynamic activity at the sediment interface in some regions.

Toxin: Around half of the approximately 30 morphologically described species of the genus are known to produce toxins (Anderson *et al*., 2012) (Table 1). These "toxins" are defined as such based upon their known association with marine faunal illnesses and mortalities via food chain accumulation and/or human cases of poisoning by ingesting toxic seafood. The tetrahydropurine saxitoxin (STX) analogs (or paralytic shellfish toxins, PSTs) are the most well-known and widely distributed neurotoxins produced by several *Alexandrium* species. Blooms of *A. catenella* on the California coast have been circumstantially linked to the classic shellfish poisoning syndrome PSP since the 1930s. Advances in analytical chemistry and structural elucidation have led to the description of approximately 50 naturally occurring STX analogs produced among *Alexandrium* strains and species (Durán-Riveroll *et al*., 2018) or as biotransformation metabolites in marine fauna. These major STX analogs synthesized by *Alexandrium* species can be structurally classified as carbamoyl, decarbamoyl, or N-sulfocarbamoyl derivatives, and in decreasing order of specific toxin potency in

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mammalian systems – an important criterion for toxin-monitoring regimes.

The capacity to produce PSTs varies widely among *Alexandrium* species and even among strains within a nominally toxigenic species (reviewed by Cembella, 1998). Within *Alexandrium* strains, the PST composition typically includes several analogs of one or more of the following major toxin subgroups: (1) carbamoyl, including saxitoxin (STX), neosaxitoxin (NEO), and the C-11 O-sulfated gonyautoxin analogs (GTX1–GTX4); and (2) N-21 sulfocarbamoyl analogs (B1 = GTX5, B2 = GTX6, C1–C4). *Alexandrium* strains produce different relative amounts of these derivatives, but the composition is a rather stable trait, and significant shifts tend to occur only under extreme change in growth regime in culture (Boczar *et al*., 1988). The amount of toxin per cell ("cell quota") varies with the growth rate and status and is directly or indirectly dependent upon abiotic factors such as light, temperature, turbulence, and nutrient supply. In natural populations, it is not known if toxin composition is subject to similar shifts in response to short-term changes in abiotic factors (light, temperature, salinity, nutrients, turbulence, etc.).

In any case, the relative composition of PST analogs within an *Alexandrium* strain is known to be genetically determined by the structure and expression of the STX gene cluster (reviewed in Durán-Riveroll *et al.*, 2018). This constitutive property of toxin biosynthesis has led to attempts to characterize species and even geographical populations of *Alexandrium* by analytical "fingerprinting" of the toxin profile. For example, *A. minutum* group populations worldwide tend to produce a relatively limited spectrum of PSP toxins (primarily carbamoyl derivatives, such as GTX1–GTX4, and sometimes NEO and/or STX) and have a low PST cell quota (Cembella *et al*., 1987). Members of the *A. tamarens*e group (now including the redefined *A. fundyense* and *A. catenella*) usually produce a broader spectrum, including both carbamoyl and N-sulfocarbamoyl derivatives, and often express higher cell toxin content (Cembella, 1998). Decarbamoyl derivatives (dcSTX, dcNEO, dcGTX1–4) are frequent metabolites of PSTs in certain shellfish, especially venerid clams, and the N-21 sulfocarbamoyl analogs C3, C4 are often found in *Gymnodinium catenatum* (Cembella and Band-Schmidt, 2018), but these latter toxin groups are rarely detected in natural bloom populations and among cultured isolates of *Alexandrium* species. Such profile differences have proven diagnostic utility for limited assessment of toxin risk in some regions, and even to infer the causative species after determining the PST profile in contaminated shellfish. Nevertheless, PST profiling at the species level must be approached with caution because of exceptions. For example, *Alexandrium* populations from western Greenland exhibit a classic *A. tamarense* morphotype (now genetically redefined as *A. catenella*) but express the toxin profile (exclusively carbamoyl toxins) more characteristic of global populations of the *A. minutum* group (Baggesen *et al*., 2012).

Within a species, early efforts to define geographical populations of the *A. tamarense* group by comparing PST profiles between and among populations were highly promising. Toxin profiles were clustered or ordinated from the northeast Pacific (British Columbia, Washington State) and compared with those from Atlantic Canada (Cembella *et al*., 1987), between the St. Lawrence estuary and Nova Scotian coast (Cembella and Destombe, 1996) and along a north–south gradient from the Bay of Fundy to the Gulf of Maine (Anderson *et al*., 1994). These studies tended to show distinct regional differences and accorded with morphospecies descriptions, but were based upon bulk toxin measurements from net tows from the field or from one or a few cultured isolates from these populations. More recent analyses of PST profiles of multiple isolates (>70 clones) within geographical populations of *A. tamarense/A. fundyense* morphotypes from the North Sea (Alpermann *et al*., 2010) have revealed high intrapopulation heterogeneity, and calls into question the representative nature of one or a few isolates to describe a population.

The toxin biosynthetic capacity of the *A. ostenfeldii* group is extremely diverse, ranging from non-toxigenesis to production of various combinations of PST, and/or cyclic imine neurotoxins, including spirolides (SPX) and gymnodimines (GYM). For example, *A. ostenfeldii* populations from New Zealand may produce rather high amounts of PST (but no SPX), whereas only SPX is produced in this species from Nova Scotia, and both groups of

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toxins can be present in certain strains from Denmark (Cembella and Krock, 2007).

Cyclic imine neurotoxins, including spirolides (SPX) and gymnodimines (GYM), are produced among a limited set of *Alexandrium* strains belonging to the *A. ostenfeldii* group (Cembella *et al.*, 2001; Krone, 2016). The association of GYM with *A. ostenfeldii* is a relatively recent discovery; these toxins were previously found only in the unrelated dinoflagellate *Karenia selliformis*. The distribution of GYM among *A. ostenfeldii* populations is now known to be widespread, including from coastal estuaries in the U.S. (reported as *A. peruvianum*) (Van Wagoner *et al.*, 2011; Borkman *et al.*, 2012), the Netherlands (Van de Waal *et al.*, 2015) and from the Baltic Sea (Salgado *et al.*, 2015).

Major frequently encountered SPX analogs found among *A. ostenfeldii* strains include SPX A, B, C and D, 13-desmethyl SPX C, 13,19-didesmethyl SPX C, 13-desmethyl SPX D and 20-methyl SPX G (Cembella and Krock, 2007). Other putative SPX analogs have been recently detected by LC-MS/MS but many of the structures have not been confirmed and the specific toxicities are unknown. The SPX toxin composition has been explored as a means of discriminating geographical populations of the *A. ostenfeldii* group, with frequent dominance of 13-desmethyl SPX C in Nova Scotia populations (Cembella *et al.*, 2000, 2001), whereas 20-methyl SPX G is more common in northern Europe along the Irish and Scottish coasts. Mediterranean isolates produce almost exclusively 13-desmethyl SPX C (cited in Anderson *et al.*, 2012). Spirolide composition of natural populations and isolates of *A. ostenfeldii* from the Gulf of Maine revealed high regional diversity among populations but could be grouped into five distinct SPX toxin clusters (Gribble *et al.*, 2005).

The cyclic imines are often called "fast acting toxins" because of the rapid convulsive response elicited in mammalian subjects. To date no human cases of shellfish intoxication have been associated with either SPX or GYM toxins, and there are no respective regulatory regimes in place for seafood safety. Inactive SPX analogs, e.g., belonging to the SPX E and F groups, are often formed by opening the imine ring via metabolism in shellfish, and similar mechanisms apply to GYM as well. Nevertheless, the strong neurotoxic symptoms observed when cyclic imine toxins are administrated intraperitoneally into laboratory rodents warrants their continued inclusion on the list of potential emerging toxins and as a caution when interpreting mouse bioassays of lipophilic toxins.

Polyether macrolide toxins known as goniodomins (GON), primarily the analog goniodomin A, are produced by certain strains of *A. monilatum* (J. F. Howell) Balech, *A. hiranoi* Kita & Fukuyo, and *A. pseudogonyaulax* (Biecheler) Horiguchi ex Kita & Fukuyo (Murakami, 1998; Hsia *et al.*, 2005). Goniodomins have not been associated with human health problems linked to seafood consumption, and hence they are not subject to regulatory monitoring. Nevertheless, they are regarded as fish-killing toxins because extracts of *A. monilatum* were reported to have caused paralysis and mortality in fish (the grey mullet *Mugil cephalus* Linnaeus) and blooms have also been associated with mass fish mortalities, e.g., along the Texas coast (Gates and Wilson, 1960).

Certain strains of some *Alexandrium* species, such as *A. catenella/tamarense* and *A. ostenfeldii*, can also produce allelochemicals with high biological activity against other plankton species (Cembella, 2003). The chemical structures of these allelochemicals remain undefined, but they are clearly distinct from the known toxins. The occurrence of these allelochemicals among *Alexandrium* species does not appear to be related to the genetic capacity to produce potent phycotoxins (PST, SPX, GYM, GON) nor is allelochemical potency correlated with the strain-specific content of these known toxins. Nevertheless, release of these compounds can cause dramatic cell lysis and/or immobilization of other plankton, and thus may have important implications for *Alexandrium* bloom dynamics by affecting competition and grazing interactions. Human health implications of their potential accumulation in seafood are unknown.

Bloom ecology and consequences: The high heterogeneity in behavior (swimming, vertical migration), *in situ* growth rate, life history transitions, and ecophysiological responses to abiotic factors (light, temperature, nutrients, stratification) within and among *Alexandrium* species poses great challenges in defining a common functional strategy to explain

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bloom dynamics. This is further complicated by differential expression of toxins and allelochemicals that may affect grazing and competitive species interactions, in susceptibility to parasite attack and the capacity for alternative nutritional modes (heterotrophy and mixotrophy). Despite the capacity for limited heterotrophy among *Alexandrium* species (Legrand and Carlsson, 1998), typical of many photosynthetic dinoflagellates, there is little evidence for an association of blooms with organic enrichment or coastal eutrophication. On the contrary, the highest magnitude blooms of the *A. tamarense* group and maximum associated PSP toxicity are often found in relatively pristine waters with little anthropogenic influence, such as in Alaska (Hall, 1982), southern Argentina (Benavides *et al*., 1995) and the lower St. Lawrence estuary (Therriault *et al*., 1985). Even in coastal embayments subject to long-term anthropogenic inputs of nutrients, the appearance of recurrent high-magnitude *Alexandrium* blooms is often linked to inorganic nutrient reduction, as is the case for blooms of the *A. catenella* morphotype in Thau Lagoon on the French Mediterranean coast (Collos *et al*., 2009).

Although physical stratification of the water column is associated with aggregations of *Alexandrium* cells and higher cell densities are typically found within low-salinity plumes at frontal zones (Therriault *et al*., 1985; Franks and Anderson, 1992), it is unclear whether this reflects biological-chemical coupled behavior, such as targeted swimming responses to organic nutrients or allelochemicals, or higher growth rates and physical retention within a density layer. The fact that *Alexandrium* taxa are capable of bloom formation in environmental regimes ranging from polar latitudes to the tropics and within shallow coastal embayments, in upwelling systems, within coastal jet currents, and at estuarine frontal plumes argues for a complex interplay of biological, chemical, and physical mediators, rather than a common "*Alexandrium* strategy" to define bloom dynamics.

In any case, the physical structure and hydrodynamics of the resident water mass are clearly major determinants of *Alexandrium* bloom initiation, development, and senescence (or advective loss). In the St. Lawrence estuary in eastern Canada, the frontal zone generated by the trans-estuarine freshwater plume of the Manicouagan and Aux-Outardes rivers generates a highly stratified water column supporting growth and retention of *Alexandrium* populations (Therriault *et al*., 1985). The bloom is subsequently transported and advected within the coastal jet Gaspé current, causing PSP toxicity in shellfish along the south shore of the estuary to the Gulf of St. Lawrence. Earlier empirical evidence linking the high cell abundance of *A. catenella* (formerly as *A. tamarense* or *A. fundyense sensu* John *et al*., 2014) during mid- to late-summer with trans-estuarine advection of the bloom, led to the development of a model incorporating meteorological and hydrodynamic factors as determinants to define bloom dynamics and consequent shellfish toxicity (Fauchot *et al*., 2008).

The role of hydrodynamic factors in driving *Alexandrium* bloom dynamics has been most intensively studied in the Gulf of Maine, USA linked to the Bay of Fundy in the north. In this region, temporal and spatial patterns of annual blooms of *A. catenella (=A. fundyense)*, causing recurrent PSP toxicity in shellfish, are driven primarily by transport within a large-scale coastal current system that traverses the Gulf (Franks and Anderson, 1992). The critical role of life cycle events, such as the timing and magnitude of resting cyst formation and hatching, are emphasized in conceptual models of *Alexandrium* bloom dynamics in the Gulf of Maine (Anderson *et al*., 2005; McGillicuddy *et al*., 2005). Such models propose bloom initiation and propagation from two major cyst "seedbeds" in the Bay of Fundy and offshore of mid-coast Maine (Anderson *et al*., 2012). In this interpretation, cyst germination within the Bay of Fundy is associated with endogenous recurrent coastal blooms in the Bay. Longshore transport of a fraction of these blooms contributes to *Alexandrium* populations in the eastern section of the Gulf of Maine coastal current (EMCC), causing shellfish toxicity within the south and westward flow. Along the way, as the bloom declines, cysts are deposited in the mid-coast Maine seedbed, where they may seed subsequent blooms in the western Gulf and possibly offshore, supplemented with freshly advected cells from the EMCC, to cause persistent and recurrent PSP toxicity.

Empirical evidence suggests that *Alexandrium* population dynamics in shallow embayments and coastal lagoons are driven more by small-scale hydrographic features coupled with life

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history transitions (e.g., cyst germination), vertical migration, and *in situ* growth than horizontal advection and longshore transport mechanisms characteristic of open coastal systems. The correlation of water temperature and stratification with growth rate and life history transitions is key to understanding the development of *Alexandrium* blooms, particularly in semi-enclosed shallow systems. In a Mediterranean lagoon, the spring bloom of *A. minutum* coincided with enhanced rainfall and freshwater runoff creating stabilization of the water column (Giacobbe *et al*., 1996). The development of high-magnitude blooms of the *A. catenella* chain-forming morphotype in Thau Lagoon on the French Mediterranean coast is known to be dependent upon reaching a lower temperature threshold (20 °C) during a calm weather period (cited in Anderson *et al*., 2012). Most *Alexandrium* blooms in Cape Cod, MA develop at apparently sub-optimal water temperatures for maximal growth (Anderson *et al*., 1983), whereas in Chinhae Bay, Korea the blooms terminate at temperatures well below those that support optimal growth in the laboratory (Han *et al*., 1992). Although it is possible that optimal growth temperatures in the laboratory may not reflect natural conditions, it is more likely that a temperature threshold triggers induction of sexuality and cyst formation even at favorable temperatures for continued vegetative growth. In shallow coastal bays, such as Cape Cod salt ponds, bloom development may be a comparatively simple function of salinity-dependent temperature regulation of growth (Watras *et al*., 1982). Such simple conceptual models, however, fail to predict *A. catenella (= fundyense)* bloom dynamics in more hydrodynamically active regimes in open, tidally mixed waters, such as the Bay of Fundy.

Alexandrium species exhibit a high degree of versatility and adaptability in exploiting ecological niches within a wide variety of habitats on a global scale. Recent evidence of advancement into new habitats ("bloom spreading") over the last few decades poses increased toxicity risk to human health and marine ecosystem functioning. The challenges of developing effective monitoring and mitigation strategies, or even reliable forecasting scenarios for bloom dynamics and ecological consequences, are expected to remain for the foreseeable future.

Opposite Page - Table 1) Species assignments based upon classical morphotaxonomic criteria and toxigenesis within the genus *Alexandrium*.

Note: Toxigenicity is indicated when at least one strain or population is associated with production of the respective toxin group. The indicated "species" are not necessarily in accordance with recent proposed reassignments based primarily upon molecular criteria (e.g., John *et al.*, 2014; Prud'homme van Reine, 2017). PST = paralytic shellfish toxins, saxitoxin analogs; SPX = spirolides; GYM = gymnodimines; GON = goniodomins.

Source: Species designations are compiled from the IOC UNESCO reference list of toxic microalgae (Moestrup *et al*., 2017) and modified after Anderson *et al.* (2012).

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Species	Toxin type	Comments
Alexandrium acatenella (Whedon & Kofoid) Balech	PST (?)	Toxin type assumed only from mouse bioassay symptoms of toxic shellfish extract associated with blooms
Alexandrium affine (Inoue & Fukuyo) Balech	PST	Typically weakly toxic or non-toxic
Alexandrium andersonii Balech	PST	Most commonly non-toxic
Alexandrium angustitabulatum Taylor	PST	Strains from the type locality weakly toxigenic
Alexandrium balechii (Steidinger) Balech	None known	Blooms coincident with mass fish mortalities in type locality probably due to oxygen depletion
Alexandrium camurascutulum MacKenzie & Todd	None known	
Alexandrium catenella (Whedon & Kofoid) Balech	PST	Often moderately to highly toxigenic; N-sulfocarbamoyl (C1/C2; B1/2) analogs frequently in high relative abundance
Alexandrium cohorticula (Balech) Balech	PST(?)	Strains from Japan reported as toxigenic, but possible misidentification of *A. tamiyavanichii*
Alexandrium compressum (Fukuyo, Yoshida & Inoue) Balech	None known	
Alexandrium concavum (Gaarder) Balech emend. Larsen & Nguyen-Ngoc	None known	
Alexandrium foedum Balech	None known	
Alexandrium fraterculus (Balech) Balech	PST(?)	Almost invariably non-toxigenic, but one questionable report of associated PST toxicity with blooms in Uruguay
Alexandrium fundyense Balech	PST	PST toxigenicity ranges from non-toxic to highly toxic; often with a highly diverse PST profile (N-sulfocarbamoyl and carbamoyl analogs); allelochemicals produced by some strains
Alexandrium gaarderae Nguyen-Ngoc & Larsen	None known	
Alexandrium globulum Nguyen-Ngoc & Larsen	None known	
Alexandrium hiranoi Kita & Fukuyo	GON	
Alexandrium insuetum Balech	None known	
Alexandrium kutnerae (Balech) Balech	None known	
Alexandrium leei Balech	None known	Typically non-toxic, but unconfirmed report of low-level STX analog from a Vietnamese strain; unknown ichthyotoxins
Alexandrium margalefii Balech	None known	
Alexandrium minutum Halim	PST	Typically toxigenic but usually low toxicity and exclusive presence of carbamoyl (GTX, STX, NEO) analogs; non-toxic strains also occur, e.g., in Mediterranean Sea
Alexandrium monilatum (Howell) Balech	GON	Strongly ichthyotoxic
Alexandrium ostenfeldii (Paulsen) Balech & Tangen	PST; SPX; GYM	Depending upon the geographical population, strains may produce PST, and/or SPX or GYM in various combinations
Alexandrium peruvianum (Balech & Mendiola) Balech & Tangen	SPX; GYM	SPX produced by strains from the Mediterranean Sea, and GYM from populations from coastal estuaries in the eastern U.S.
Alexandrium pseudogonyaulax (Biecheler) Horiguchi ex Yuki & Fukuyo	GON	
Alexandrium satoanum Yuki & Fukuyo	None known	
Alexandrium tamarense (Lebour) Balech	PST	As classic morphospecies, PST profile and cell toxin content overlap with *A. fundyense*, but as redefined species (John *et al.*, 2014) considered non-toxigenic; allelochemicals/ichthyotoxins may be produced
Alexandrium tamiyavanichii Balech	PST	
Alexandrium tamutum Montresor, Beran. & John	None known	
Alexandrium taylori Balech	PST	Usually non-toxic, but PST produced by a cultured strain from Malaysia; also known to produce undescribed non-proteinaceous exotoxin
Alexandrium tropicale Balech	None known	

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Alexandrium

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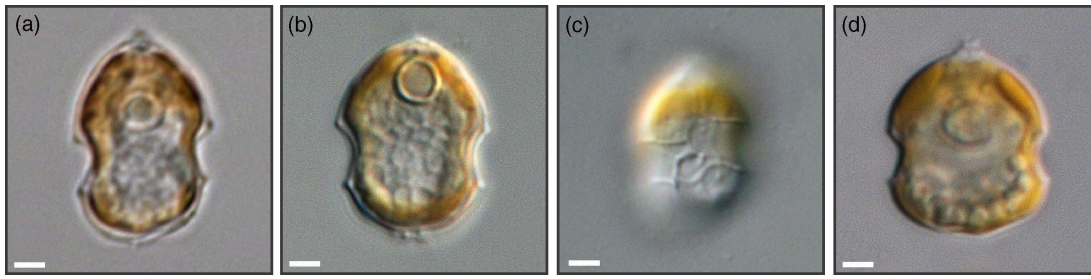

Figure 1 LM micrographs of *Azadinium spinosum* (a), *Az. poporum* (b), *Az. dexteroporum* (c) and *Amphidoma languida* (d). Scale bars = 2 μm.

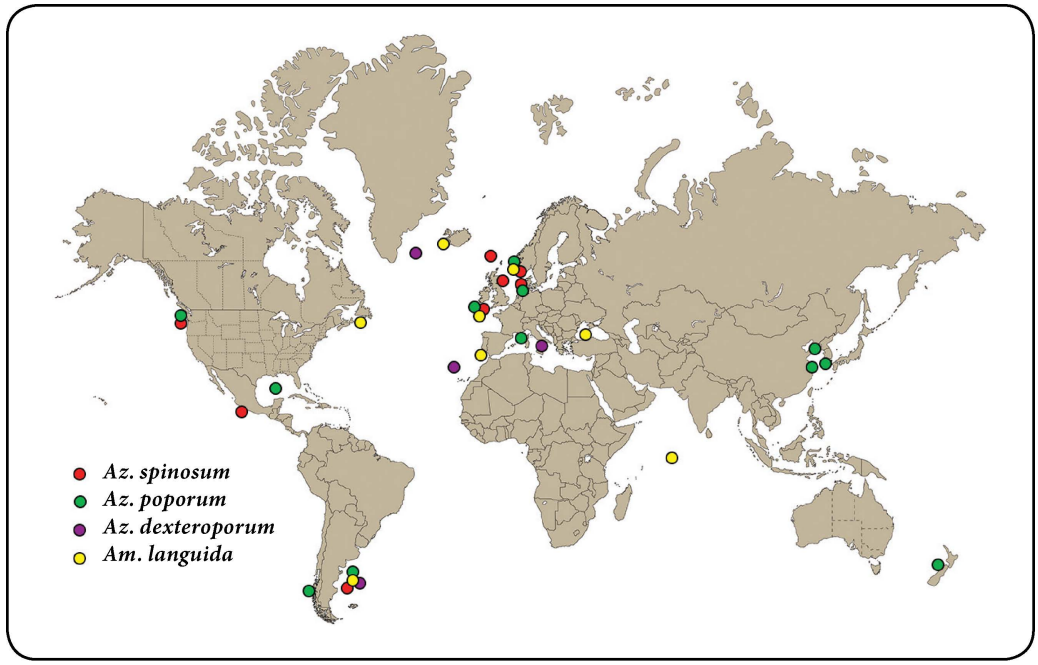

Figure 2 Global records of the four species of Amphidomataceae known to produce azaspiracids (AZA).

Table 1 Members of Amphidomataceae and status as AZA producers.

AZA producer	no AZA found	not analysed yet
Azadinium spinosum	*Azadinium obesum*	*Azadinium caudatum* var. *caudatum*
Azadinium poporum	*Azadinium polongum*	*Azadinium luciferelloides*
Azadinium dexteroporum	*Azadinium caudatum* var. *margalefii*	*Amphidoma nucula*
Amphidoma languida	*Azadinium dalianense*	*Amphidoma acuminata*
	Azadinium trinitatum	*Amphidoma curtata*
	Azadinium cuneatum	*Amphidoma depressa*
	Azadinium concinnum	*Amphidoma elongata*
	Azadinium zhuanum	*Amphidoma laticincta*
	Amphidoma parvula	*Amphidoma obtusa*
		Amphidoma steinii

Amphidomataceae

General: Azaspiraids (AZA) are a group of lipophilic polyether toxins first detected and described in the late 1990s. With the description of *Azadinium spinosum* in 2009, the first source organism has been identified. Currently, there are four out of 22 species of the genera *Azadinium* and *Amphidoma* (merged in the family Amphidomataceae) that have been shown to produce AZA. However, it has to be kept in mind that there is only a limited number of cultured strains available, and it is thus not clear if and to what extent AZA production is a species-specific stable phaenotypic trait.

General Morphology: All AZA-producing species of *Azadinium* and *Amphidoma languida* are small (size of about 10–16 µm) and ovoid to elliptical in shape with a hemispherical hyposome. In all these species, the episome is larger than the hyposome, with slightly convex sides ending in a distinctly pointed apex. The cingulum is deep and wide, accounting for roughly 1/5 to 1/4 of the cell length. A central or more posteriorly located large nucleus is visible, which generally is round to elliptical but may become distinctly elongated in shape close to cell division. All species are photosynthetic and possess a presumably single chloroplast, which is parietally arranged, lobed, and normally extends into both the epi- and hyposome. For all of the AZA-producing species, stalked pyrenoid(s) are visible in the light microscope because of a distinct starch cup. *Azadinium* spp. and *Amphidoma languida* have delicate thecal plates difficult to detect in light microscopy (LM), so that live cells are sometimes difficult to differentiate from small athecate gymnodinoid species. Plate pattern and thecal plate details are important for determination of the genus and species, but require scanning electron microscopy (SEM). Species of *Azadinium* are characterised by the Kofoidean plate pattern of Po, cp, X, 3–4′, 2–3a, 6″, C6, 5S, 6‴, 2⁗, whereas *Amphidoma languida* has six apical plates and no anterior intercalary plates. A very characteristic feature among the AZA-relevant species is the prominent apical pore complex visible in LM, which is composed of an X-plate and a pore plate with a central round pore covered by a cover plate. Plate details important for species identification include the presence/absence and/or location of a single antapical spine and primarily the position of a ventral pore. Morphology, and in particular the plate tabulation with five different rows of plates, undoubtedly classified the family Amphidomataceae as a member of the dinophycean subclass Peridiniphycidae (Tillmann *et al.*, 2009). The relation to one of the two orders of the subclass (i.e. Gonyaulacales and Peridiniales), however, is less clear as some morphological traits imply affinity to Peridinales and others to Gonyaulacales. Using a concatenated alignment of LSU and SSU, the Amphidomataceae have been placed on the peridinean branch remote from the Gonyaulacales, but the true relation to Peridiniales could not be identified reliably (Tillmann *et al.*, 2014). It thus remains to be determined whether they are part of the Peridiniales or represent a distinct lineage that would deserve the recognition at a higher taxonomic level.

Known Distribution: Although the first species of *Azadinium* were initially described from the North Sea, there is increasing evidence that AZA-producing species have a wide geographical distribution. Nevertheless, knowledge on the biogeography of the genus or of certain species currently is rather limited and patchy. It is based on the troublesome procedure of isolating, cultivating and fully characterizing local strains; on a very few records of species detected by scanning plankton samples by electron microscopy; or on positive signals using species-specific molecular detection methods.

Cysts: Knowledge on the life cycle of *Azadinium* and/or *Amphidoma* is quite incomplete. Successful isolation of *Az. poporum* by incubating sediment samples (Potvin *et al.*, 2012; Gu *et al.*, 2013) made the presence of cysts quite likely for that species, and that has been confirmed by Gu *et al.* (2013): in one out of 25 cultured strains, they observed the presence of a few distinct cysts. These cysts are ellipsoid, around 15 µm long and 10 µm wide, and are filled with pale granules and a yellow accumulation body. Likewise, the species *Az. polongum* (a non-AZA producer) has been described to produce cysts in culture,

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round cells of 10–16 µm in diameter and with pale white inclusion. No cyst-like cells have been reported for other species, including *Az. spinosum*, *Az. dexteroporum* and *Am. languida*. Clearly, more data and observations are needed to clarify the whole life cycle of Amphidomataceae.

Toxin: Species of Amphidomataceae are the source of azaspiracids (AZA), a class of polyether toxins discovered almost 20 years ago. Azaspiracids are polyketides with a highly hydroxylated carbon chain that is cyclised by ether bridges, and they contain a six-membered cyclic secondary amino ring. To date more than 50 AZA analogs are known. These include about 20 of dinoflagellate origin, and the others are thought to be produced by bioconversion in shellfish (Hess *et al*., 2014). Azaspiracids are known to be responsible for gastrointestinal disorders with the consumption of AZA-contaminated shellfish, with symptoms quite similar to those of DSP, such as nausea, vomiting, diarrhea and stomach cramps. Preliminary studies of AZA suggested that these compounds are highly toxic with multi-organ damage in mice and teratogenic potential to developing fish, along with a wide array of cellular-level effects, ranging from cytotoxicity to apoptosis and to effects on the hERG potassium channel (reviewed by Twiner *et al*., 2014). Minimal lethal doses (i.p. mice) for the most dominant AZA in mussels have been determined as 200, 110, and 140 µg/kg for AZA-1, -2, and -3, respectively (Satake *et al*., 1998; Ofuji *et al*., 1999). Consequently, a regulatory limit of 160 µg/kg mussel meat for AZA-1 to AZA-3 was implemented in 2002 into the EU biotoxin legislation. More recent studies yielded similar results for mouse toxicity for AZA-2 and AZA-3, but a distinctly lower dose (higher toxicity) of 74 µg/kg for AZA-1 (Kilcoyne *et al*., 2014a). Oral mouse studies indicated no additive or synergistic effects when AZA was administered in combination with okadaic acid or yessotoxin (Kilcoyne *et al*., 2014a).

Around 20 AZA analogs are currently described to be of dinoflagellate origin. Among the dominant AZA found in shellfish, AZA-1 and AZA-2 are produced by *Azadinium*, whereas no planktonic source of AZA-3 is known yet. An increasing number of new AZA are discovered in the Amphidomatacean cultures. Initial mass spectral data (Krock *et al*., 2012) as well as structural elucidation by nuclear magnetic resonance (NMR) spectroscopy (Kilcoyne *et al*., 2014b; Krock *et al*., 2015) showed that some of the new AZA discovered in dinoflagellates are structurally unique from previously reported analogues by having a modification of the nitrogen-containing I-ring of the molecule, which consists of either a missing methyl group at C39 or an additional double bond.

All four described European strains of *Az. spinosum* have the same toxin profile consisting of AZA-1, -2, and -33 (Tillmann *et al*., 2012b), and a few minor compounds have additionally been found in the Scottish strain (Kilcoyne *et al*., 2014b). For *Az. poporum*, a larger number of strains from different areas around the globe have been described, and this is reflected by a considerable diversity within this species in terms of toxin profiles. Whereas all three available North Sea strains produce AZA-37, *Az. poporum* from the Asiatic Pacific region produces more complex AZA profiles, including AZA-2, -11, -36, -40, -41 in different combinations, and also strains without any known AZA have been described.

Azaspiracid-2 (AZA-2) is the major AZA produced by *Az. poporum* from Argentina and by a strain from the Mediterranean, whereas strains from the Pacific coast of Chile produce AZA-11. Most recently, the new AZA-59 was identified from *Az. poporum* strains isolated from Puget Sound, WA (Kim *et al*., 2017). A feature that is shared among some Asian Pacific and Argentinean strains of *Az. poporum* is the production of minor amounts of AZA-related compounds with higher molecular masses. For the Argentinean strains, one of these compounds has been identified as AZA-2 phosphate, which is the first report of a phosphated marine algal toxin (Tillmann *et al*., 2016).

The presence of AZA has also been unambiguously described for the Mediterranean strain of *Az. dexteroporum* (Percopo *et al*., 2013), and detailed LC-MS analysis confirmed the presense of six novel AZA and AZA-35 (Rossi *et al*., 2017). A new strain of *Az. dexteropo-*

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rum, isolated from the subarctic Irminger Sea, however, clearly lacked any of these or other known AZA (Tillmann *et al*., 2015).

The type strain of *Amphidoma languida* isolated from Ireland and a strain originating from the Iceland area produce AZA-38 and -39 (Krock *et al*., 2012; Tillman *et al*., 2015). In contrast, *Am. languida* from the Atlantic coast of southern Spain produce AZA-2 and -43 (Tillman *et al*., 2017).

Cell quotas of AZA were found to be variable within and among strains and species but are typically in the range of 5–20 fg cell^{-1}. A maximum value of 220 fg cell^{-1} for *Azadinium spinosum* grown at 10 °C was reported (Jauffrais *et al*., 2013).

In vitro toxicity along with structure elucidation for some of the new AZA detected in *Az. spinosum* (Kilcoyne *et al*., 2014b) and *Az. poporum* (Krock *et al*., 2015) have recently been determined, and they showed both lower and higher cytotoxicity compared to AZA-1. For other compounds (e.g. AZA produced by *Am. languida* [AZA-38, -39] and *Az. dexteroporum*), specific toxicity is not known yet.

Methods for Toxin Identification: In 2011, the EU replaced the mouse bioassay with LC-MS/MS as the primary monitoring method for the analysis of AZA (and other lipophilic toxins) in shellfish. A number of validated LC-MS/MS methods for detection and quantification of AZA in shellfish have been described (Hess *et al*., 2014). Work on alternative detection methods for AZA has been limited. An antibody-based ELISA assay, as a rapid analytical technique using inexpensive instrumentation, has recently been described as a suitable tool for shellfish toxin analysis (Samdahl *et al*., 2015).

Ecological Observations: As species of Amphidomataceae have only recently been detected and identified, knowledge on their biology and ecology is rather limited. A first set of growth experiments indicated that *Az. spinosum* was fairly easy to grow with a number of standard culture media (indicating no special nutritional requirement) and at a wide range of different salinities, temperatures, and light conditions. Quantitative abundance data of toxic Amphidomataceae are hardly available, but dense blooms (> 10^6 cells L^{-1}) from a species of *Azadinium* from the Argentinean shelf have been observed (Akselman and Negri, 2012). Pathway and transfer kinetics of AZA into bivalve molluscs are just getting started to be explored. Azaspiracid accumulation in mussels following direct feeding on *Az. spinosum* has been proven experimentally, but *Az. spinosum* also had a significant negative effect on mussel feeding behavior and slightly increased mussel mortality compared to a control food (Jauffrais *et al*., 2012). Azaspiracids have been detected in a number of micrograzers (e.g., *Protoperidinium crassipes*, *Favella ehrenbergii*), so that a role of plankton vectors for mussel intoxication needs to be explored.

General Notes: With their small size, their distinctive and species-specific morphological characteristics that are hardly or not at all visible at the LM level, and with the close resemblance of toxigenic and non-toxigenic species, the AZA-producing Amphidomataceae are a good example for the necessity of applying molecular detection methods in monitoring and early warning systems. Molecular probes have been developed for the first three described species, *Az. spinosum*, *Az. poporum*, and *Az. obesum* (Toebe *et al*., 2013), but specific probes for other AZA-producing species (*Az. dexteroporum* and *Am. languida*) are still missing. In addition, it has to be kept in mind that there probably are more AZA-producing species that are not yet identified. *Am. languida*, for example, is the only species of the genus *Amphidoma* known so far for AZA production, and there are eight more species described, for which AZA production cannot be excluded. A general probe recently developed to detect a broad range of Amphidomataceae will be helpful to screen field samples and to aid in the detection, isolation and characterisation of AZA-producing species (Smith *et al*., 2016).

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Amphidomataceae

Azadinium spinosum Elbrächter et Tillmann (Tillmann *et al.*, 2009)

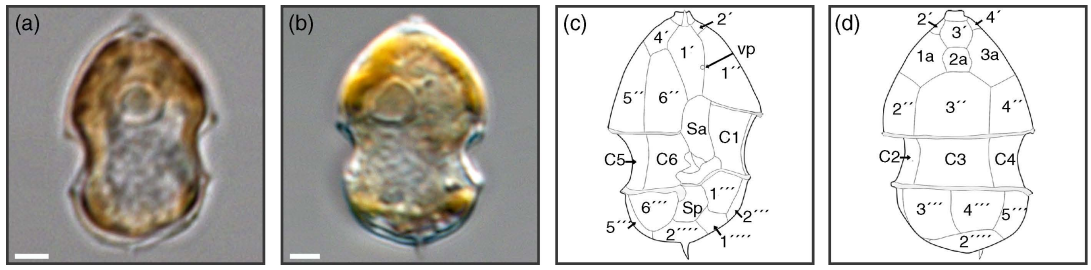

Figure 3 *Az. spinosum* LM micrographs (a, b) and schematic drawings (c, d) including the thecal plates in Kofoidean notation (vp = ventral pore). Scale bars = 2 µm.

Synonyms: None.

Morphology: *Azadinium spinosum* is a small (12–16 µm length and 7–11 µm width), slender (length-width ratio = 1.6), and slightly dorsoventrally compressed thecate, photosynthetic dinoflagellate. The conical episome with convex sides ends with a conspicuous apical pore complex (APC) and is larger than the hemispherical hyposome. It has a wide and descending cingulum, which is displaced by about half its width. In the light microscope, one large pyrenoid visible by its starch sheath is located in the episome. Eponymous for the species is the presence of a single small antapical spine located slightly asymmetrically at the right side of the cell.

Plate pattern and thecal plate details are important for determination of the genus and species, but require SEM. The Kofoidean thecal tabulation of *Az. spinosum* is Po, cp, X, 4′, 3a, 6″, 6C, 5S, 6‴, 2⁗. *Az. spinosum* has a distinct ventral pore located on the left side of the first apical plate.

Distribution: *Azadinium spinosum*, the type of the genus, has been isolated off the Scottish coast, the coast off Denmark, the Shetland Islands, the Norwegian coast, and from coastal Atlantic waters in Ireland. A species of *Azadinium* most likely *Az. spinosum* has been recorded in SEM samples from coastal Pacific waters off Mexico. *Az. spinosum* has also been identified in SEM field samples from the Argentinean shelf (South Atlantic). Recently *Az. spinosum* was detected by qPCR from Puget Sound, WA, U.S.

Azadininium poporum Tillmann et Elbrächter (Tillmann *et al.*, 2011)

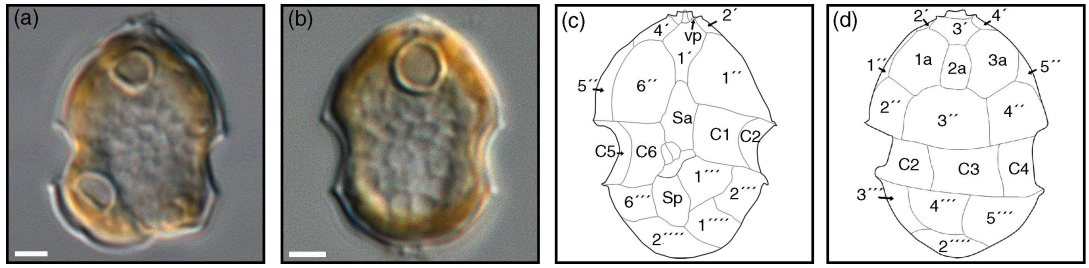

Figure 4 *Az. poporum* LM micrographs (a, b) and schematic drawings (c, d) including the thecal plates in Kofoidean notation (vp = ventral pore). Scale bars = 2 µm.

Synonyms: None.

Morphology: *Azadininium poporum* is small (11–16 µm length, 8–12 µm width), ovoid (length-width ratio = 1.3), slightly dorsoventrally compressed, with a broad and slightly descending cingulum, and with a hyposome slightly smaller than the episome ending in a conspicuous APC. In *Az. poporum*, there may be several (up to four) pyrenoids with a starch sheath visible in LM located in both the epi- and hyposome. The most distinctive morphological feature of *Az. poporum* requires SEM; it is the characteristic position of the ventral

Amphidomataceae

pore, which is located anterior at the cell's left side of the pore plate at the junction with the first two apical plates.

Distribution: *Azadinium poporum* was described based on strains from the North Sea off Denmark and has also been recorded in Ireland and along the Norwegian coast. A number of strains have been obtained from outside Europe. *Az. poporum* obviously is quite widely distributed in the Asian Pacific. As a first record of *Azadinium* in Pacific waters, *Az. poporum* has been isolated from Shiwha Bay in Korea, and subsequently, 25 different strains of *Az. poporum* originating from China covering the Bohai Sea and the East and South China Seas were established. Most recently, *Az. poporum* was detected in New Zealand both by qPCR and by establishing a culture. Likewise, *Az. poporum* cultures were obtained from samples from the South Atlantic (Argentina), the South Pacific (Chile), and the Gulf of Mexico. Most recently *Az. poporum* was identified by qPCR and isolated strains from Puget Sound, Washington.

Azadinium dexteroporum Percopo et Zingone (Percopo *et al.*, 2013)

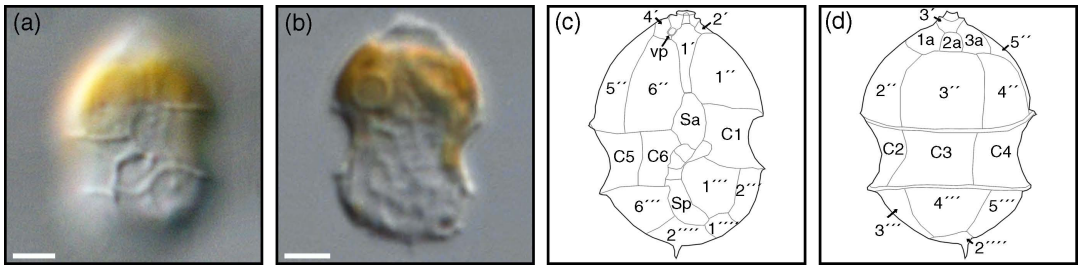

Figure 5 *Az. dexteroporum* LM micrographs (a, b) and schematic drawings (c, d) including the thecal plates in Kofoidean notation (vp = ventral pore). Scale bars = 2 µm.

Synonyms: None.

Morphology: *Azadinium dexteroporum* is the smallest species of *Azadinium* (7.0–10.0 µm in length and 5.0–8.0 µm in width). Cells are slightly elongated (length-width ratio = 1.4) and dorso-ventrally compressed, with the episome longer and slightly larger than the hyposome. The hyposome is slightly asymmetrical, with a small spine located in its posterior right side. The cingulum is deeply excavated and notably wide. One pyrenoid visible by its starch cup is present in the episome. Species-specific morphological details visible at the SEM level include the characteristic arrangement of the ventral pore, which is located at the right posterior end of the markedly asymmetric pore plate. A pronounced concavity of the median intercalary plate 2a has been highlighted as a peculiar feature of the Mediterranean type material, but this plate was plain for a subarctic strain originating from the Irminger Sea.

Distribution: *Azadinium dexteroporum* was initially described from the Mediterranean (Naples), but a new strain representing the species was recently obtained from the Subarctic (Irminger Sea). *Az. dexteroporum* was also identified in SEM preparation of spring bloom samples from the South Atlantic (Argentinean shelf) and is on a species list (as *Az.* cf. *dexteroporum*) of Madeira (North Atlantic off Morocco).

Amphidomataceae

Amphidoma languida Tillmann, Salas et Elbrächter (Tillmann *et al.*, 2011)

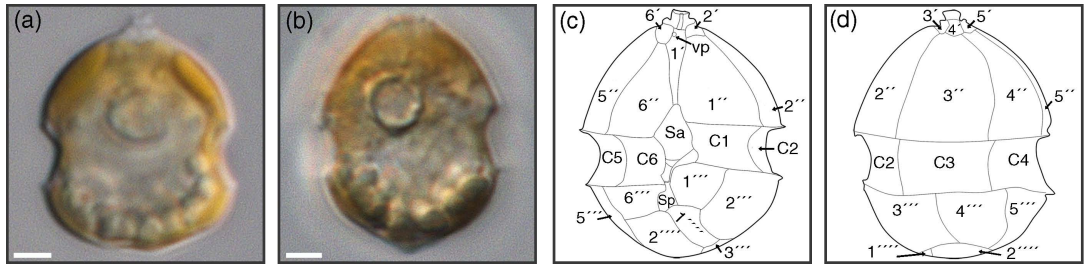

Figure 6 *Am. languida* LM micrographs (a, b) and schematic drawings (c, d) including the thecal plates in Kofoidean notation (vp = ventral pore). Scale bars = 2 µm.

Synonyms: None.

Morphology: Cells of *Amphidoma languida* are ovoid to slightly elliptical (length-width ratio = 1.3), with a conical episome and a distinctly pointed APC. Cells are small (12.9–15.5 µm in length and 9.7–14.1 µm in width). The episome is slightly larger than the spherical hyposome, which ends in a pointed antapex. At the light microscope level *Am. languida* is very similar to small species of *Azadinium*. Electron microscopy, however, reveal major differences in plate pattern, with a Kofoidean plate pattern of Po, cp, X, 6′, 0a, 6″, 6C, 5S, 6‴, 2′′′′. *Am. languida*, as other species of the genus *Amphidoma*, has thus 6 apical and no anterior intercalary plates, whereas *Azadinium* has 3–4 apical plates and 2–3 anterior intercalary plates. Other specific details visible with SEM are the presence of a large antapical pore (which in fact is a field of a number of small pores) and the location of a ventral pore on the anterior right side of the first apical plate.

Distribution: The AZA-producing species *Amphidoma languida* has first been isolated from a bay in Ireland, but definitely has a much wider distribution. Sequence data from plankton samples of the Skagerrak area and strains of this species from the Norwegian coast and from Iceland indicate the presence of *Am. languida* in the North Sea and the North Atlantic as well. More recently, it has been observed in SEM from a seawater sample collected at Saint-Pierre and Miquelon in 2012 and at several sampling locations along the southern coast of the Black Sea in 2014. In contrast to the shallow coasts of Ireland and the Subarctic near Iceland, cells most likely determinable as *Am. languida* have been observed in SEM from a sample collected at the open West Indian Ocean as well. Moreover, *Am. languida* was present in a 1991 bloom sample from the Argentinean shelf. Finally, a culture of *Am. languida* has been established from water off the Atlantic coast of southern Spain.

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Author: Urban Tillmann

Aureococcus anophagefferens Hargraves et Sieburth & *Aureoumbra lagunensis* DeYoe et Stockwell – Brown Tides

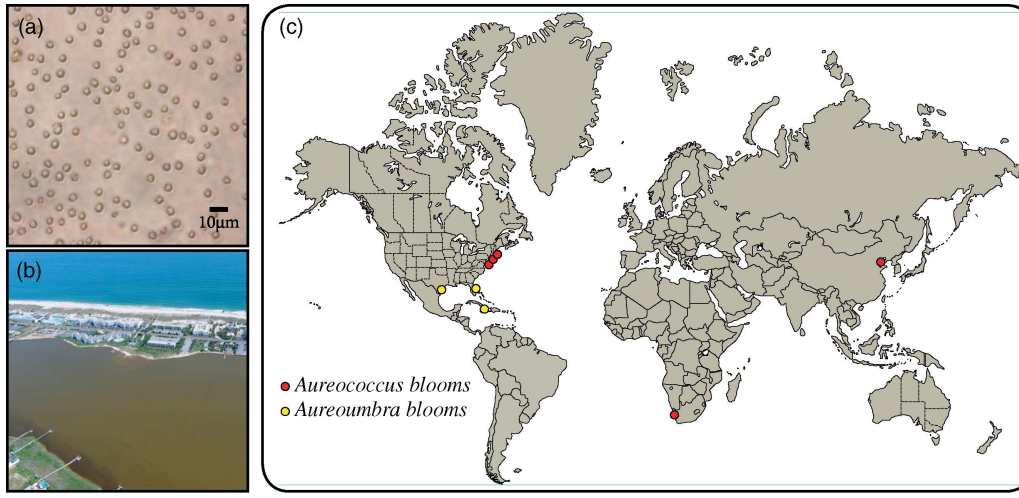

Figure 1 *Aureoumbra lagunensis* (a), brown tide from *Aureococcus anophagefferens* (b). Known distribution of brown tides (c).

Synonyms:

- *Aureococcus anophagefferens* Hargraves et Sieburth, 1988
- *Aureoumbra lagunensis*, DeYoe et Stockwell, 1987

Morphology: *Aureoumbra lagunensis* and *Aureococcus anophagefferens* are both small (4–5 µm for *Aureoumbra* and 2–3 µm for *Aureococcus*), spherical, non-motile cells without flagella containing the pigment 19′-butanoyloxyfucoxanthin. Cells typically each have a slight "dimple," making them not perfectly round.

Known Distribution: In the U.S., *Aureococcus* blooms have occurred in Narragansett Bay, Rhode Island; Great South Bay and the Peconic Estuary on Long Island, NY; Barnegat Bay, NJ; Little Assawoman Bay, Delaware; Chincoteague Bay, Maryland; and Virginia. During the past decade, blooms have been most common along the south shore of Long Island, NY, and in Chincoteague Bay, Maryland. Globally, blooms have been documented in South Africa and China. Cells of *Aureococcus* have been detected from Florida to Maine in the U.S. Global DNA sequencing studies have documented this species as being present globally.

Blooms of *Aureoumbra* have occurred in Laguna Madre and Baffin Bay, Texas, as well as the Indian River Lagoon and Mosquito Lagoon, Florida. Blooms have also been confirmed in Cuba. Low concentrations of *Aureoumbra* cells have also been found in coastal bays across Florida, Texas, and Mexico.

Cysts: Neither species is known to create cysts. Both species may have resting stages. *Aureococcus* has been found in ships' ballast water and has been shown to survive 40 days of darkness. *Aureoumbra* has been shown to form resting cells under stress capable of surviving multiple months in the dark. *Aureoumbra* resting cells have been described as more rounded than vegetative cells and larger (~10 µm).

Toxin: Both species have adverse impacts on invertebrates and zooplankton, however, no toxin has been characterized from either species. Neither species is harmful to humans. Both species secrete excessive amounts of extracellular polysaccharides. These substances are thought to contain or to be the toxic principle in these species. *Aureococcus* adversely impacts the growth, survival, and reproduction of many bivalves, including larval, juvenile, and adult northern quahog (=hard clam) (*Mercenaria mercenaria*), larval and adult bay scallops (*Argopecten irradians*), adult and juvenile blue mussels (*Mytilus edulis*), and larval oysters (*Crassostrea virginica*), and inhibits grazing by zooplankton. Both types of brown tides achieve extremely high cell densities, often exceeding one million cells per milliliter. Extreme

Aureococcus anophagefferens & Aureoumbra lagunensis

levels of biomass block out light and thus contribute to the mass loss of seagrass.

The ecological impacts of *Aureoumbra* appear to be similar to those of *Aureococcus*. Blooms of *Aureoumbra* cause a substantial increase in light attenuation, and the resulting shading of the bottom decreased the abundance of the once extensive sea grass beds. *Aureoumbra* has been associated with slowed growth and/or mortality of northern quahogs (*Mercenaria mercenaria*), eastern oysters (*Crassostrea virginica*), and the dwarf surfclam (*Mulinia lateralis*). *Aureoumbra* has also been shown to cause mortality in polychetes and multiple species of zooplankton.

Methods for Toxin Identification: Toxins have yet to be assayed quantitatively in these species.

Ecological Observations: *Aureococcus* brown tides occur in shallow estuaries with long residence times and high salinities (> 25). These estuarine characteristics may foster the accumulation of algal biomass and a nutrient environment (high dissolved organic matter and low dissolved inorganic nitrogen) as well as a reduced light regime that encourages rapid cellular growth of *A. anophagefferens*. *Aureococcus* brown tides can lose dominance when inorganic nutrient levels are high. A lack of sufficient grazing control by benthic and pelagic suspension feeders during the initiation phase of blooms is also implicated in the development of brown tides.

Conditions for blooms of *Aureoumbra* share some similarities with *Aureococcus* blooms. *Aureoumbra* blooms occur in shallow lagoons with long residence times and have high levels of dissolved organic nitrogen and low levels of nitrate. *Aureoumbra* prefers ammonium as a nitrogen source and is capable of proliferation when phosphate levels are very low. A key and unique characteristic of *Aureoumbra* blooms is that they frequently occur under hypersaline conditions or when salinities exceed 40.

General Notes: The extremely small and nondistinct nature of these cells makes their identification in the wild extremely difficult if not impossible with a light microscope. Immunofluorescent antibodies have been developed for each species that permits confirmation and quantification of each species via epifluorescent microscopy or flow cytometry. A quantitative PCR assay has also been developed for *Aureococcus*.

Blooms of *Aureococcus* have been particularly damaging to shellfisheries. In NY, combined annual losses of the bay scallop fishery alone have amounted to more than $100M during the past three decades. In China, a single bloom event was responsible for more than $30M in lost shellfish.

Blooms of both species often develop during the late spring. *Aureococcus* blooms often dissipate in late summer when temperatures exceed 25 °C, but can re-bloom in the fall when temperatures drop below 20 °C.

The first recorded bloom of *Aureoumbra* persisted continuously for seven years in Laguna Madre, TX, 1990–1997.

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Author: Christopher J. Gobler

Ceratium furca (Ehrenberg) Claparede & Lachmann

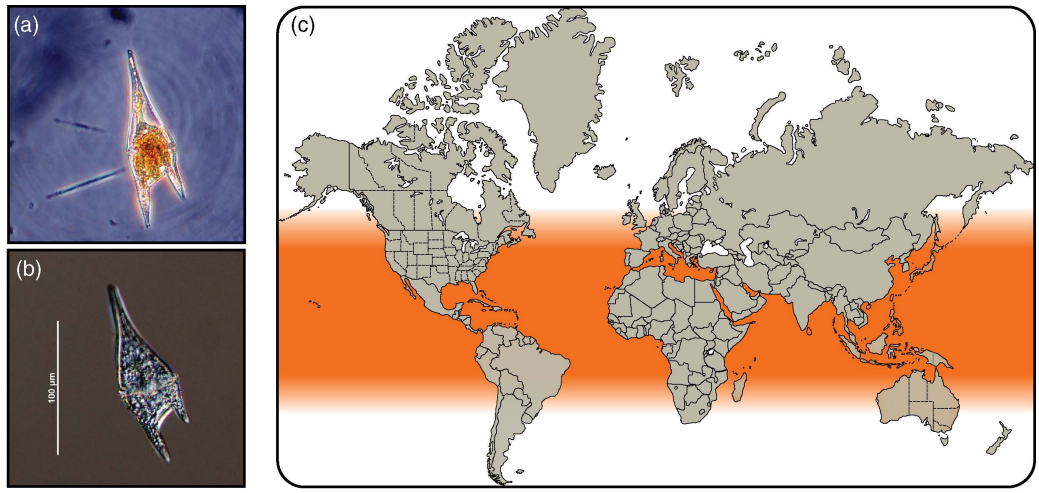

Figure 1 (a–b) Light micrographs of *Ceratium furca*. (c) Distribution of *C. furca* blooms – global, primarily in temperate and tropical waters.

Synonyms:

- *Peridinium furca* Ehrenberg, 1834

Morphology: This species is characterized by the presence of three horns, one apical horn and two antapical horns, where the left antapical horn is well developed while the right antapical horn is reduced. The thecal plates are thick with ridges and pores. Cells are 70 to 200 µm long and 30–50 µm wide.

Known Distribution: Cosmopolitan in cold temperate to tropical waters; planktonic.

Cysts: No cysts are known.

Toxin: No known toxin produced.

Methods for Toxin Identification: Not applicable.

Ecological Observations: *Ceratium furca* is known to cause anoxic red tides, which have direct effects on fish by damaging gills or promoting low-dissolved-oxygen concentrations. Persistent blooms have been reported in the North Sea, North Atlantic Ocean, Indian Ocean, and Southeast Asia area.

General Notes: N/A.

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Author: Steve L. Morton

Chattonella marina

Synonyms:

- *Chattonella marina* (Subramanian) Hara & Chihara 1984
- *Chattonella marina* var. *marina* Demura & Kawachi 2009
- *Hornellia marina* Subramanian 1954

Morphology: Cells pyriform with a blunt posterior and tightly packed chloroplasts surrounding a central nucleus. Two flagella, emerging from a subapical groove. Some cells spherical with little surface definition. Color normally golden brown depending on previous light history. Cell dimensions 30–50 µ long and 20–30 µ wide. Cells exhibit positive phototaxis in low turbulence environments. Form nondescript cyst stages.

Known Distribution: Found in enriched temperate to tropical waters at temperatures from 15 to 25 °C. Eastern U.S.: Eastern Shore, Maryland; New River, North Carolina; Pt. Aransas, Texas; Redondo Beach, California.

World Distribution: Inland Sea, Japan, India, South Australia, Tasmania, New Zealand, Mexico, Gulf of California.

Cysts: Cysts of *Chattonella marina* are usually small, unbound by surface structures, and often attached to detrital material such as empty diatom frustules. They remain an important part of the life cycle and are major elements needed for recurrent blooms of this species (Imai and Yamaguchi, 2012).

Toxin: Reactive oxygen species (superoxide, peroxide, and hydroxide radicals), polyunsaturated fatty acids, brevetoxin-like compounds.

Methods for Toxin Identification: Chemiluminescent methods, LC-MS.

Biological Observations: The effects of *Chattonella marina* are particularly notable on fish exposed to bloom populations. Symptoms including cardiac irregularities of fish, and disorientation with fish swimming upside down, are commonly associated

Chattonella marina

with a neurotoxic component. There is a notable production of mucous about the gills with potential water channel blockage when fish are exposed to high densities of this species. Allelopathic effects on other phytoplankton species were noted, including competition with other harmful algal species.

General Notes: Like most of the raphidophytes, *Chattonella marina* is considered a noxious species lacking a strong single toxin. Its effect on fish is notable and has caused large losses of fish farmed in open pens resulting in complete loss of stock. No effective control mechanism has been devised to prevent the damage from the blooms.

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Cochlodinium – Rust Tide

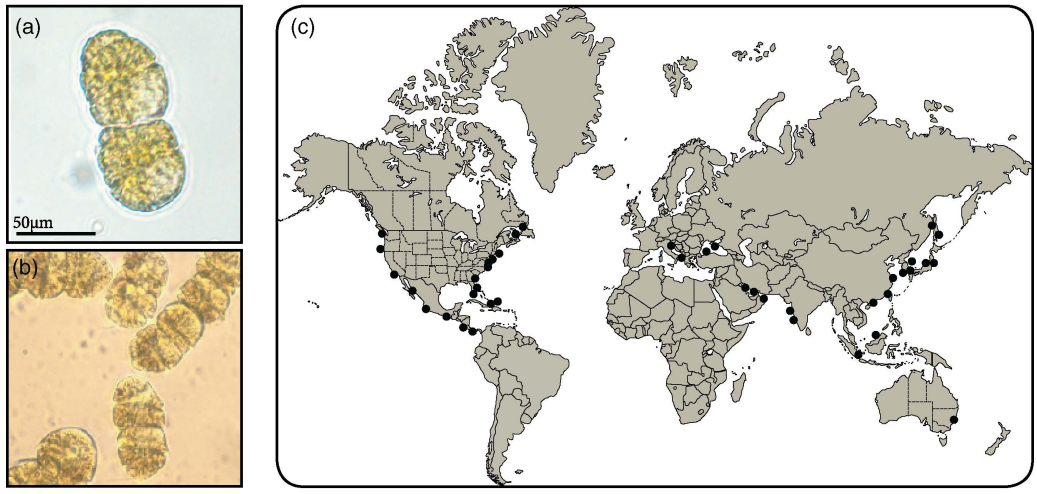

Figure 1 Light micrographs of *Cochlodinium*. (a) *C. fulvescens* obtained from a red tide event in coastal waters of Avila Beach, California, U.S. (b) *C. polykrikoides* isolated from estuarine waters of the U.S. east coast. (c) Known distribution of *Cochlodinium* blooms.

Synonyms: It is noted that a new name for this genus has recently been proposed (Margalefidinium). This name has yet to be widely accepted by the scientific community. More than 40 species of *Cochlodinium* have been identified, but two species are confirmed, common HAB species:

- *Cochlodinium polykrikoides* Margalef, 1961
- *Cochlodinium fulvescens* Iwataki, Kawami & Matsuoka, 2007

Morphology: Both dinoflagellates are large (25–45 µm) naked dinoflagellates composed of oblong cells that commonly form chains of 2 to 8 cells. Cells are typically filled with brownish pigments with *C. polykrikoides* containing rod-like chloroplasts and *C. fulvescens* containing granular chloroplasts. A cingulum encircling cells more than 1.5 times is a defining morphological feature of cells. Cells typically contain a reddish-orange eyespot in the anterior dorsal region.

Known Distribution: In the U.S., *C. polykrikoides* blooms along the U.S. east coast from Massachusetts to Florida as well as in Puerto Rico. Blooms have been specifically noted in Nantucket and other regions of coastal Massachusetts; Point Judith Pond, Rhode Island; Long Island Sound, the Peconic Estuary, Shinnecock Bay, and Great South Bay in New York; Barnegat Bay, New Jersey; the York and James Rivers and other tributaries of Chesapeake Bay, Virginia; Skidaway Estuary, Georgia; the Indian River Lagoon, Florida; and the bays of Puerto Rico. While blooms in New Jersey, Rhode Island, Virginia, and Puerto Rico were noted decades ago, blooms in Massachusetts, New York, Georgia, and Florida were all documented for the first time in the past decade.

C. fulvescens has formed harmful blooms in Monterey Bay, California, and unknown *Cochlodinium* species have been detected in La Jolla, California.

Cysts: Recently, conclusive evidence of cyst formation in *C. polykrikoides* has been established in both laboratory and field studies. In New York, cysts of *Cochlodinium* appear as similar in size to *Cochlodinium* cells, rounded, yellowish-brown in color, and containing one or sometimes two red accumulation bodies, with a cyst wall that is relatively thin (<2 µm), possibly composed of two layers. Cyst surfaces are smooth, without rough projections seen in cysts of other unarmored dinoflagellates. In contrast, cysts of *C. polykrikoides* from Korea have been shown to have external ornamentation, although this represents a different ribotype of *C. polykrikoides* and environmental conditions, such as anoxic sediments, can alter external cyst morphology.

Toxin: These blooms are strongly ichthyotoxic and can also kill many other marine organisms, although the compounds responsible for these impacts have yet to be identified and bloom-associated toxins are not known to affect human health. U.S. east coast isolates

Cochlodinium

of *C. polykrikoides* have been shown to cause rapid mortality in multiple species of fish, with smaller and early life stage fish being more vulnerable than larger fish. Importantly, however, even large fish in enclosed regions (pens, tanks, creeks) exposed to *C. polykrikoides* can perish during exposure to blooms of this algae. Other organisms shown experiencing mortality during these blooms include larval and juvenile bay scallops (*Argopecten irradians*), juvenile eastern oysters (*Crassostrea virginica*), and larval stages of northern quahogs (=hard clam) (*Mercenaria mercenaria*). *C. polykrikoides* can also cause rapid mortality in zooplankton and competing phytoplankton.

There have been two, not mutually exclusive, explanations for the toxicity of *C. polykrikoides*: reactive oxygen species (ROS) and extracellular polysaccharides. There have been substantially more data generated describing putative toxins as behaving like the ROS than like extracellular polysaccharides. Importantly, no study has comprehensively and conclusively demonstrated the precise toxic action in *C. polykrikoides*.

Methods for Toxin Identification: Toxins have yet to be quantitatively assayed in this species. Multiple methods are available to quantify ROS, although these compounds have yet to be fully confirmed as the toxic principal in either species.

Ecological Observations: Blooms of *C. polykrikoides* on the U.S. east coast have been shown to occur over a broad range of temperatures (15–30 °C) and salinities (19–30). *C. fulvescens* seems well-adapted to warm (>20 °C) moderate (30–33) salinities often associated with offshore waters. The ability of *C. polykrikoides* to cause rapid mortality in a variety of zooplankton, bivalves, and planktivorous fish may promote blooms by minimizing population losses. The ability of *C. polykrikoides* to cause rapid mortality in other phytoplankton may promote blooms by minimizing competition. On the U.S. east coast, *C. polykrikoides* blooms occur in moderately eutrophied estuaries, meaning blooms may be linked, in part, to excessive nutrient loads. Blooms and isolates of *C. polykrikoides* from the U.S. east coast have displayed nutritional flexibility, capable of growing rapidly via the use of multiple nitrogen sources and changing to use the most abundant nitrogen source available within differing regions. Some strains of *C. polykrikoides* have been shown to be phagotrophic, capable of consuming other phytoplankton for nutrition.

General Notes: *Cochlodinium* blooms often manifest as spatially large (10s to 100s of kilometers) and dense (>1000 cells mL^{-1}) cell aggregates or patches that are heterogeneous in their vertical and horizontal distributions. This species vertically migrates, and thus bloom patches may migrate to depths at night. The reddish-brown coloration of bloom patches has lead to the common name for these blooms, rust tide.

C. polykrikoides blooms can been particularly damaging to aquacultured fish that cannot escape dense bloom patches. In Korea, fisheries losses due to *C. polykrikoides* were noted as exceeding $100M in a single year.

There has been modest success in mitigating the impact of *C. polykrikoides* on caged fish via the application of modified clays to surface waters.

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Author: Christopher J. Gobler

Cyanobacteria

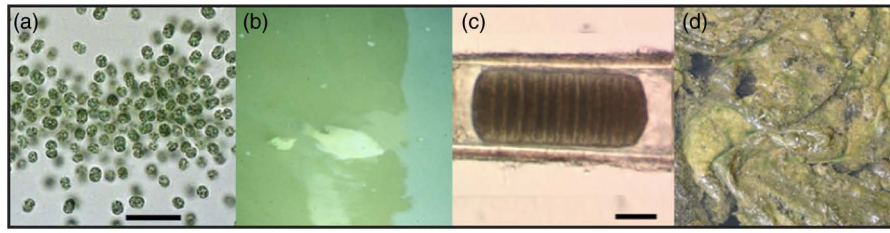

Figure 1 Examples of toxigenic (potentially toxic) cyanobacteria and their outbreaks or "blooms": (a) planktonic *Microcystis* (*photo credit*: West Bishop, North Carolina State University [NCSU]); (b) toxic bloom of *Microcystis aeruginosa* with floating dead bluegill sunfish (*Lepomis macrochirus*) (*photo credit*: the late Robert Moeller); (c) a hormogonium (short, asexually reproductive trichome or filament) of benthic *Lyngbya wollei* (*photo credit*: Elle Allen, NCSU Center for Applied Aquatic Ecology [CAAE]); and (d) floating scum from a toxic bloom of *P. wollei* (*photo credit*: Elle Allen, NCSU CAAE). Scale bars = 50 µm.

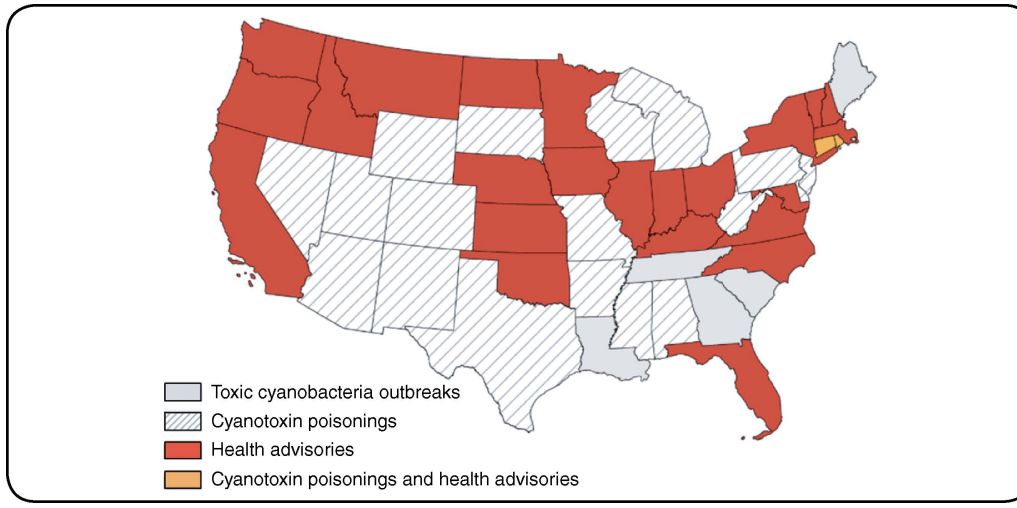

Figure 2 Map showing general locations of toxic cyanobacteria outbreaks or blooms, which have occurred in every state on the U.S. mainland. Some of these blooms have been associated with cyanotoxin poisonings of wildlife, domestic stock, pets, and/or humans; others have resulted in health advisories or, in two states, both cyanotoxin poisonings and health advisories. *Source*: Modified from Graham and Loftin (2014) to include information from Lewitus *et al.* (2008) and the U.S. Environmental Protection Agency (U.S. EPA, 2009). Note that toxic cyanobacteria blooms also have been documented in Alaska and Hawaii (U.S. EPA, 2016).

Synonyms: The division "Cyanobacteria" follows the bacteriological code. As summarized in Burkholder (2002 and references therein), synonyms include:

- Cyanophyta (botanical code)
- Blue-green algae
- Prochlorophyta (botanical code)
- Mixophyta ("slime plants")

General: Toxic cyanobacterial blooms are widespread, especially in nutrient over-enriched freshwaters during warm seasons. Because strains within a given species can vary from nontoxic to highly toxic, and because toxicity status can change over the course of a bloom, the only way to assess whether a bloom is toxic is to analyze samples to verify the presence or absence of, at least, common cyanotoxins such as microcystins.

Morphology: The three basic cyanobacteria morphs include coccoid unicells, colonies of coccoid cells, and unbranched or branched filaments (trichomes) (Burkholder, 2002 and references therein). Filaments may be uniseriate or multiseriate. They may be unbranched, or they may have "false" or true branching. Most cyanobacteria have copious outer mucilage that is often visible under light microscopy. These few morphs occur across cyanobacterial taxa, making species identifications based on morphology and light microscopy difficult or impossible.

Cyanobacteria

Molecular techniques increasingly have been relied upon for identifications.

Cyanobacteria are prokaryotes, meaning that their cells lack organelles. Thus, their pigments are not compartmentalized, and the cells appear uniformly pigmented in light microscopy. The cells generally are small (~0.4–3.0 µm, biovolume < 1 to 10 μm^3), although some unicellular and coccoid taxa are much larger (maximum dimension up to 60 µm, biovolume > 10,000 μm^3). Thick-walled specialized cells in cyanobacteria include heterocytes (heterocysts) for nitrogen fixation (conversion of nitrogen gas into ammonia), although nitrogen fixation can also occur in non-heterocytous cyanobacteria (e.g., *Trichodesmium*; *Lyngbya*; Bergman *et al*., 1997) and in akinetes, which are resting stages of vegetative cells packed with food reserves.

Life Stages, Cysts: Cell division in cyanobacteria occurs by primitive fission. Vegetative (asexual) reproductive cells found in some taxa include baeocytes (endospores) produced by subdivision of a cell into multiple cytoplasmic units; exospores, which bud from the apex of a filament; and akinetes, the specialized resting stages or cysts mentioned above (Burkholder, 2002 and references therein). There is no known sexual reproduction in cyanobacteria. Transfer of short DNA fragments has been documented in some rapidly growing, unicellular strains (populations), but the mechanism for DNA uptake has not been determined, and transduction or conjugation (mating) has not been confirmed.

Unless otherwise noted, the following information is taken from Chorus and Bartram (1999), Burkholder (2009), O'Neil *et al*. (2012), and Gobler *et al*. (2016).

Toxicity, Toxin, and Effects: At least 50 taxa within 20 genera, about one-third benthic and the rest planktonic species, are toxigenic (potentially toxic) – that is, within each species, some strains can produce cyanotoxins, defined as chemical substances that cause animal and human poisonings or health risks. Half of these species are within six genera – *Anabaena, Dolichospermum, Microcystis, Nostoc, Phormidium*, and *Planktothrix*. Most toxigenic species are found in freshwaters, but some taxa, such as *Nodularia spumigena* (planktonic) and *Lyngbya majuscula* (benthic), are brackish or marine. Many cyanobacteria also produce bioactive substances with antimicrobial and cytotoxic effects, the latter often referred to as *cytotoxins*.

At least 150 cyanotoxins are known, including neurotoxins, hepatotoxins, and cytotoxins with selective bioactivity. There are also numerous cyanotoxins that have not yet been identified, including various cytotoxins, lipopolysaccharide endotoxins, and volatile sulfur substances. Most known cyanotoxins are multiple variants of cyclic peptides, alkaloids, and lipopolysaccharides. For example, there are more than 90 congeners (forms) of microcystin cyanotoxins. Many strains produce multiple toxins depending upon various molecular and environmental controls. The toxins vary from hours to months in the time required for degradation in the environment. The most commonly reported cyanotoxins in the U.S. are microcystins, cylindrospermopsins, anatoxins, saxitoxins, and acutiphycins. A likely addition to this list may also be the more recently characterized β-methyl-amino-L-alanine (BMAA), which is produced by a wide array of freshwater, estuarine/marine, and terrestrial cyanobacteria (see review in Holtcamp, 2012).

Cyanotoxins can cause disease and death of zooplankton, fish, birds, and wildlife. Among fish species, there is high variation in sensitivity to cyanotoxins, but impacts have included damage to liver, kidney, heart, gills, skin, and spleen. These toxins also can cause human disease and, rarely, death. Humans are most commonly exposed to cyanotoxins by ingesting water that contains a toxic bloom, but also can be exposed by contact with concentrated cyanobacterial biomass ("scums") on the water surface or along shores, by contact with contaminated water, or by inhaling aerosols over or near blooms. Scums can contain especially high levels of cyanotoxins. Based on mouse/rat models, research on birds, wildlife, and livestock, and more limited information on humans, sublethal exposure to cyanotoxins can cause nausea, vomiting, asthma-like symptoms, liver hemorrhaging and failure, and central nervous system dysfunction. Cyanotoxins may be a cause of the amyotrophic lateral sclerosis–parkinsonism–dementia complex (Holtcamp, 2012 and references therein). They can also promote malignant hepatic, abdominal, uterine, and thoracic tumors.

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Cyanobacteria

Methods for Toxin Identification and Detection: Cyanotoxins have been identified using chromatographic methods, including gas chromatography with flame ionization detection (GC/FID) or with mass spectrometry (GC/MS); thin layer chromatography (TLC); and various types of liquid chromatography (LC): high-performance liquid chromatography/ultraviolet-visible detection (HPLC/UV), LC/fluorescence (FL), LC ion trap mass spectrometry (LC/IT MS), LC time-of-flight mass spectrometry (LC/TOF MS), LC single quadrupole mass spectrometry (LC/MS), or LC triple quadrupole mass spectrometry (also LC/MS). Less costly biological assays are available for some toxins, such as animal tests (e.g., mice), enzyme-linked immunosorbent assays (ELISAs), protein phosphatase inhibition assays (PPIAs), and neurochemical assays (e.g., acetylcholinesterase-based) (Loftin *et al*., 2010; U.S. EPA, 2014).

Ecological Observations: As prokaryotes, cyanobacteria tend to have small cells and rapid growth rates. Planktonic species are widespread in neutral and alkaline habitats, and often dominate the late summer plankton of eutrophic, temperature-stratified lakes and reservoirs, slowly flowing lower rivers, and stratified marine waters. Many species are diazotrophs, meaning that they can "fix" or convert dinitrogen gas (N_2) to inorganic nitrogen as ammonia, which gives them a competitive advantage over other algae when N becomes limiting in surface waters. Cyanobacteria harmful algal blooms (cyanoHAB) can form major water discoloration with up to several billion cells mL^{-1}. They commonly deplete dissolved oxygen due to high respiration at night, causing massive fish kills. Nutrient (phosphorus [P] and N) over-enriched conditions stimulate cyanobacteria.

While both P and N are important, recent findings indicate that the growth and toxicity of some non-diazotrophic cyanobacteria such as *Microcystis* spp. can be controlled by N, and that cyanobacteria biomass can often be more strongly related to inorganic N than P concentrations. Shifts in the N:P supply ratio due to management actions that focus on reducing one but not both nutrients also tend to favor cyanobacteria.

In recent decades, the incidence and intensity of cyanobacteria blooms have increased. There are new discoveries of cyanotoxins, and newly reported genera capable of producing cyanotoxins reported on a continual basis. These changes have been linked to increasing nutrient pollution, food web alterations such as overfishing, and increasing temperatures from global warming. Toxic cyanobacteria blooms are expected to continue to increase in magnitude and frequency as warming and nutrient pollution continue.

As important harmful algae in many freshwaters, tidal fresh habitats, estuaries, and marine waters, cyanobacteria and their ecology are discussed throughout much of this *Compendium*, including emphasis on benthic filamentous species across the salinity gradient (Chapter 15), and other taxa (e.g., Chapter 1). Among many diverse, predominantly planktonic harmful taxa, two notable examples, *Cylindrospermopsis raciborskii* and *Microcystis aeruginosa*/*Microcystis* spp., are briefly described here. Major toxins CYL and MCs, which these and various other cyanobacteria produce, are under consideration by the U.S. EPA (2016) for recommended numeric criteria to protect recreationists. Both taxa are sensitive to mixing and low light, and under stratified conditions they use buoyancy regulation via internal gas vacuoles to control their vertical station in the water column.

***Cylindrospermopsis raciborskii*:** Temperate to tropical freshwaters (see de la Cruz *et al*., 2013; U.S. EPA, 2016). This filamentous, akinete- and heterocyte-forming diazotroph has been likened to an invasive species because it has extended its geographic range within the past two decades. Toxic strains produce CYL, deoxy-CYL, and/or anatoxin. Blooms and CYL production generally occur when phosphate concentrations are very low; CYL levels have been positively correlated with total P concentrations; and in P-limited cultures during exponential growth, CYL production rates have been positively correlated with growth rates. Contrasting effects of N have been reported for toxin production versus growth: growth in culture has been more rapid with N as ammonium, followed by nitrate and then urea. Highest intracellular CYL has been found at low N, whereas lowest intracellular CYL has occurred at saturating ammonium. Blooms generally form deeper in the water column, and can be more difficult to detect.

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Cyanobacteria

***Microcystis aeruginosa*/*Microcystis* spp.:** Fresh and brackish waters – notorious bloom former on every continent except Antarctica (see Figure 3; O'Neil *et al*., 2012; Gobler *et al*., 2016; U.S. EPA, 2016). Toxic *Microcystis* strains most commonly occur as colonies of small coccoid or oval cells within a thin mucopolysaccharide matrix. Their blooms commonly occur at the water surface and can accumulate along shorelines as toxic scums. Various *Microcystis* spp. can produce MCs (more than 100 congeners or forms known – Meriluoto and Spoof, 2008), anatoxin-a, BMAA, cyanoginosins, cyanoviridin, toxic lipopolysaccharides, and toxic unidentified volatile sulfur compounds (Fristachi and Sinclair, 2008; Rastogi *et al*., 2014). As they are non-diazotrophs, N appears to be as important as, or more important than, P in controlling toxic blooms. Increasing N levels generally increase growth and toxicity, and toxic blooms often occur in high-N waters. Bloom populations tend to shift from dominance by toxic to nontoxic strains as N (nitrate and/or ammonium) declines through the summer and as maximum cell levels occur. MCs are N-rich, and toxin synthesis is N-demanding; thus, toxic cells appear to have a higher N requirement than nontoxic cells, and toxic strains have higher growth rates at high N concentrations. Some forms of dissolved organic N can be used efficiently by *Microcystis*, such as urea and amino acids.

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Figure 3 **(facing page)** Recent examples of massive freshwater toxigenic cyanobacteria outbreaks: (a) Satellite image of Lakes St. Clair and Erie, U.S.–Canada, April 2005, showing all of Lake St. Clair (1114 km^2 or 430 $mi.^2$) and about 80% of the Lake Erie, U.S.–Canada (25,719 km^2 or 9930 $mi.^2$), covered by *Microcystis aeruginosa*, including toxic strains – note that these lakes have sustained such blooms, sometimes over much of the year, for more than 15 years. (b) Satellite image of Lake Okeechobee, Florida, U.S., July 2016, where a toxic bloom of *M. aeruginosa* covered about one-third of the surface area (619 km^2 or 239 $mi.^2$) in summer 2016. (c) The Ohio River, U.S., August–September 2015, where a 1046-km (650-mi.) segment spanning Indiana, Ohio, and Kentucky waters sustained a toxic *M. aeruginosa* bloom; and (d) Utah Lake, Utah, U.S., July–August 2016, where 90% of the lake surface area (~346 km^2 or ~133 $mi.^2$), as well as an 82-km (51-mi.) segment of downstream Jordan River and canals, were impacted by a mixed cyanobacterial bloom. *Photo credits*: (a) European Space Agency and MODIS/TERRA/NASA; (b) NASA Earth Observatory, by J. Stevens using USGS Landsat data; (c) West Virginia Department of Environmental Protection; (d) Valente, *Daily Herald*, Salt Lake City, Utah.

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Dinophysis

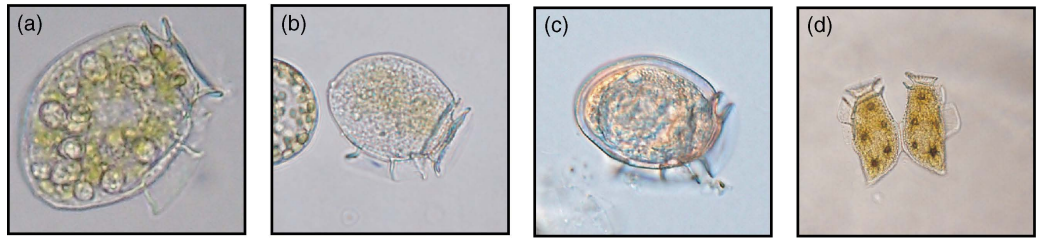

Figure 1 Examples of *Dinophysis* spp. *Source*: Photographs courtesy of Steve L. Morton.

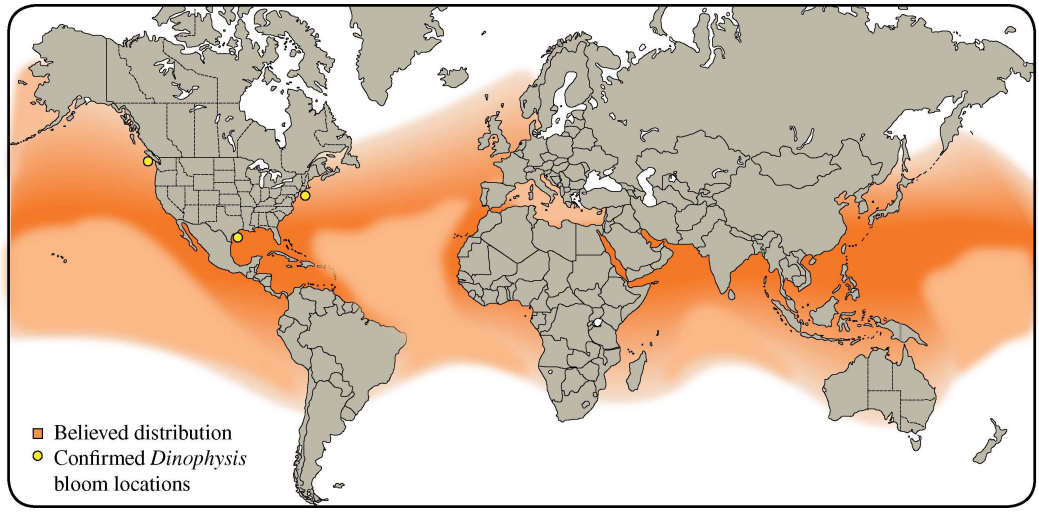

Figure 2 Known distribution of the *Dinophysis* sp. dinoflagellates, with suspected circumtropical distribution in orange and known bloom locations indicated by filled yellow circles.

Synonyms:

- *Dinophysis elipsoides Kofoid 1907*
- *Dinophysis lacmannii Paulsen 1949*
- *Dinophysis skagii Paulsen 1949*
- *Dinophysis borealis Paulsen 1949*
- *Dinophysis boehmii Paulsen 1949*
- *Dinophysis lacmanii Solum 1962*

Morphology: *Dinophysis acuminata* is characterized by a high girdle with lists, giving the form of a collar. Very reduced epitheca. Cells are relatively small, oval in shape. Left sulcal list supported by three ribs. Thecal surfaces are areolated, but these surface markings are dependent on the age of the cell. Cells are between 35–50 µm long and 30–35 µm wide. Usually dorsally and ventrally flattened.

Known Distribution: Neritic, cold to warm temperate regions. Toxic blooms in the United States have been observed in New York and Washington.

Cysts: No cysts have been observed.

Toxin: Known producer of okadaic acid, dinophysis toxin(s), and pectenotoxin.

Methods for Toxin Identification: Okadaic acid and *Dinophysis* toxins can be identified using commercially available ELISA test kits or the protein phosphatase assay in field laboratories. Regulatory methods rely on LC-MS/MS. Pectenotoxin can only be monitored using LC-MS/MS.

Ecological Observations: *Dinophysis acuminata* is a mixotrophic dinoflagellate that feeds using a peduncle. This species can maintain actively photosynthetic plastids from prey items for several

Dinophysis acuminata

generations. The ciliate *Mesodinium rubrum* has been identified as the primary prey species. In culture, *Mesodinium* is required to feed on various species of cryptophytes before being preyed on by *Dinophysis acuminata*.

General Notes: There are several potentially toxin-producing species of *Dinophysis*, including D. *acuminata*, *D. acuta*, *D. caudata*, *D. fortii*, *D. infundibula*, *D. miles*, *D. norvegica*, *D. ovum*, *D. sacculus*, and *D. tripos*. In the Gulf of Mexico, a similar species, *Dinophysis ovum*, is responsible for diarrheic shellfish poisoning (DSP) events. It is difficult to determine the difference between these two species using a light microscope; thus, some observations call these species the "*Dinophysis acuminate* species complex."

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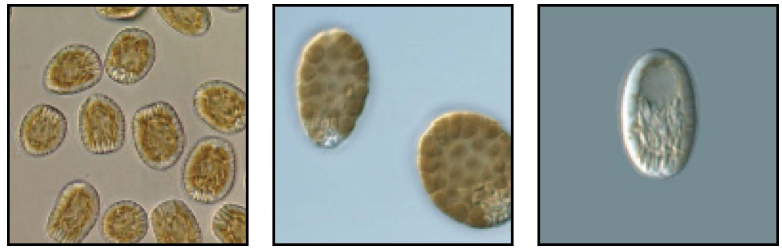
Author: Steve L. Morton

Fibrocapsa japonica



Synonyms:

- *Fibrocapsa japonica* (Toriumi et Takano)
- *Chattonella japonica* Loeblich III & Fine 1977
- *Exuviella* sp. Iwasaki, 1971
- *Exuviella* sp. (isolated by Jordan, Pt. Loma, CA 1970)
- *Chattonella japonica* UTex LB 2162

Morphology: Shape varies from oval to round to rectangular, and is rarely seen as a pyriform (teardrop)-shaped cell. Size varies in the range of 20–30 µm long × 15–17 µm wide. As with all raphidophytes, *Fibrocapsa* has two flagella inserted in a subapical groove. The forward oriented flagellum is 1 to 1½ body length, while the posteriorly oriented one is usually carried alongside the cell and is much shorter (< body length). This species is a relatively weak swimmer, but has distinctly dark-brown plastids throughout the cell body. A distinguishing feature is the concentration of mucocysts appearing as a colorless or transparent area in the posterior of the cell. Mucocysts are commonly ejected with the addition of fixatives.

Known Distribution: Observed in Massachusetts (Falmouth salt ponds); Narragansett Bay, Rhode Island; Long Island Sound; Delaware Inland Bays; Maryland Eastern Shore Bays; York River, Virginia; Neuse River, North Carolina; New River and Cape Fear River basins, and Kiawah Island, South Carolina; Savannah River, Georgia; St. Johns River, Bayboro Harbor (Tampa Bay), Florida; Corpus Christi Bay, Texas; Redondo Beach pier, California.

World distribution: Japan, France, Italy (Adriatic), Germany, Holland, Mexico, Brazil, Uruguay.

Cysts: Spherical cysts of *Fibrocapsa japonica* are reported from sediment samples and culture. The cysts appear to be well formed and defined. Viable cells have been released from cysts in laboratory conditions.

Fibrocapsa japonica

Toxin: Confirmation of toxins from *Fibrocapsa japonica* indicate their effect as hemolysins as measured by the erythrocyte lysis analysis. These presumably are related to the PUFAs present in this species. There is additional evidence of the presence of polyether neurotoxins as measured by HPLC and LC-MS.

Methods for Toxin Identification: Hemolysins – erythrocyte lysis analyses, PUFAs, GC-MS, NMR spectroscopy; polyether neurotoxins – lipid extraction and then observed by LC-MS and NMR spectroscopy, cell-based bioassays.

Biological Observations: Along with the other raphidophytes, *Fibrocapsa japonica* has an occurrence consistent with temperatures above 18–20 °C and a preference for enriched coastal areas. It often appears in association with other raphidophyte species (*Heterosigma* and *Chattonella*); in some instances, it is the last to appear in a succession of these species. Visually, it produces a dark-brown bloom and can manifest impacts by making organisms susceptible to secondary infections. When cells come in contact with fixatives such as Lugol's solution, strands of ejected mucocysts can be observed.

General Notes: There is some mention of *Fibrocapsa japonica* lacking mucocysts. To date, this is rare and not verified.

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Author: Carmelo R. Tomas

Gambierdiscus

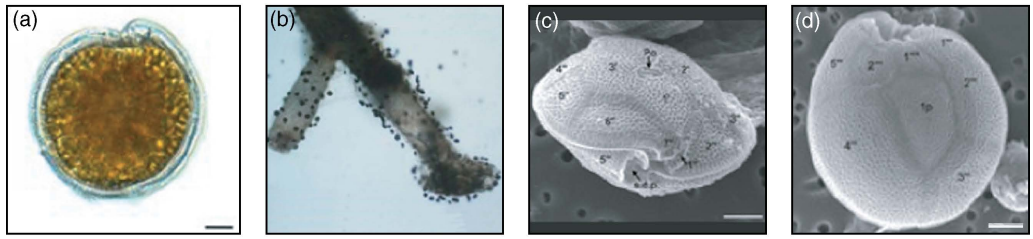

Figure 1 (a–d) Light micrograph of *Gambierdiscus belizeanus* collected from the central Saudi Arabian Red Sea (a). Image of *Gambierdiscus* cells colonizing filamentous algae (b). Cells attach to algal surfaces using mucoid threads. Scanning electron microscopy (SEM) micrograph of *Gambierdiscus belizeanus*; ventral view (c). SEM micrograph of *Gambierdiscus belizeanus*; hypothecal view (d). Scale bars are 10 µm (except for d, where the scale bar is 20 µm). *Source*: Photographs taken by M. Richlen (a), M. Parsons (b), and courtesy of Steve Morton (c and d).

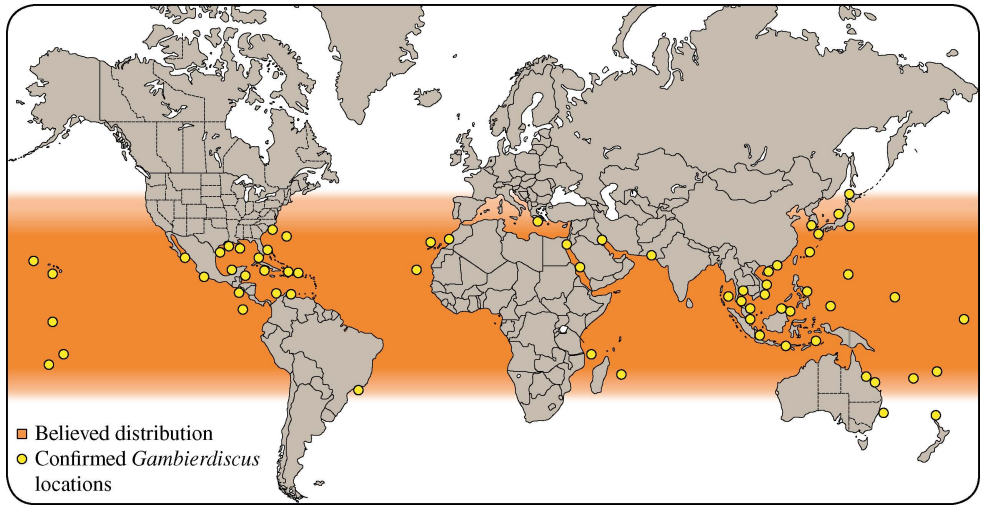

Figure 2 Known distribution of the *Gambierdiscus* sp. dinoflagellates, with suspected circumtropical distribution in orange and known locations indicated by filled yellow circles.

Synonyms: None.

Morphology: There are 15 species currently described in this genus: *G. toxicus* (type), *G. australes*, *G. balechii*, *G. belizeanus*, *G. caribaeus*, *G. carolinianus*, *G. carpenteri*, *G. cheloniae*, *G. excentricus*, *G. honu*, *G. lapillus*, *G. pacificus*, *G. polynesiensis*, G. *scabrosus*, and *G. silvae*. Cells are anterioposteriorly compressed, lenticular in shape. The epitheca of some species (e.g., *G. silvae*) is taller than the hypotheca. Cells are 45–150 µm long (ventral to dorsal), 24–60 µm deep (apical–antapical), and 42–140 µm wide (transdiameter). The modified Kofoidian plate formula is Po, 4′, 0a, 6″, 6c, 6s?, 5‴, 0p, 2⁗.

Known Distribution: Circumtropical. Known poleward extent: 36°S; 33°N (approx.).

Cysts: Unknown.

Toxin: Ciguatoxin (congeners, precursors, and metabolites) and maitotoxin. Ciguatoxins are lipophilic cyclic polyether molecules that are extremely stable. The toxins produced by key *Gambierdiscus* species have been shown to be regionally specific. Pacific ciguatoxins (P-CTXs) are characterized by a 13-ring backbone (e.g., P-CTX1), which has a molecular formula of $C_{60}H_{92}O_{19}$ and a molecular weight of 1110.3 Da. The left-hand wing of P-CTX1 has been associated with the toxic mechanism on voltage-gated sodium channels. Caribbean ciguatoxins (C-CTXs) have been identified in reef fish in the Western Atlantic, Caribbean Sea, and Gulf of Mexico, and precursors are thought to be produced by select species and biotransformed to C-CTX1 and C-CTX2 via trophic transfer. C-CTX1 is a 14-ring structure with a molecular formula of $C_{62}H_{92}O_{19}$ and

Gambierdiscus

a molecular weight of 1140.6 Da. The right-hand wing of C-CTX1 has been reported as the most critical for binding and toxic action at the voltage-gated sodium channel. Ciguatoxins from the Indian Ocean (I-CTXs) have also been characterized but have yet to be structurally elucidated. Based on the chemical data available, including accurate mass (1140.6 Da) and chromatographic separation, these toxins were reported as being similar to C-CTXs in structure. Toxicity data suggest that I-CTXs are less potent than P-CTXs and slightly more potent than C-CTXs based on mouse bioassay, and are therefore deemed a new class of CTXs.

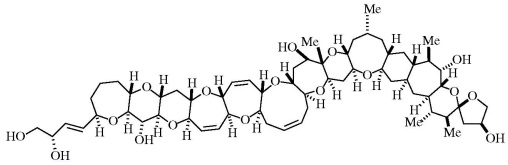

Figure 3 Structure of Pacific ciguatoxin-1b (P-CTX1b).

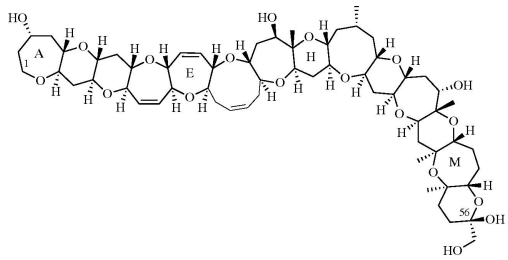

Figure 4 Structure of Caribbean ciguatoxin-1 (C-CTX1). Caribbean ciguatoxin-2 (C-CTX2) is the epimer of C-CTX1 at carbon 56, noted on the right-hand wing of the structure.

Maitotoxin (MTX) is the largest non-biopolymer known, and is thought to be involved in ciguatera fish poisoning resulting from consumption of herbivorous fish. MTX disodium salt has a chemical formula of $C_{164}H_{256}O_{68}S_2Na_2$ and a molecular weight of 3422 Da. The molecule is characterized by 32 ether rings, 28 hydroxyl groups, and two sulfate esters. The MTX molecule is thought to be similar to glycolipids and other membrane-associated components, and based on the presence of many hydrophobic rings, it may be capable of penetrating plasma membranes. Due to the presence of hydrophilic and hydrophobic functionalities and uneven distribution of polar groups, MTX is considered amphiphilic in nature. The large structure of MTX contributes to a broad suite of toxic mechanisms being reported and resulting in generalized cytotoxicity, icthyotoxicity, and hemolytic activity at very low doses.

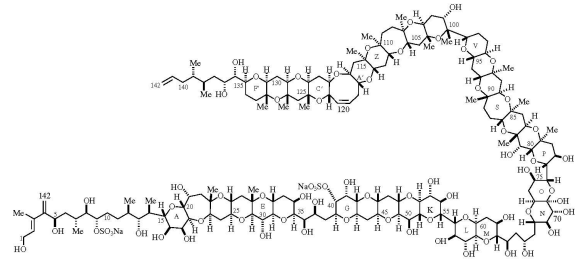

Figure 5 Complex structure of maitotoxin.

Methods for Toxin Identification: Detection methods for CTXs largely have been based on the primary mechanism of action at the voltage-gated sodium channel and chemical detection methods. Mouse bioassay has been the regulatory standard used in many countries, but other methods have proved effective and more sensitive for composite CTX detection. For instance, a sodium channel dependent (ouabain–veratridine dependent) cytotoxicity assay utilizing mouse neuroblastoma cells (N2A) has been widely used and is highly sensitive for quantification of composite sodium channel toxins present in phytoplankton, fish, and shellfish, including sodium channel activators such as ciguatoxins and brevetoxins, in addition to sodium channel blockers such as saxitoxin and tetrodotoxin. While sensitive, this method takes approximately 72 hours to obtain results. A more rapid radioligand binding assay utilizing tritiated brevetoxin has also been developed and appears to be selective for ciguatoxins and brevetoxins. This method, however, has been reported to be at least 20 times less sensitive than the N2A method using current protocols. An alternative fluorescence-based receptor binding assay was recently reported for site 5 sodium channel toxins and, once validated, may provide a useful method for screening of CTXs in algal and fish extracts. Immunoassay methods, such as the sandwich enzyme-linked immunosorbent assay (ELISA), are in development by several laboratories worldwide and may also provide a sensitive and rapid alternative for CTX detection once validated. In all cases, these bioassay methods require additional chemical methods to confirm the presence of specific CTX congeners. The preferred method

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for detection, quantification, and confirmation of CTX has been reverse-phase liquid chromatography coupled to tandem mass spectrometry. While these methods are sensitive, reliable, and robust, efforts to standardize these approaches in regulatory settings have been hampered by the lack of commercially available standards and reference materials for the various CTX congeners.

Ecological Observations: *Gambierdiscus* cells are epiphytic, growing on macroalgae and seagrass, and also have been observed in sand (*G. belizeanus*) and detritus on dead coral. They are slow-growing (~0.3 div. d^{-1}) and will tether to host macrophytes via mucus threads if not directly attached or swimming nearby (Figure 1B). *Gambierdiscus* spp. are generally thought to be more abundant in calm, protected habitats (e.g., lagoons), and are sensitive to light, generally preferring intensities <300 µmol photons m^{-2} s^{-1}. *Gambierdiscus* cells co-occur with other benthic dinoflagellates, including *Ostreopsis* spp., *Prorocentrum* spp., *Amphidinium* spp., and *Coolia* spp., as well as diatoms, cyanobacteria, and other microalgae; and they are frequently found at low abundance compared with other members of the benthic assemblage. Multiple *Gambierdiscus* species co-occur and differ significantly in toxicity, thus determining the potential for ciguatera at a particular location.

Ciguatera outbreaks caused by *Gambierdiscus* spp. have long been associated with ecological disturbances to reef environments, including shipwrecks, reef dredging, coral bleaching, and hurricanes. It has been postulated that such disturbances create new habitats for attachment of host macroalgae, thus enabling the proliferation of *Gambierdiscus*. However, the functional relationship between *Gambierdiscus* and algal cover is yet unknown.

General Notes: *Gambierdiscus toxicus* was named after the place where it was first collected and identified in 1975, the Gambier Islands, French Polynesia. Subsequent studies identified and described additional species in the genus (currently 15) and several ribotypes.

The various species of *Gambierdiscus* differ physiologically, with some strains better adapted to cooler water and others to warmer temperatures. Similar distinctions are evident regarding salinity and light tolerances. Further work is needed, however, to determine which species are the most significant producers of ciguatoxins.

Gambierdiscus abundance is closely linked with seawater temperature, and ocean warming associated with climate change may result in shift in the geographic range of *Gambierdiscus* spp., as temperatures exceed thermal tolerance thresholds in some areas, while promoting growth in others.

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Gambierdiscus

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Gymnodinium catenatum

Figure 1 *Gymnodinium catenatum* live cyst. *Photo credit*: C. Band-Schmidt.

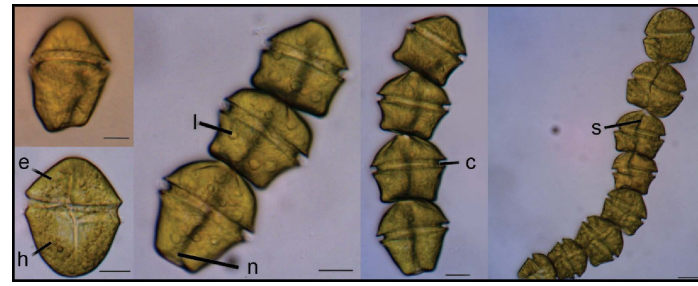

Figure 2 Light micrographs of individual cells and chains of *Gymnodinium catenatum*. Cingulum (c); sulcus (s); notch (n); epicone (e); hypocone (h); internal lipid globules (l). *Photo credit*: D.V. Ramírez-Rodríguez.

Figure 3 Global distribution of *Gymnodinium catenatum*. Red circles: vegetative cells reported; C: cysts reported.

Synonyms: None.

Morphology: Cells are unarmored and can be found as individual cells, but more typically occur in chains of 4 to 10 cells and occasionally up to 64 cells in nature (Graham, 1943; Balech, 1964). The chains can be easily broken by handling, and cultured isolates frequently form only smaller chains or exist as individual cells. Cells are usually wider than long, with length ranging from 22–65 µm and width from 30–46 µm (Morey-Gaines, 1982; Estrada *et al.*, 1984; Blackburn *et al.*, 1989). The cell body is circular to squarish, rounded anteriorly, and truncate posteriorly with a deep notch at the sulcus (longitudinal groove in ventral view). Single cells are generally elongate-ovoid with slight dorso-ventral compression. The epicone (top hemisphere) is smaller than the hypocone (bottom hemisphere). In general, cells forming chains are squarish-ovoid with anterior-posterior compression, whereas individual cells are usually longer (average: 59 ± 8 µm) and wider (38 ± 5 µm). Cells from eight-cell chains are shorter (28 ± 5 µm) and narrower (30 ± 3 µm). Individual cells are more bi-conical, and as the length of the chain increases, cells become more spherical (Graham, 1943; Blackburn *et al.*, 1989; Band-Schmidt *et al.*, 2008). In the middle of the cell, there is a clearly visible groove with sharply defined rims (the cingulum or "girdle") that surrounds the cell with a slight displacement to the left. There is a narrower groove (the sulcus) extending almost the full length of the cell (Graham, 1943; Balech,

Gymnodinium catenatum

1964; Morey-Gaines, 1982). The cell surface is covered with roughly hexagonal membrane sacs (amphiesmal vesicles), and the cell terminates with a horseshoe-shaped apical groove.

Cells are greenish-yellow-brown in color, with numerous chloroplasts, and internal lipid globules are common; the large single nucleus is centrally located (Graham, 1943; Blackburn *et al*., 1989). The swimming motion of the chains is snake-like, in relatively wide spirals.

Under microscopic observation of specimens fixed with formalin, paraformaldehyde, or Lugol's iodine solution, chains usually appear curved. Microscopic morphological analysis could confuse *G. catenatum* with similar species such as *G. nolleri* Ellegaard & Moestrup, *G. microreticulatum* Bolch, Negri & Hallegraeff, *G. impudicum* (Fraga & Bravo) Hansen and Moestrup; or *G. trapeziforme* Attaran-Fariman & Bolch. These species, however, can be discriminated by cell size, chain length, size and shape of the nucleus, as well as by toxigenicity. Among these species, *G. catenatum* has the largest cells, and is the only one that forms long chains and that produces saxitoxin analogues. *Gymnodinium impudicum* is smaller and only forms chains as long as four cells, whereas *G. nolleri* never forms chains longer than two cells (Ellegaard *et al*., 1993; Nehring, 1995). *Gymnodinium trapeziforme* can be differentiated by a large nucleus, ovoid to teardrop-shaped, positioned on the right side of the cell and is not a chain-forming species (Attaran-Fariman *et al*., 2007). At the molecular genomic level, there are also consistent differences among these species (Ellegaard and Oshima, 1998; Attaran-Fariman *et al*., 2007).

Known Distribution: After the original species description in 1939 from the Gulf of California, *G. catenatum* Graham (1943) was found near Mar del Plata, Argentina, in 1962 (Balech, 1964), and later in southern Japan in 1967 (Hada, 1967). To date, vegetative cells of this species have been reported from warm-temperate regions in the North Pacific, Southern Gulf of Mexico, South East Pacific, Atlantic, Mediterranean Sea, South Caribbean Sea, East Arabian Sea, China Sea, South East Indian Ocean, and the Tasman Sea, including adjacent Tasmanian coastal waters (Cuellar-Martínez and Mariona-Castillo, 2007; Hallegraeff *et al*., 2012; Licea *et al*., 2013; Gu *et al*., 2013, Poot-Delgado *et al*., 2015).

Cysts: Benthic resting (sexual) cysts (or hypnozygotes) are spherical and 38 to 60 µm in diameter. The cell wall is brown, with numerous reticulations over the entire surface (Anderson *et al*., 1988). These cysts are fossilizable, but are usually present in low numbers in the sediments. Live cysts of *G. catenatum* were found and germinated for the first time from the Ria de Vigo, Galicia, Spain (Bravo, 1986). In North America, live cysts have been reported for Bahía Concepción, Mexico (Morquecho and Lechuga-Devéze, 2003), the Atlantic coast of USA (Zonneveld *et al*., 2013), El Salvador (Cuéllar-Martínez and Mariona-Castillo, 2007), and in the Gulf of Nicoya, Costa Rica (Víquez and Hargraves, 1995). Cysts have also been reported in Venezuela (La Barbera-Sánchez *et al*., 1994), Argentina (Zonneveld *et al*., 2013), Uruguay (Zonneveld *et al*., 2013), the North Sea and the Mediterranean Sea (Zonneveld *et al*., 2013), Greece (Giannakourou *et al*., 2005), Spain and Portugal (Iberian Peninsula) (Amorin and Dale, 2006), southern Japan (Matsuoka and Fukuyo, 1994), Korean waters (Kim *et al*., 1995), China (Bolch and de Salas, 2007), the Arabian Sea off India (Godhe *et al*., 2000; Bolch and de Salas, 2007), New Zealand (Mackenzie and Beauchamp, 2002), Australia (Bolch and Reynolds, 2002) and Angola (Zonneveld *et al*., 2013). Highest cyst abundances of *G. catenatum* (up to 49% of total) occur in the Yellow Sea, China Sea and off NW Africa (Zonneveld *et al*., 2013).

The distribution of fossil cysts is widespread and not always in accord with the biogeography of extant *G. catenatum* blooms. In North America, fossil cysts have been reported in the Pacific from Saanich Inlet, Vancouver Island, Canada (8000–9000 BP, Mudie *et al*., 2002), and in Pescadero Basin in the southern Gulf of California (dated from ~1483) (Flores-Trujillo *et al*., 2009). Other regions from which fossil cysts have been reported include Omura Bay, Japan (from ~1700 AD, Matsuoka *et al*., 2006), and the Atlantic coastal slope near Lisbon, Portugal (dated 1898 AD, Amorim and Dale, 2006).

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Gymnodinium catenatum

Toxin: *Gymnodinium catenatum* is the only gymnodinoid dinoflagellate among the three genera of marine dinoflagellates (*Alexandrium*, *Pyrodinium*, and *Gymnodinium*) known to produce neurotoxic analogs of the tetrahydropurine alkaloid saxitoxin, also called paralytic shellfish poisoning (PSP) toxins. Whereas *Alexandrium* and *Pyrodinium* are quite closely related phylogenetically, *G. catenatum* is distant from both genera and tends to produce a distinct and characteristic toxin profile, now comprising at least three dozen naturally occurring analogs among various isolates and geographical populations. Although most strains produce the low-potency N-sulfocarbamoyl toxins as dominant toxins, these are readily converted to higher potency carbamoyl derivatives via metabolism in shellfish or thermal processing of seafood – hence posing an increased human health risk.

Earlier studies on PSP toxin profiles among *G. catenatum* blooms and cultured isolates and shellfish contaminated by this species were typically based upon analysis by liquid chromatography with fluorescence detection (LC-FD). This work was hampered by the lack of a complete suite of analytical standards for toxin identification, possible false-positive identification of "toxins" as peaks of coincident retention time, and inability to yield unequivocal confirmation of toxin structures. Nevertheless, such work served to demonstrate the stable and unique toxin compositional differences among geographical populations of *G. catenatum*.

First evidence for an unusual PSP toxin profile in both cultured cells and natural phytoplankton blooms was provided for inshore Tasmanian waters (Australia) (Oshima *et al*., 1987). In this geographical region, the dinoflagellate toxins were dominated by low-potency N-sulfocarbamoyl derivatives, particularly toxins C1 and C2, but also included unusual analogs C3 and C4. Mussels and oysters contaminated by the dinoflagellate showed a similar toxin profile, but with a higher proportion of C3 and more potent carbamoyl toxins (7–23 mole% total). Australian populations are known to express a wide spectrum of PSP toxins, including the N-sulfocarbamoyl analogs (C1–C4 and B1/B2), the gonyautoxins (GTX1–GTX4), decarbamoyl gonyautoxins (dcGTX2/3), decarbamoyl saxitoxin (dcSTX), and saxitoxin (STX), plus deoxydecarbamoyl toxins. Nevertheless, comparison of cultured isolates from Australia (Tasmania), Spain (Galicia) and Japan revealed that they could be discriminated on the basis of toxin profiles, including the presence of 13-deoxydecarbamoyl toxins found only in the Australian population (Oshima *et al*., 1993). This toxin profile is roughly consistent with the composition found by LC-FD in *G. catenatum* from northeast Spain (Galicia), and in contaminated shellfish (allowing for biotransformation). In Venezuela, in contrast, the dcSTX and dcGTX analogs were dominant in shellfish exposed to a *G. catenatum* bloom (La Barbera-Sánchez *et al*., 2004).

The advent and application of liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) to PSP toxin analysis have yielded both confirmatory identification of toxins and expanded the known toxin composition among *G. catenatum* populations and associated contaminated shellfish. This technique successfully confirmed a unique toxin profile in an isolate from Singapore (Holmes *et al*., 2002), which comprised primarily the highly potent carbamoyl gonyautoxins (GTX1/4) with lesser amounts of GTX2, GTX3, neosaxitoxin (NEO) and saxitoxin (STX). This is in contrast to *G. catenatum* populations from Australia, China, Japan, New Zealand, the Philippines, Portugal, Spain, and Uruguay, for which N-sulfocarbamoyl, decarbamoyl, and/or deoxydecarbamoyl toxins are most often characteristic.

The application of LC-MS/MS has served to confirm the presence of a new class of PSP toxins, the benzoyl derivatives, which to date are known only from the species *G. catenatum*. The expanded toxin profile of natural populations and cultured isolates of *G. catenatum* now comprises up to 18 STX analogs, highlighting the dominance of the less potent sulfocarbamoyl toxins (C1/2), but indicating the absence of STX, GTX 2/3, and NEO in Mexican strains (Bustillos-Guzmán *et al*., 2015). At least seven

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putative benzoyl toxin analogs are known from Mexican populations, but these toxins are also common in strains from Iberia (Vale, 2008). The high similarity (>80%) of the toxin composition of *G. catenatum* from the Mexican Pacific coast to toxin profiles reported from other regions around the world suggests low genetic variability of the toxin biosynthetic genes among global populations.

Bloom ecology and consequences: Bloom dynamics of *G. catenatum* have been studied mainly in three geographical regions: Tasmania, Iberia, and the Mexican Pacific coast. In Tasmania, the first bloom of *G. catenatum* was recorded in 1980 (Hallegraeff *et al*., 2012). Extensive cyst beds are found in southern Tasmania (Bolch and Hallegraeff, 1990), suggesting autochthonous blooms. Blooms have had a significant interannual variability, with high shellfish toxicity in 1986, 1991, 1993, 1999, 2002, and 2011 (Hallegraeff *et al*., 2012). The *G. catenatum* blooms in Tasmania tend to occur mainly from December to June, when water temperatures range from 12 to 18 °C and salinities range from 28–34. Blooms decline when temperature falls below 12 °C. Sources of inorganic nitrogen as well as strong rainfall trigger blooms of *G. catenatum* in this region (Hallegraeff *et al*., 2012).

In North Atlantic waters, *G. catenatum* was first observed in the rias of Galicia in the autumn of 1976 (Estrada *et al*., 1984), followed by another record in 1981. Since then, this dinoflagellate was commonly observed in this area until 1995, resulting in prolonged shellfish toxicity and shellfish quarantine closures. Blooms in this region have been correlated with upwelling relaxation (Fraga *et al*., 1988), advection of populations from outside the ria (Blanco, 1995; Gómez *et al*., 1995), and excystment of benthic cysts (Figueiras and Pazos, 1991; Blanco, 1995). Between 1996 and 2004, there were no blooms of *G. catenatum* along the North Atlantic coasts of Portugal and Spain, but blooms reappeared in this region in 2005, 2007, and 2008 (Costa *et al*., 2010; Vale, 2013). Álvarez-Salgado *et al*. (1993) reported a long-term decrease (over decades) in upwelling intensity off the Iberian Peninsula that corresponded with an ecosystem shift that appeared to favor dinoflagellates such *Dinophysis*, a stratification- and low-nutrient adapted organism, as opposed to *G. catenatum* with affinity for turbulence and high nutrient concentrations.

The *G. catenatum* populations from the Mexican Pacific tolerate a wide range of temperature, salinity, and N:P ratios (Band-Schmidt *et al*., 2004, 2014; Bustillos-Guzmán *et al*., 2012). Blooms are most frequent during March–April and have been associated with nutrient increase, mainly by nitrogen compounds, from upwelling events or transitional periods and temperatures from 18 to 25 °C (reviewed in Band-Schmidt *et al*., 2010). Sea surface temperature above 24 °C seems to limit bloom formation. This observation is supported by reports from the south-eastern part of the Gulf, which indicate that *G. catenatum* tends to disappear during warm El Niño conditions. Palynological records show that *G. catenatum* cysts have been present in the Gulf of California since ~1483 (Flores-Trujillo *et al*., 2009), with higher abundances from 1888 to 1920, and from 1945 to 1965, but showing a steady decrease in the latter part of the century (1965–1994). Cyst abundances seem to increase during La Niña conditions and decrease during warmer El Niño events. Abundances were also inversely related with sea surface temperature (SST), decreasing steadily from 1972 to 1994 as the SST increased in this area.

Blooms of *G. catenatum*, along the coast of Sinaloa, Mexico, have caused the death of nauplii and adult shrimp in shrimp farms (Cortés-Altamirano *et al*., 1997; Alonso-Rodríguez and Páez-Osuna, 2003), although the exact nature of the toxins responsible could not be specified. A major bloom of this species has been recorded in the north of the Gulf of California in 2015, causing substantial mortalities of birds, dolphins, and fish, as well as shellfish harvest closures due to high levels of PSP toxins (244–1015 µg STX eq. 100 g^{-1}) (Bustillos-Guzmán *et al*., 2016; García-Mendoza *et al*., 2016).

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Records of blooms on the Pacific coast of southern Mexico indicate that before 1987, *G. catenatum* was a common component of red tide events, but was succeeded by the tropical PSP–toxigenic dinoflagellate *Pyrodinium bahamense*. The latter species is now widely distributed in low cell numbers along the south coast of the Gulf of California (Martínez- López *et al*., 2007; Morquecho-Escamilla, 2008; Gárate-Lizárraga and González-Armas, 2011), but blooms of these species have also recently co-occurred, e.g., in Acapulco Bay and adjacent waters. This putative range extension suggests that changes in oceanographic conditions may have already led to regime shifts affecting the distribution and dynamics of populations of *G. catenatum* and *P. bahamense*, thereby posing a double threat and enhanced risk of PSP toxicity.

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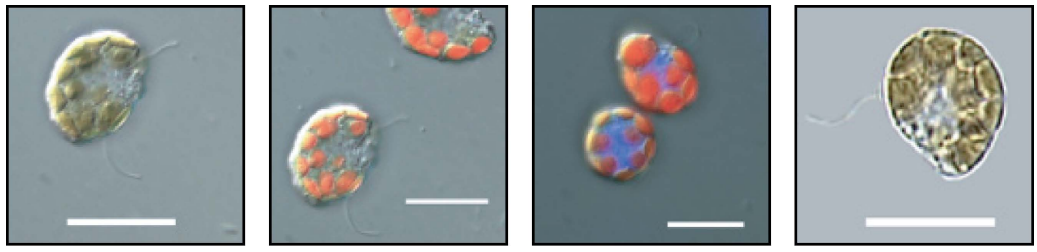
Heterosigma akashiwo



Synonyms:

- *Heterosigma akashiwo* Hara and Chihara 1985
- *Note*: Prior to 1985, this species was mistakenly reported as *Olisthodiscus luteus.*
- New species described as *Heterosigma minor* (Engesmo *et al*., 2016).

Morphology: Highly variable, pleomorphic (having no firm cell wall), appearing as spherical, ovoid, or long elliptical cells with two flagella emerging from a subapical groove. Cells from 15 to 30 µm. Chloroplasts small and numerous throughout the cell, numbering from 3 to over 40 with an average of 25. Nucleus center and surface appearing irregular with minute mucocyst projections. Color normally dark green to brown with major pigments consisting of chlorophyll-*a*, c1 and c2, fucoxanthin, and β-carotene.

Cells distort with commonly used fixatives. Samples normally need to be observed live for positive identification.

Known Distribution: Normally found in enriched, temperate coastal waters with blooms occurring when temperatures are between 15 and 22 °C.

Eastern U.S.: Massachusetts: Falmouth, Woods Hole area; Rhode Island: Narragansett Bay and coastal salt ponds; New York: Long Island Sound, Long Island South facing ocean bays; New Jersey: South Shore; Delaware: Inland Bays and Delaware Bay; Maryland: Eastern Shore, St. Martin's River, Bishopville Broad, Chesapeake Bay; Virginia: York River; North Carolina: Pamlico Sound, Neuss River, Gales Creek, New River, Cape Fear River; South Carolina: Bulls Bay, Kiawah Island; Georgia: Savannah Marshes, Altamaha River.

South: Florida: St. Johns River, Florida Bay, Charlotte Harbor, Tampa Bay, Bayboro Harbor, Anclote Key, Perdido Bay; Louisiana: Cocodrie; Mississippi: Mississippi River mouth region; Texas: Galveston, Offatts Bayou, Corpus Christi Bay, Laguna Madre.

West coast: California: Monterey Bay, Santa Barbara, Los Angeles; Washington: Seattle, Puget Sound Region, Hood Canal.

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World Distribution: Norway, UK, Ireland, France, Spain, Portugal, Italy, Croatia, Greece, Turkey, Japan, China, Syria, Thailand, Singapore, Saudi Arabia, South Korea, Namibia, Egypt, South Africa, New Zealand, Australia, Russia, Mexico, Bermuda, Brazil, Peru, Uruguay, Chile, Argentina.

Cysts: Aggregated masses as benthic stages (Tomas, 1978); detailed cyst described in Kim *et al*. (2015). Cysts are difficult to identify and require collection of sediment and separation methods from detrital material. Cyst germination can occur at temperatures above 15 °C and can remain viable for up to 3 months in the dark and cold (Tomas, 1978).

Toxin: Several toxins have been identified or implicated in the action of *Heterosigma* on fish, brine shrimp, and other test animals. Originally, *Heterosigma* (as *Olisthodiscus luteus*) was noted in producing phenolic-like compounds similar to brown macroalgae. Later, this species was identified as being directly involved in the production of reactive oxygen species (ROS; e.g., hydroxide, superoxide, and peroxide radicals). In addition, raphidophytes in general were identified as producing polyunsaturated fatty acids (PUFAs). Hemolytic activity using human red blood cells was detected in cultures of *Heterosigma*, possibly related to the presence of PUFAS or glycolipids. The potency of any toxin-like compound may not be an excessive combination. Other indications include interference with calcium regulation in cells and potentially neurotoxins, although these have not been defined chemically.

Methods for Toxin Identification: Hemolysis: erythrocyte lysis method (ELA); PUFAs, LC-MS; ROS: FAA spectrophotometric absorbance.

Biological Observations: Blooms of *Heterosigma akashiwo* are episodic, often appearing without notice, remaining for a period of time, and disappearing just as quickly. Cells do not preserve well, and monitoring must rely on live cell observations or the assistance of molecular probes designed specifically for the organism. Water discoloration in the form of "brown" water remains when cell concentrations are above 10^5 cells/L. Since this organism is phototactic and accumulates in the surface waters, or has very patchy distributions, estimates from these blooms may often be overestimates (e.g., the 300+ million cells/L recorded for some blooms). Relative to toxicity and fish mortality, confusing evidence occurs. Lacking a strongly dominating toxin, modes of mortality often take a scenario of synergistic agents as a combination of ROS, PUFAs, and hemolytic glycolipids. This is not to mention the effect of dense blooms in oxygen depletion. The fact that these blooms are termed *nuisance blooms* results from the uncertainty of a central toxic agent.

General Notes: Raphidophytes are generally ubiquitous in coastal temperate waters and are particularly problematic in areas of elevated eutrophication. Given that the cells of all raphidophytes are delicate and not subject to conserving with the standard fixatives, a live sample is advisable along with one fixed with Lugol's iodine. The live one is used for species identification. Once comfortable with the morphology of fixed cells, the fixed sample can be used for quantitative purposes.

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Author: Carmelo R. Tomas

Karenia brevis (Davis) Hansen et Moestrup – Florida Red Tide

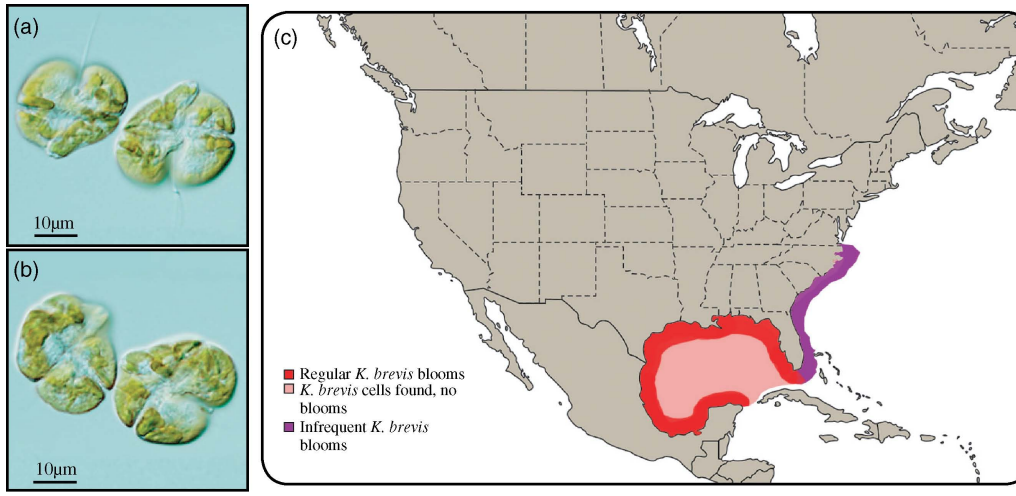

Figure 1 *Karenia brevis* cells (a,b). Known distribution of *Karenia brevis* (c).

Synonyms:

- *Gymnodinium breve* Davis 1948
- *Ptychodiscus brevis* (Davis) Steidinger 1979
- *Karenia brevis* (Davis) Hansen et Moestrup 2000

Morphology: *Karenia brevis* is an unarmored dinoflagellate with two flagella. The lack of a cell wall leads to it being highly pleomorphic and polymorphic. In general, it is shaped somewhat like a cloverleaf, flattened and concave. It is around 18–45 μm wide, 18–45 μm long, and 10–15 μm thick.

Known Distribution: *Karenia brevis* lives throughout the Gulf of Mexico in low abundance. Near the coastline, it can occasionally generate large blooms. The most frequent blooms occur along the west coast of Florida, but blooms can also occur along the coastline of Mexico and Texas, and elsewhere along the Gulf Coast. While most blooms occur on the continental shelves, blooms can occasionally occur in bays and estuaries as well, if the salinity is not below around 15.

Occasionally, blooms have occurred along the east coast of Florida and North Carolina. This is apparently the result of the Gulf Stream picking up blooms from the Gulf of Mexico and transporting them around the southern tip of Florida and up the east coast of North America. Blooms have occurred in Florida and North Carolina where the Gulf Stream comes closest to the coastline. While these blooms can last for a few months, they do not appear to be indigenous.

Cysts: *Karenia brevis* is known to produce cysts in culture and in nature, but the nature of these cysts is still not well known. At least some are the result of sexual reproduction. It is not known at this time whether the cysts are a short-term part of the life cycle of *Karenia brevis* or a long-term resting cyst that could germinate years later to initiate a bloom.

Toxin: While *Karenia brevis* produces a number of compounds that can be considered toxic, brevetoxin is the primary one of concern because of its effects on human health. "Brevetoxin" is actually a suite of around 12 congeners that are all lipid-soluble cyclic polyether polyketides with a molecular weight of around 900. The two parent compounds are PbTx1 and PbTx2, and the additional ten congeners are the result of small chemical changes to these two parent compounds.

They are tasteless, odorless, and heat stable, thus making them a difficult problem for human health. They cause the Na channels of nerve cells to remain open, leading to the depolarization of nerve cells. This can result in the disturbance of respiratory, cardiac, neuromuscular, and thermoregulatory control in humans. During times of large blooms of *Karenia brevis* along the west coast of Florida, it has been documented that there is approximately a 50% increase in hospital admittances for various respiratory and gastrointestinal disorders.

Brevetoxin can reach humans by two pathways: through the food chain and as an aerosol. Being lipid soluble and a large complex molecule, most

Karenia brevis

organisms have difficulty breaking it down and/or excreting it, so it tends to accumulate and biomagnify in the food chain. Filter-feeding molluscs that feed on blooms of *Karenia brevis* accumulate high concentrations of brevetoxin. The brevetoxin does not seem to affect the molluscs, but can be highly toxic to humans or other vertebrates that consume them. This is the basis for the term neurotoxic shellfish poisoning (NSP).

Because *Karenia brevis* is unarmored, it is rather delicate and easily broken apart by turbulence, particularly by waves at the surface and at the shoreline. This leads to the release of the brevetoxin as an aerosol, which leads to respiratory distress in humans and other air breathers.

During blooms of *Karenia brevis*, large numbers of fish, turtles, seabirds, manatees, and dolphins can be killed. In addition to these widespread deaths, one can also find sublethal concentrations of brevetoxin in the food chain. It is not known at the present time what the health effects of these sublethal concentrations in fish may be for humans who consume them. There is evidence, however, that some dolphins have died in areas of no obvious bloom, but rather from consuming fish with sublethal concentrations of brevetoxin in them.

Methods for Toxin Identification: One complicating factor for toxin analysis is that there are approximately 12 congeners, each with different levels of toxicity and chemical characteristics. The suite of congeners changes as the brevetoxins are metabolized and transferred through the food chain. A standard method that has been used for many years to assess brevetoxin toxicity in seafood is the mouse bioassay in which a mouse is injected with an extract from shellfish or other seafood. Because of the time involved, number of mice needed, and nonspecificity, numerous other methods have been developed, each with their own advantages and disadvantages.

Various types of high-performance liquid chromatography coupled with mass spectrometry (HPLC/MS) can be used to identify and quantify the individual congeners. Monoclonal antibody enzyme-linked immunosorbent assays (ELISAs) can be used to detect the suite of brevetoxins. Receptor site binding using neuroblastoma cells, synaptosomes, or hippocampal slices have been used to detect the presence of brevetoxins. In general, these techniques are more specific, but also more expensive and useful only in specialized laboratories. They tend to be used for research or confirmation once there is strong evidence of brevetoxin contamination, but not for general seafood monitoring.

Ecological Observations: Blooms of *Karenia brevis* are notoriously difficult to predict. Statistically, most blooms occur during late summer or the fall months, although they can occur at any time. The most frequent blooms occur along the west coast of Florida from about Tampa Bay to Sanibel Island. Less frequently, blooms occur along the coast of Mexico and Texas, and elsewhere along the Gulf Coast. At the present time, there is no accepted general hypothesis that can explain when and where these blooms will occur. Concentrations of *Karenia brevis* are higher inshore where nutrients from land runoff are higher. High inputs of nutrient-rich land runoff do not inevitably lead to blooms of *Karenia brevis*, but they appear to make them larger if they already exist.

General Notes: The general approach to avoid human health problems caused by *Karenia brevis* has been to monitor for blooms using water sampling and satellite imagery before there is any seafood contamination. The seafood industry and public can then be warned when blooms approach areas where human seafood is harvested. A variety of bioassays and chemical assays can then be used if seafood contamination is suspected. Similarly, the public can be warned if there is a likelihood of brevetoxin aerosol along beaches.

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Author: Larry E. Brand

Ostreopsis

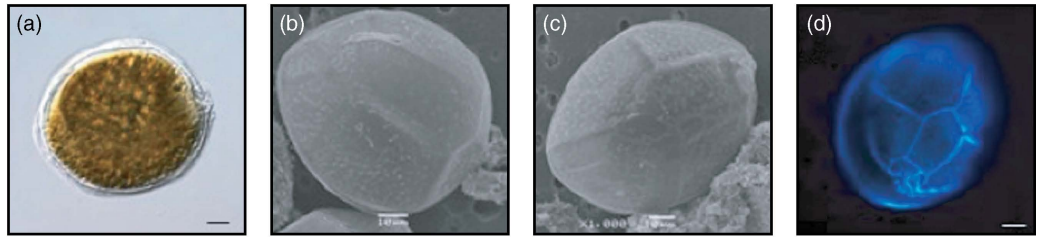

Figure 1 (a–d) Micrographs of *Ostreopsis* spp. isolated from the coastal waters of the island of Hawaii. (a) Light micrograph; (b) scanning electron microscopy (SEM) micrograph of epithecal view; (c) SEM micrograph of hypothecal view; (d) epifluorescent light micrograph of hypothecal view. All scale bars are 10 µm. *Source*: Photographs courtesy of Steve Morton.

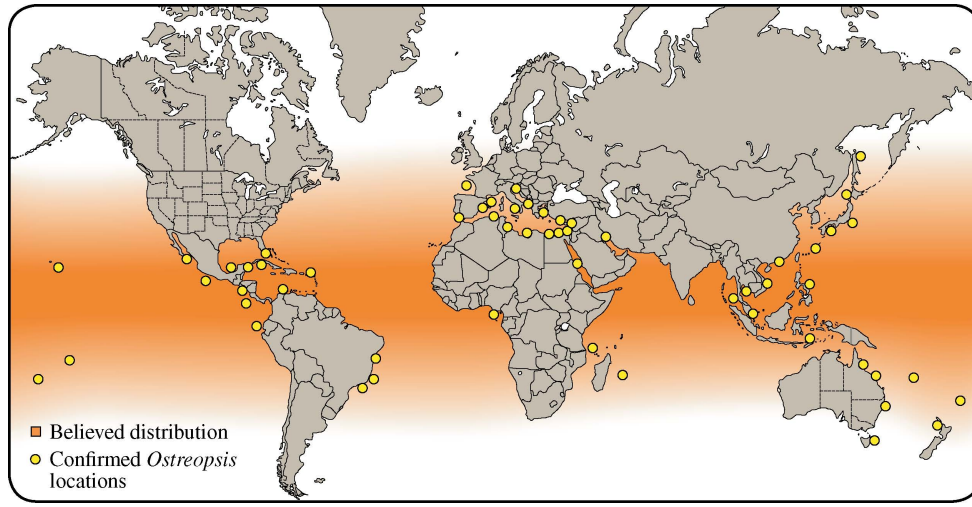

Figure 2 Known distribution of the *Ostreopsis* sp. dinoflagellates, with suspected circumtropical distribution in orange and known locations indicated by filled yellow circles.

Synonyms: None.

Morphology: There are eleven described species and an additional five "ribotypes" in this genus: *O. siamensis* (type), *O. belizeanus*, *O. caribbeanus*, *O. fattorussoi*, *O. heptagona*, *O. labens*, *O. lenticularis*, *O. marina*, *O. mascarenensis*, *O. ovata*, and *O. rhodesiae*. Ribotypes include, *O.* cf. *siamensis*, *O.* cf. *ovata*, *Ostreopsis* sp. Lanzarote type, *O. lenticularis* (?), and *O. labens* (?). Cells are anterioposteriorly compressed, teardrop-shaped (apical view), and tapering ventrally. Cells are 47–166 µm long (ventral to dorsal) and 26–86 µm wide (transdiameter). The modified Kofoidian plate formula is Po, 4′, 0a, 6″, 6c, 6s?, 5‴, 0p, 2″.

Known Distribution: Circumtropical. Known poleward extent: 35°S; 45°N (approx.).

Cysts: Unknown.

Toxin: Palytoxin analogs including putative palytoxin, ostreocins, ovatoxins, and mascarenotoxins.

Palytoxin is one of the most complex and toxic, non-proteinaceous natural products known, being only slightly less toxic than maitotoxin. Palytoxins are heat stable and water soluble, although due to the large size (2652 Da) and complexity of the structure, they contain both hydrophobic and hydrophilic functionalities. Palytoxin was first isolated from the soft coral *Palythoa toxica*, and a range of palytoxin analogs have since been isolated from other *Palythoa* species and several analogs are produced by benthic dinoflagellates of the genus *Ostreopsis*. The putative palytoxin is characterized by a long aliphatic backbone containing cyclic ethers, 64 chiral centers, more than 40 hydroxyl groups, and two amides.

Ostreopsis

Palytoxins' primary mode of action involves the disruption of the Na^+, K^+-ATPase pump.

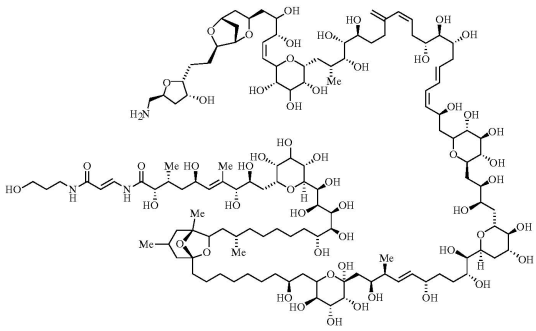

Figure 3 Structure of palytoxin.

Ostreopsis siamensis has been shown to produce ostreocin-D (the 3,26 bisdesmethyldeoxy analog of putative palytoxin), while *O. mascarenensis* produces mascarenotoxin A and B, which were determined to be palytoxin analogs based on structural fragmentation by mass spectrometry. *Ostreopsis* cf. *ovata* was found to produce a suite of palytoxin analogs known as ovatoxins A–F. In all cases, the toxin profile depends on the species and strain of *Ostreopsis* in the water column, environmental conditions, and geographical origin, leading to wide variations in toxin cell quotas and toxin profiles.

Palytoxin analogs have been shown to bioaccumulate in shellfish and fish, and therefore pose a human health risk via seafood consumption. The ovatoxin analogs have also been detected in marine aerosols during a bloom of *O. ovata*, demonstrating possible recreational and occupational health risk via inhalation.

Methods for Toxin Identification: Currently, there are no accepted regulatory methods for palytoxin analysis, but many instrumental and biological assays have been used. While chemical methods are needed to confirm the presence of palytoxin/analogs, biological tests often allow a quantitative assessment of composite toxicity. As is the case for many marine phycotoxins, the mouse bioassay (MBA) has been used to detect palytoxin analogs in phytoplankton and shellfish extracts, but due to ethical considerations for animal care and use, these are less desirable. Several cell-based assays have been utilized for the detection of palytoxins, including the erythrocyte hemolysis assay, the hemolysis neutralization assay (which has reported the lowest detection limits of all methods), the Neuro 2A cytotoxicity assay, and a cytotoxicity assay using the MCF7 breast cancer cell line. These assays allow many samples to be analyzed at one time, but due to the nature of these functional cell-based assays, they cannot provide any information on the toxin profile in a given sample, and interference from other toxins/compounds in the environment is possible. Immunoassays such as the enzyme-linked immunosorbent assay have been reported, but antibodies and standards are quite difficult to obtain, so to date no commercial, validated kit is currently available.

Many chemical methods have been used for the detection of palytoxin/analogs. High-performance liquid chromatography–fluorescence detection (HPLC-FLD) and liquid chromatography–tandem mass spectrometry (LC-MS/MS) methods have proved to be extremely useful. The latter is the only method available that can provide quantitative toxin profiling and is extremely useful for verification of analogs, but is not very sensitive due to the complexity of the molecule, which can have multiple charged states and other complications that can lead to ambiguities in identification in environmental samples. The use of pre-oxidation and/or high-resolution MS methods appears to provide the best compromise. These chemical methods do not provide an assessment of toxicity (including the presence of nontargeted toxins), so they are best paired with a suitable functional or cytotoxicity assay. Like many marine toxins, the development and implementation of these methods are currently hindered by the need for standards and reference materials.

Ecological Observations: *Ostreopsis* cells are predominately benthic, growing not only on macroalgae and seagrass, but on sand and other hard substrates as well. Significant densities ($>10^4$ cells L^{-1}) of *Ostreopsis* spp. have been found the water column, suggesting that this genus may be more planktonic than other benthic dinoflagellates, including *Gambierdiscus*. Much is still unknown regarding *Ostreopsis* ecology and physiology. Field and laboratory studies examining the influence of environmental factors on growth have yielded contradictory findings, which likely reflect species and geographic differences. More research is needed to better

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Ostreopsis

characterize the factors influencing *Ostreopsis* spp. abundance and distribution, particularly the influence of water motion and temperature, and to examine species-specific growth responses to temperature, salinity, and nutrient concentrations.

General Notes: The type species *O. siamensis* was first identified from samples collected in the Gulf of Thailand (also known as the Gulf of Siam) in 1901, followed by an expanded description of this species, along with *O. lenticularis* and *O. ovata*, by Fukuyo (1981). The taxonomy of this genus is currently under review, due in part to apparent ambiguities in defining the morphological characters used for species classification, as well as the availability of genetic data.

Impacts of *Ostreopsis* spp. (primarily *O.* cf. *ovata*) are particularly well known in the Mediterranean Sea, where intense and frequent blooms have occurred along the coasts of Italy, Spain, Greece, Algeria, Egypt, Tunisia, Lebanon, Turkey, and Morocco. In the Tyrrhenian Sea, blooms were suspected of causing respiratory illness and skin irritation in tourists and workers at beaches. Additionally, *Ostreopsis* spp. were associated with negative health impacts (including mortalities) to marine invertebrates and fishes at several locations around the world, including New Zealand, Brazil, and the northern Adriatic Sea.

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Ostreopsis

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Pfiesteria piscicida Steidinger & Burkholder and *Pfiesteria shumwayae* Glasgow & Burkholder

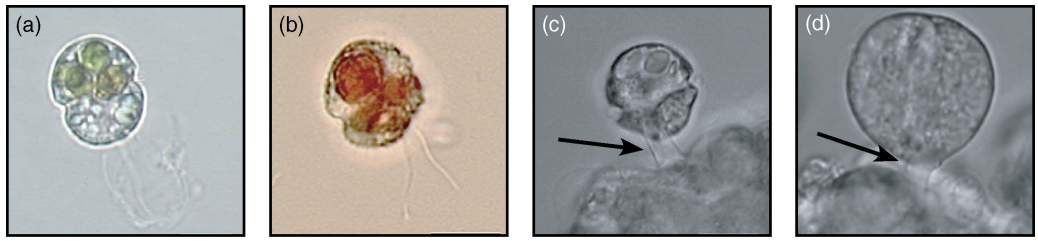

Figure 1 (a–c) Examples of flagellate stages of *Pfiesteria* spp., which are the stages most commonly found in the water column: (a) Excysted zoospore of *Pfiesteria piscicida* (greenish contents in food vacuoles are from consumed *Dunaliella* prey), which remained momentarily attached by its longitudinal flagella to the discarded hyaline temporary cyst wall (from Parrow and Burkholder, 2002, fig. 2d, reproduced with permission of Elsevier); (b) planozygote of *P. piscicida* after feeding on cryptomonads (reddish contents in food vacuoles; from Parrow and Burkholder, 2004, fig. 2d, reproduced with permission of Wiley); (c,d) sequential micrographs showing observations on an individual flagellate cell of *Pfiesteria shumwayae* (c) at time zero feeding on sterile fish cells (*Oncorhynchus tshawytsha* cell line), and (d) 120 seconds after attachment to the fish cells, engorged from ingestion; flagella are not evident. Note, however, that the feeding tube (arrows) was detached from the fish cells and the cell swam away less than 10 seconds after (d) was photographed. Scale bar = 10 μm for all micrographs. *Source*: From Parrow *et al.* (2005, fig. 2). Reproduced with permission of Inter-Research.

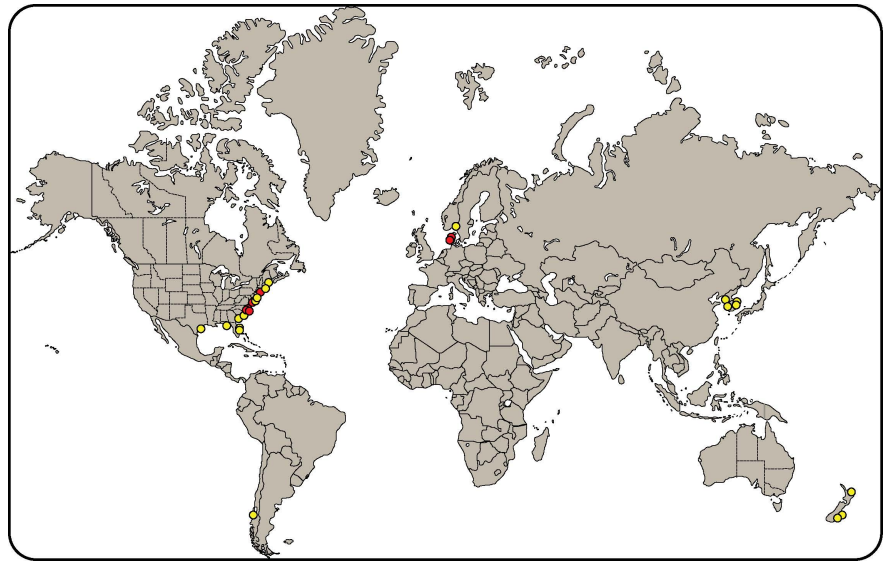

Figure 2 Known distribution of the dinoflagellates *Pfiesteria piscicida* and *Pfiesteria shumwayae* in estuaries along the U.S. mainland. Compiled from Burkholder *et al.* (2001), Rublee *et al.* (2001, 2005), Jeong *et al.* (2006), Park *et al.* (2007), references cited in Burkholder and Marshall (2012) and Moestrup *et al.* (2014). Note that *Pfiesteria piscicida* has also been documented from a saline lake in Antarctica (Park *et al.*, 2007).

Synonyms:

- *Pfiesteria* species B (Kempton 1999), for *Pfiesteria shumwayae*
- *Pseudopfiesteria shumwayae* Litaker, Steidinger, Mason, Shields et Tester, 2005

The genus *Pfiesteria* includes two species, *Pfiesteria piscicida* and *Pfiesteria shumwayae*. The latter species was transferred to a new genus, *Pseudopfiesteria*, but was reclassified back into the redefined genus *Pfiesteria* by Marshall *et al.* (2006). These dinoflagellates (phylum Dinophyta) are within the order Peridiniales (Marshall *et al.*, 2006). Flagellate stages are heterotrophs and, thus, lack chloroplasts, but can appear pigmented from consumption of algal prey (Figure 1A and 1B). They can also temporarily retain kleptochloroplasts from cryptophyte algal prey (Lewitus *et al.*, 1999). The information below is from Burkholder and Marshall (2012 and references therein), unless otherwise noted.

Pfiesteria piscicida & *Pfiesteria shumwayae*

Morphology: The predominant asexual flagellate stage, zoospores (Figure 1A and 1C), have a thin outer covering (theca). The zoospores are referred to as "armored" due to the presence of thin cellulosic structures called thecal plates. The Kofoidian thecal plate formula for the genus is Po, cp, X, 4′, la, 5–6″, 6c, p.c., ?s, 5‴, 0p, 2″ (Marshall *et al*., 2006). There is a difference of only one plate between the two species: There are five precingular plates in *P. piscicida*, versus six precingulars in *P. shumwayae*; thus, the anterior intercalary plate is triangular in *P. piscicida*, versus somewhat diamond-shaped (four-sided) in *P. shumwayae*. Flagellate cells range in size from 9 to 17 µm (*P. piscicida*) or from 9 to 25 µm (*P. shumwayae*) (Marshall *et al*., 2006).

Some clonal cultures of *Pfiesteria* spp. also have been observed to have single flagellate cells transforming into amoebae of varying sizes, and amoeboid cells that produced flagellate cells. Amoeboid morphs mostly have been found within the first several months after toxic populations were isolated from field material at in-progress fish kills.

Known Distribution: One or both *Pfiesteria* species have been documented from Maine to Texas in brackish waters (Figure 2). The genus is considered to be cosmopolitan with the exception of the Arctic.

Cysts: The genus *Pfiesteria* has been described to have a complex life cycle with multiple flagellate and amoeboid stages, based on toxic cultures recently isolated from fish kills. Marshall *et al*. (2006) isolated a vahlkampfia-like amoeba (Gymnamoebae) into clonal culture from estuarine sediments, and fed it cryptomonad prey for three months. Other stages/cells were not observed in the culture. Purified DNA from the clonal amoebae (208 bp consensus sequence) had 100% identity with the corresponding region of sequence data for all *P. piscicida* GenBank submissions that had been obtained from flagellate stages, providing field evidence for *Pfiesteria* amoebae. Temporary cysts, zygotic cysts, reproductive cysts, and other cysts varying in morphology also have been documented from *Pfiesteria* spp.

As the dominant stage in the life history is expected to vary depending on culture conditions, prey type and availability, and strain, more simplistic life histories lacking amoeboid stages have commonly been reported in nontoxic *Pfiesteria* cultures. Additional work is needed to document the life history of *Pfiesteria* spp. using freshly isolated toxic strains.

Toxin: Toxins from *Pfiesteria* (PfTxs) have been documented from axenic *Pfiesteria piscicida* cultures and monoxenic *Pfiesteria shumwayae* cultures (grown on a sterile fish cell line; Burkholder *et al*., 2005 and references therein). Much less PfTxs were found in those cultures than in xenic cultures containing prey and bacteria. PfTxs from *P. piscicida* fed algal prey, and including bacteria, were characterized by Moeller *et al*. (2007). Similar water-soluble PfTxs were found in all of these culture conditions (Burkholder *et al*., 2005).

PfTxs identified thus far include radical-forming, toxic organic-ligated copper complexes (Moeller *et al*., 2007). The toxicity of multiple toxin congeners is due to carbon–sulfur–metal-based radical production. Other metals could substitute for copper, and the ligands would also be expected to vary depending on the organics present. Moeller *et al*. (2007) hypothesized that the rapid, free radical–mediated toxicity of PfTxs occurs by production of a redox-cycling metal center and free radicals that can lead to specific reactions with "pro-toxins," and that these, in turn, can produce more active toxic species. Metal-mediated free radical production explains *Pfiesteria* toxicity as well as previously reported difficulty in observing the molecular target, due to the ephemeral nature of radical species. The toxins are highly labile in purified form, maintaining activity for only 2–5 days before all activity is lost.

The multiple toxin congeners in active extracts are also susceptible to decomposition in the presence of white light, pH variations, and prolonged heat. Other PfTxs remain to be characterized, such as aerosolized toxins based on health impacts sustained by laboratory workers. PfTxs, produced by environmentally relevant densities of flagellate cells found at estuarine fish kills, have adversely affected mammalian cells (GH4C1 rat pituitary cells) as well as fish (Burkholder *et al*., 2005 and references therein).

Methods for Toxin Identification: As for various other harmful algae, obstacles preventing identification and quantification of *Pfiesteria* spp. toxins have included extraction difficulties, instability of the toxins as described, the need for very large volumes

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Pfiesteria piscicida & *Pfiesteria shumwayae*

of dense culture that dependably retains toxicity (considering that *Pfiesteria* spp. have both toxic and nontoxic strains, and can rapidly lose toxicity in culture), and the lack of available pure standards.

Moeller *et al.*'s (2007) work was based on corroborating data from five different methods, including nuclear magnetic resonance spectroscopy, inductively coupled plasma mass spectrometry, liquid chromatography particle beam glow discharge mass spectrometry, electron paramagnetic resonance spectroscopy, and X-ray absorption spectroscopy.

Ecological Observations: *Pfiesteria* spp. are heterotrophic prey generalists that typically feed by phagotrophy. Nutrient (N, P) enrichment can affect *Pfiesteria* spp. directly, but the linkage most commonly is indirect, mediated through increased abundance of prey.

Toxicity is highly variable among strains, ranging from apparently nontoxic (non-inducible, or NON- IND) to actively toxic (TOX-A) in the presence of live fish. Toxicity and its detection in the two *Pfiesteria* spp. thus depend on the strain and the use of reliable assays, and there have been many misrepresentations and misinterpretations of toxic *Pfiesteria* research. Toxic strains show strong chemosensory attraction to live fish or their fresh tissues. They can kill fish by toxins alone, and by toxins together with physical attack from feeding upon epidermis and other tissues. Non-inducible strains do not produce sufficient toxin to kill fish, but some are capable of causing larval fish death by physical attack. The TOX-A functional type is mainly a fish predator with little attraction for other prey. Toxic strains have been isolated from in-progress kills. In contrast, most NON-IND populations have shown strong attraction to algal prey and high feeding activity on those prey, but little attraction to fish. Toxic flagellate cells that have been separated from live fish for a short period (days to weeks – TOX-B functional type) have shown an intermediate response.

General Notes: Both *Pfiesteria* spp. have been linked to major fish kills (10^3 to 10^9 fish) in quiet, eutrophic estuaries, especially the two main tributaries of the Albemarle-Pamlico Estuarine System, and in aquaculture facilities – regarding the latter, most recently in Scandinavia (Moestrup *et al.*, 2014). Various fish and shellfish species have been affected. The abundance of flagellate cells during *Pfiesteria*-related kills typically has ranged from 300 to 1200 mL^{-1}, rarely at 10^4 mL^{-1}. Between kills, cell abundance usually is low in the water column (fewer than 10 cells mL^{-1}), and the available evidence suggests that the populations are mostly in the lower water column and within surficial sediments.

Fish kills linked to toxic *Pfiesteria* spp. mostly have involved juvenile Atlantic menhaden (*Brevoortia tyrranus*) with diffuse surficial lesions as well as deep focal (ulcerated) lesions. Sublethal exposure to *Pfiesteria* weakens fish and likely renders them more susceptible to attack by other microbial pathogens. Test fish have developed similar lesions when exposed to filtrate (0.22 µm pore size) from TOX-A *Pfiesteria shumwayae* that was causing fish death in bioassays (Burkholder *et al.*, 2005). Affected fish have shown neurological signs within minutes to hours after exposure to TOX-A *Pfiesteria* spp., including depression, loss of equilibrium, episodic hyperexcitability, and decreased respiration.

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Pfiesteria piscicida & Pfiesteria shumwayae

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Prorocentrum

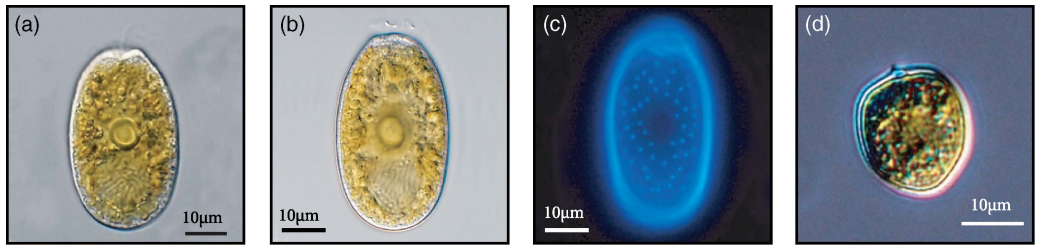

Figure 1 (a–d) Examples of potentially toxic *Prorocentrum* spp., including the benthic species *Prorocentrum lima* (a, b, c); and (d) the planktonic species *Prorocentrum minimum*. *Photo credits*: *P. lima* – The World Register of Marine Species at http://www.marinespecies.org/photogallery.php?album=1033&pic=33854); and *P. minimum* – Gert Hansen, Phyto'pedia.

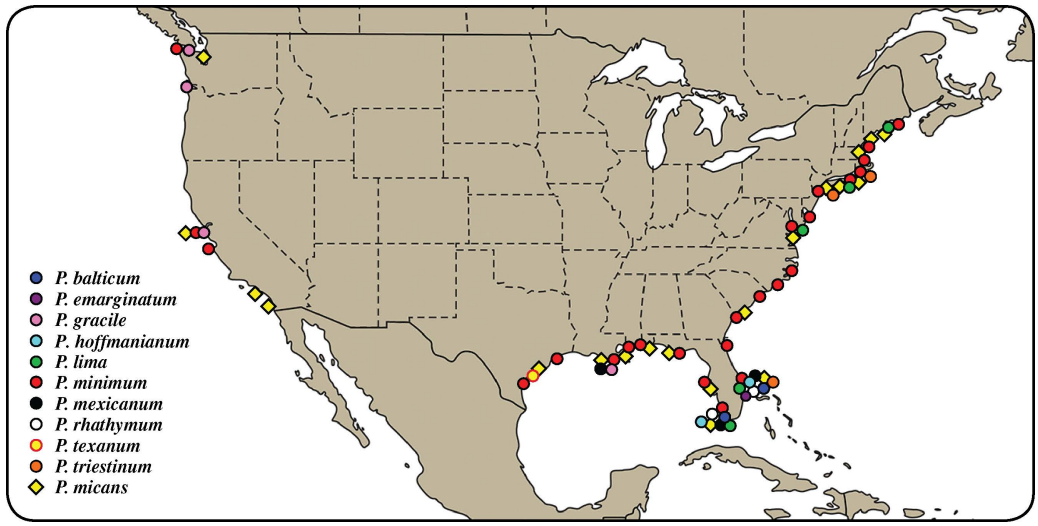

Figure 2 Map showing general locations of potentially harmful *Prorocentrum* spp. (filled circles) in and near the U.S. Note that *P. micans* (filled diamonds) is not toxigenic, but can form high-biomass blooms (see Appendix at end of this fact sheet for references).

Synonyms: Various harmful species within the dinoflagellate genus *Prorocentrum* (Ehrenberg, 1834) have synonyms, some of which are included within the genus *Exuviaella*. The taxonomy of some harmful *Prorocentrum* species is uncertain. In addition, the toxic *P. lima* species complex is believed to contain other cryptic species that will be detected as molecular tools become more advanced (Glibert *et al.*, 2012 and references therein).

Morphology: Species within this genus have small to medium-sized vegetative cells (predominant life history stage; maximum dimension, 15–100 µm) that are bilateral and thecate ("armored" with cellulose plates), with two apically inserted flagella (Elbrächter, n.d.). The cell covering (amphiesma) includes two large pieces (cellulose valves) that may be smooth, or may have pores or spines. The apical flagellar region contains 7–14 small cellulose plates, depending on the species.

Known Distribution: Bloom-forming and toxic *Prorocentrum* spp. distributions are shown in Figure 2. Of ten species (see Glibert *et al.*, 2012; Henrichs *et al.*, 2013), nine have been reported as toxigenic, although toxicity has not been confirmed in *P. balticum*, *P. gracile* (synonym: *P. sigmoides*), or *P. triestinum*, and toxicity is questionable in *P. mexicanum*. Species *P. micans* is not toxigenic, but can form high-biomass blooms.

Figure 2 provides an indication of general occurrence. For example, based on the various reports considered, *P. minimum* and/or other *Prorocentrum* spp. likely occur in virtually all estuaries along the U.S. Atlantic and Gulf Coasts. Note that the cluster of species along eastern and southwestern Florida

Prorocentrum

have been reported to occur in the Indian River Lagoon and Florida Bay, respectively.

Cysts: Asexual reproduction in *Prorocentrum* occurs by binary fission (Elbrächter, n.d.). Sexual reproduction involves haploid isogametes that form a fertilization tube through which the nucleus of the male gamete is injected into the plasma of the female gamete (Elbrächter, n.d.). The diploid planozygote (swimming zygote) replicates its DNA, becoming tetraploid, followed by two successive meiotic divisions resulting in tetrads of haploid vegetative cells. Benthic species can form cysts. For example, *P. lima* produces a pale-colored, round, resting cyst (diameter: 70–75 µm) with a smooth, triple-layered wall (Faust, 1993).

Toxin: The following information is taken from Glibert *et al.* (2012 and references therein) unless otherwise indicated. Of ~17 toxigenic *Prorocentrum* species worldwide, at least ten have been reported from estuarine and coastal marine waters in and near the U.S. (Figure 1). Their toxicity is highly strain (population)-dependent. Among the predominantly planktonic species, several *P. minimum* strains have produced an uncharacterized, water-soluble neurotoxin that has rapidly (i.e., in minutes) caused mouse death at high doses. Toxin production has been stimulated by bacteria, but an axenic clone also tested positive for toxin(s). Laboratory experiments testing responses of grazing molluscs to *P. minimum* cultures have yielded results ranging from tissue pathologies, systematic immune responses, and mortality (scallops, oysters) to normal growth (oysters; reviewed by Wikfors, 2005). Shellfish have varied in response to *P. minimum* blooms, from no apparent harm to adverse impacts on survival and settling (oysters; Wikfors, 2005 and references therein). Immune responses and tissue pathologies of shellfish (eastern oysters, bay scallops) exposed to some *P. minimum* cultures have also been shown. Variable observations may reflect transient toxin expression in *P. minimum* (Wikfors, 2005), or perhaps relatively few strains are toxigenic (Heil *et al.*, 2005). There are no definitive data indicating *P. minimum* toxicity to humans.

Variable toxicity is more commonly associated with certain benthic *Prorocentrum* species. Some strains produce diarrhetic shellfish poisoning (DSP) toxins, including water-soluble "fast-acting toxins" (FATs); the lipid-soluble polyether compounds okadaic acid (OA, which can accumulate in fish tissues), methyl-okadaic acid, and/or dinophysistoxin (DTX1); other OA derivatives; and/or prorocentrolides (Glibert *et al.*, 2012 – Table 1). Highly potent toxins such as OA and its derivatives are protein phosphatase inhibitors, tumor promoters, and possibly tumor inducers in mammals (Dominguez *et al.*, 2010 and references therein). The benthic habitat of these *Prorocentrum* species may make their toxins relatively unavailable as food resources for filter-feeding shellfish (which could become contaminated seafood for humans) except when they occasionally are suspended in the water column, thus perhaps minimizing their role in causing DSP in humans.

Some toxins from benthic *Prorocentrum* species, such as OA and derivatives, are also thought to be involved in ciguatera finfish poisoning, although biomagnification to the level required to affect human health has not yet been documented. Pearce *et al.* (2005) reported negative effects on digestion of oyster spat when exposed to *P. rhathymum*. Ajuzie (2008) exposed juvenile European sea bass (*Dicentrarchus labrax*) to cell-free culture medium that had contained a toxic strain of *P. lima*, and to live cultures of the strain, and found that fish exhibited stress-related behaviors such as hyperactivities, poor feeding reflexes, and, after about three weeks, cessation of feeding. Fish that directly ingested *P. lima* cells or *Artemia* (brine shrimp) that had been feeding on *P. lima* died, and histopathology analysis showed that their gill and liver tissues were damaged. Secreted mucus "overwhelmingly covered" the respiratory epithelium of gill lamellae, causing the aorta blood to become hypoxic. Ajuzie (2008) suggested that the *P. lima* complex may cause fish kills in natural habitats but are not detected because of their cryptic nature; and that chronic exposure to toxic *P. lima* may cause wild fish to cease feeding and die.

Methods for Toxin Identification: Purified chemical standards, (toxin standards) are available commercially for some *Prorocentrum* toxins. Toxin analysis generally is carried out using liquid chromatography–tandem mass spectrometry (LC-MS/MS) with multiple reaction monitoring or selected ion monitoring (Suzuki and Quilliam, 2011 and references

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Prorocentrum

therein). This method is highly sensitive and accurate, but its use is limited to relatively few research laboratories due to the high instrument costs. Detection methods have also included mouse bioassays and various biochemical methods such as an enzyme assay using protein phosphatase. A protein phosphatase inhibition assay (PPIA) kit is available for the OA toxins group (OA, DTX1, DTX2) in molluscan shellfish. Enzyme-linked immunosorbent assays (ELISAs) are also available. As a general characteristic, these PPIA and ELISA assays are relatively rapid and inexpensive but limited; they generally do not detect the suite of toxins and toxin derivatives with equal sensitivity.

Ecological Observations: The following information is taken from Glibert *et al.* (2012 and references therein) unless otherwise indicated. *Prorocentrum* species are phototrophs, and those that have been tested (the plankters) are also mixotrophic, grazing on cryptomonads and many other microbes. High-biomass blooms of planktonic *Prorocentrum* species, especially *P. minimum* in U.S. waters, are among the most commonly recognized harmful algae that are increasing in frequency, duration, and magnitude globally in response to increasing nutrient loads.

Blooms of these taxa can be sustained for long periods under excessive nitrogen (N) conditions and, therefore, at proportions of N:P that are well in excess of Redfield proportions (molar, 16:1; by weight, 7:1). The large *P. minimum* blooms that develop in eutrophic environments can also indirectly affect food webs through food resource imbalances, low-oxygen stress from bloom respiration and decomposition, and habitat loss.

Much less is known about the ecology of harmful benthic *Prorocentrum* species. Best studied is the *P. lima* species complex; their toxin production has been shown to increase when nutrient ratios are above Redfield proportions. A toxic *P. lima* strain had viable cells after passage through the gut tract of bay scallops, suggesting that viable toxic populations could be spread through shellfish transport in the aquaculture industry. Allelopathic effects of toxic *Prorocentrum* species toward other algae have been shown experimentally. Toxic strains of the *P. lima* complex can negatively affect the behavior of juvenile fish and cause their mortality (Ajuzie, 2008).

General Notes: Harmful *Prorocentrum* species occur in brackish and marine coastal waters worldwide, especially under poorly flushed, nutrient-enriched conditions. Given the projection for land-based nutrient pollution to continue to increase, increased bloom magnitude and frequency of planktonic *Prorocentrum* species are expected, as well as more intensive toxic benthic occurrences.

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Prorocentrum

Appendix

Information considered in making the map of harmful *Prorocentrum* spp. distributions as shown in the *Prorocentrum* summary (Figure 2).

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Prymnesium parvum (Carter) – "Golden Algae"

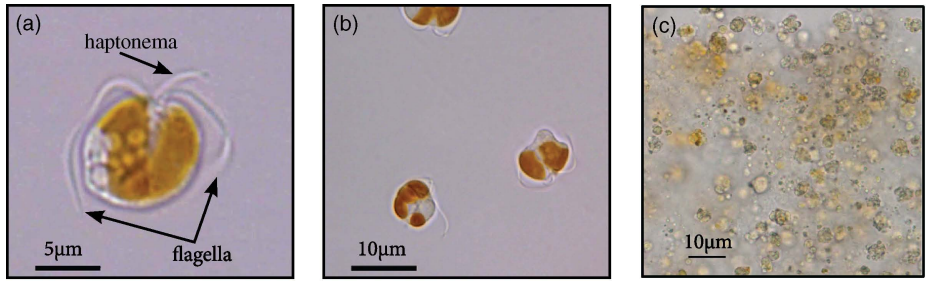

Figure 1 *Prymnesium parvum* are irregularly shaped unicellular algae; cells have 2 smooth flagella, equal in length, and a haptonema (a). Cell dimensions range in length from 8 to 16 µm and in width from 4 to 10 µm (b). In cultures, when in senescence, golden sediments accumulate that appear to be resting stages, here shown among a bacterial matrix (c). *Photo credits*: Schonna R. Manning.

Figure 2 Location of documented lethal *Prymnesium parvum* blooms.

Synonyms:

- *Prymnesium parvum* N. Carter, 1937
- *Prymnesium parvum f. patelliferum* (J.C. Green, D.J. Hibberd & R.N. Pienaar) A. Larsen, 1999

Morphology: *Prymnesium parvum* is a unicellular microalga with cells that are oblong and irregular in shape (length 8–16 µm and width 4–10 µm; Figure 1) (Green *et al.*, 1982). Two smooth flagella are inserted subapically from a groove and beat with heterodynamic movements. A third structure, the haptonema, emerges between the flagella and can be used for attachment to substrata (Green and Jordan, 1994). The haptonema is somewhat stiff in *P. parvum* and is typically shorter than or equal in length to the flagella when compared to other haptophytes. Cells of *P. parvum* have two golden-brown chloroplasts and abundant fucoxanthin. The cells do not have an outer wall, but they are covered with two layers of plate-like, ornate cellulosic scales. Scale morphologies can be discerned with scanning electron microscopy. They are considered species-specific and are the primary feature considered in species identification.

Known Distribution: This euryhaline, eurythermal organism tolerates salinities ranging from 1 to more than 35, and temperatures ranging from 2 to 32 °C (Watson, 2001 and references therein). It is cosmopolitan with the exception of Antarctica, and blooms in many inland and coastal waters (Figure 2; Granéli *et al.*,

Prymnesium parvum

2012; Roelke *et al.*, 2016 and references therein). It is most often associated with estuarine or marine habitats, but it is also common in some inland water bodies. In at least one instance, mineral extraction activities resulted in *P. parvum* blooms in a mountain stream habitat (Brooks *et al.*, 2011). Interestingly, blooms have occurred in open coastal marine ecosystems in some regions, but thus far not in large U.S. estuaries, bays, and coastal waters (Roelke *et al.*, 2016).

Cysts: The life history of *P. parvum* is believed to be haplodiplontic, including four morphologically distinct stages: two are flagellated haploid cells, one stage is a flagellated diploid cell, and one is a nonmotile form considered to be a resting stage or cyst (Edvardsen and Medlin, 2007). Asexual reproduction predominates. Confusion about the life history is reflected in the taxonomy: what has been called *P. parvum f. patelliferum* is haploid, while *P. parvum* is diploid. The life history requires further resolution.

Toxin: The many toxic substances of *P. parvum* have been reported to include lipopolysaccharides (hemolysins), a galactoglycerolipid, polene polyethers, cyclo-amines ("fast-acting ichthyotoxins"), reactive oxygen species, dimethylsulfonio-propionate, polyunsaturated fatty acids, and fatty acid amides with fish-killing, cytotoxic, hemolytic, hepatotoxic, neurotoxic, and/or antimicrobial activities (Burkholder, 2009; Bertin *et al.*, 2012a, 2012b and references therein). As examples, the complex polyketides prymnesin-1 and -2 have been isolated from *P. parvum* (Igarashi *et al.*, 1996), and they have been reported to have ichthyotoxic, neurotoxic, hemolytic, and cytotoxic properties (Manning and La Claire, 2010). However, some studies are contradictory regarding the effects and functions of polyketide prymnesins, at least partly because various *P. parvum* toxins form micelles and require activation by cofactors such as monovalent and divalent cations, antibiotics, or polyamines. Thus, after the toxins are released into the environment, multiple abiotic factors can increase their toxicity (Bertin *et al.*, 2012a, 2012b). At least some of the fish-killing substances from *P. parvum* are light-sensitive, and are rendered ineffective at pH 7 or lower.

Blooms of *P. parvum* have caused death of gill-breathing organisms such as fish, mussels, and larval amphibians. A common mode of action of *P. parvum* toxins is to destroy the selective permeability of cell membranes and disrupt ion regulation in gills. Affected fish typically bleed from the gills and may develop a heavy mucus layer. They often swim slowly, lie on the bottom, gather near shore or near a fresh source of water, or actively leap onto shore. Some bioactive substances produced by *P. parvum* have also suppressed growth or lysed other algae, and/or have killed zooplankton or suppressed their feeding and reproduction (Granéli *et al.*, 2012; Roelke *et al.*, 2016).

A suite of fatty acid amides from *P. parvum* cultures were toxic to two mammalian cell lines (Neuro 2A and GH4C1 rat pituitary cells) (Bertin *et al.*, 2012a, 2012b), but there is no further information on the potential toxicity of these substances to mammals.

Methods for Toxin Identification: As for various other harmful algae, obstacles preventing identification and quantification of *P. parvum* toxins have included extraction difficulties, toxin(s) instability in light, the need for very large volumes of dense culture that dependably retains toxicity (as *P. parvum* has both toxic and nontoxic strains, and can change in culture over time), the lack of available pure standards, and the lack of available molecular probes for the toxins. Mass spectrometry is essential for verification of prymnesin-1 and -2 presence and purity. Streamlined methods were recently developed for co-isolation of prymnesin-1 and -2 from modest amounts of cultured cells and from culture supernatants for the identification of the metabolic fingerprint for these compounds by liquid chromatography and mass spectrometry (Manning and La Claire, 2013). Chemifluorescent methods are now available for semi-quantitative detection of prymnesin-1 and -2 using spectrophotometry (La Claire *et al.*, 2015). Various other toxic substances reported from *P. parvum* have been analyzed using combinations of high-performance liquid chromatography, electrospray ionization mass spectrometry, and nuclear magnetic resonance.

Ecological Observations: *P. parvum* is photosynthetic, but it can also be a strong mixotroph, consuming dissolved organic substances or ingesting bacteria, various protists, fish materials, and other prey.

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Prymnesium parvum

Thus, *P. parvum* is able to "kill and eat" many of its competitors and predators, which in turn enables near-monospecific blooms. Mixotrophy increases under inorganic nutrient limitation (Granéli *et al*., 2012; Roelke *et al*., 2016).

Most blooms of this organism have occurred in nutrient-enriched waters. Cell production can be stimulated by inorganic nutrient enrichment. In contrast, nitrogen or phosphorus limitation can enhance toxin production and/or release. Blooms usually develop under suboptimal conditions (i.e., lower temperatures and salinities). The toxins appear to be essential for bloom development; amelioration of toxicity quickly leads to decimation of *P. parvum* populations by grazers (Granéli *et al*., 2012; Roelke *et al*., 2016).

As for various other harmful algae, the conditions promoting blooms are poorly understood. Physical and chemical conditions conducive for blooms have commonly occurred in the south-central U.S., for example, with and without bloom development. Altered salinity, altered hydrology, and nutrient over-enrichment are most commonly invoked as having promoted *P. parvum* blooms. Other factors that have been noted include changes in water hardness, herbicide use, pH, and the presence of toxin-resistant and/or *P. parvum*–inhibiting plankton (Roelke *et al*., 2016).

General Notes: Because *P. parvum* blooms are often nearly monospecific and populations sometimes reach very high densities, waters take on a golden color and are often accompanied by large fish kills and death of other gill-breathing organisms (Granéli *et al*., 2012). Thus, the blooms are commonly referred to as *golden alga* or *golden algae blooms*. The blooms can also be large, stretching along watersheds hundreds of kilometers. High cell densities are not always an indicator of ichthyotoxicity, for two reasons: first, *P. parvum* has nontoxic as well as toxic strains; and, second, toxic compounds produced by *P. parvum* can be potent at nanomolar concentrations and when cell densities are low.

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Pseudo-nitzschia – *seriata* group; *delicatissima* group

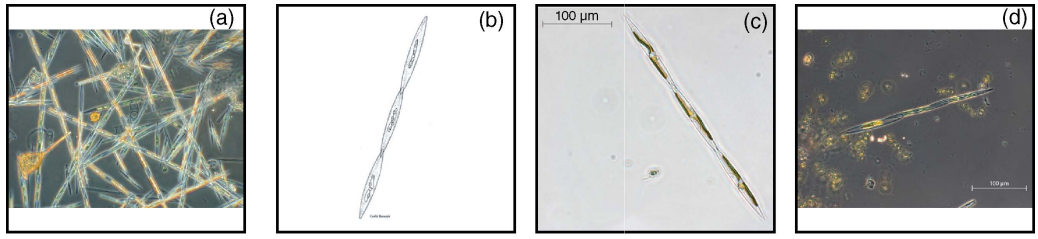

Figure 1 Images of *Seriata* class *Pseudo-nitzschia* from Monterey Bay, California. (a) Mixed assemblage of *Pseudo-nitzschia*, with *Ceratium* and *Eucampia*; (b) line drawing of *Pseudo-nitzschia australis*; (c) light field image of *P. australis*; (d) dark field image of *P. australis* with *Chaetoceros* in the background.

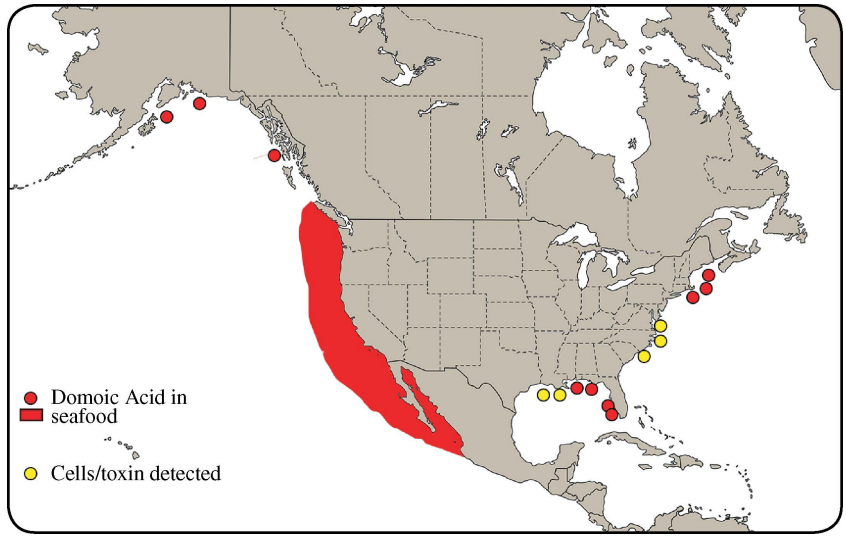

Figure 2 Distribution of *Pseudo-nitzschia* cells and toxin in seawater (yellow) and presence of domoic acid in seafood (red) for North America, based on literature reports through 2015.

Synonyms:

- *P. abrensis* Pérez-Aicua & Orive, 2013
- *P. amanii* Amato & Montesor, 2008
- *P. americana* (Hasle) Fryxell, 1993
- *P. arenysensis* Quijano-Scheggia, Garcés, & Lundholm, 2009
- ***P. australis* Frenguelli, 1939**
- *P. batesiana* Lim, Teng, Leaw & Lim, 2013
- ***P. brasiliana* Lundholm, Hasle, & Fryxell, 2002**
- *P. caciantha* Lundholm, Moestrup & Hasle, 2003
- ***P. calliantha* Lundholm, Moestrup & Hasle, 2003**
- *P. circumpora* Lim, Leaw, & Lim, 2012
- ***P. cuspidata* (Hasle) Hasle, 1993**
- *P. decipiens* Lundholm & Moestrup, 2003
- ***P. delicatissima* (Cleve) Heiden, 1928**
- *P. dolorosa* Lundholm & Moestrup, 2006
- ***P. fraudulenta* (Cleve) Hasle, 1993**
- *P. fryxelliana* Lundholm, 2012
- *P. fukuyoi* Lim, Teng, Leaw & Lim, 2013
- ***P. galaxiae* Lundholm & Moestrup, 2002**
- ***P. granii* (Hasle) Hasle, 1974**
- *P. hasleana* Lundholm, 2012
- *P. heimii* Manguin, 1957
- *P. inflatua* (Hasle) Hasle, 1993
- ***P. kodamae* Teng, Lim, Leaw, & Lim, 2015**
- *P. linea* Lundholm, Hasle, & Fryxell, 2003
- *P. lineola* (Cleve) Hasle, 1965

Pseudo-nitzschia

- *P. lundholmiae* Lim, Teng, Leaw, & Lim, 2013
- *P. micropora* Priisholm, Moestrup & Lundholm, 2002
- ***P. multiseries* (Hasle) Hasle, 1995**
- *P. multistriata* (Takano) Takano, 1995
- *P. obtusa* (Hasle) Hasle & Lundholm, 2005
- *P. plurisecta* Orive & Pérez-Aicua, 2013
- *P. prolongatoides* (Hasle) Hasle, 1993
- ***P. pseudodelicatissima* (Hasle) Hasle, 1993**
- ***P. pungens* (Grunow ex Cleve) Hasle, 1993**
- *P. pungiformis* (Hasle) Hasle, 1993
- *P. roundii* (Hernández-Becerril), 2006
- ***P. seriata* (Cleve) Pergallo, 1899**
- *P. sinica* Qi, Ju, & Lei
- *P. subcurvata* (Hasle) Fryxell, 1993
- *P. subfraudulenta* (Hasle) Hasle, 1993
- *P. subpacifica* (Hasle) Hasle, 1993
- ***P. turgidula* (Hustedt) Hasle, 1993**
- *P. turgiduloides* (Hasle) Hasle, 1993

Morphology: *Pseudo-nitzschia* species are difficult to identify by light microscopy, and are commonly grouped by cell width into the *seriata* group (>3 µm width) and the *delicatissima* group (<3 µm width). They are sometimes further divided into the *multiseries/pungens* group, *australis/fraudulenta/heimii* group, and the *pseudodelicatissima/delicatissima* group. All are pennate diatoms with longitudinal symmetry. They form chains (except *P. americana*) of variable length. Numbers and spacing of fibulae, striae, and poroids allow morphological determination of species, but this typically requires scanning or transmission electron microscopy (SEM or TEM) or application of molecular methods.

Known Distribution: *Pseudo-nitzschia* exhibits cosmopolitan distribution. Despite that distribution, most oceanic species are weakly toxic or nontoxic, and most toxic blooms have been restricted to eastern boundary current upwelling systems. Focusing specifically on the large, toxic bloom events, blooms are common along the west coast of the United States (California, Oregon, and Washington, including Puget Sound) and have been reported in Alaska; British Columbia, Canada; as well as Baja California, Mexico. Cells and toxin are commonly reported in the Gulf of Mexico (particularly Louisiana) and the east coast of the U.S. (and eastern Canada) from Maine to Florida. Shellfish closures occur annually on the U.S. west coast and have occurred in Prince Edward Island, Bay of Fundy, and Gulf of St. Lawrence, Canada; British Columbia, Canada; Portugal; Spain; Ireland; Scotland; Denmark; New Zealand; and Brazil.

Toxin: Known producers of the toxin domoic acid are listed in bold (several species have yet to be tested). Domoic acid is a 311 Da water-soluble amino acid, containing three carboxyl groups. It is an analog of the neurotransmitter L-glutamic acid, and kainic acid. There are multiple isomers (isodomoic acids A through H) and the diastereoisomer epidomoic acid. Epidomoic acid is usually summed with domoic acid, while the isomers are generally much less toxic and are often excluded from analysis. Domoic acid affects the nervous system of birds and mammals by binding to receptors in the central nervous system with greater affinity than glutamic or kainic acid. In mammals, including humans, this results in damage to the hippocampus, causing short-term memory loss (which is why domoic acid poisoning is referred to as amnesic shellfish poisoning [ASP]). Intoxication leads to gastric distress (cramps, nausea, diarrhea). confusion, headaches, breathing difficulties, disorientation, dizziness, and memory loss. In some cases, it led to coma and death. There is no known antidote. Human poisoning is rare, but widespread poisoning of marine mammals and birds is common. Domoic acid does not appear to directly impact (behaviorally or physiologically) invertebrates (crabs, shellfish) or fish at typical environmental concentrations, but domoic acid is transferred through the food web over relatively short time scales and typically through three or less trophic transfers. As a water-soluble toxin, domoic acid does not bioaccumulate easily, but depuration rates vary widely, ranging from hours (*Mytilus edulis*) to months (*Siliqua patula*).

Methods for Toxin Identification: Numerous analytical methods for detection of domoic acid exist. The most common are based on high-performance liquid chromatography with UV absorption or derivatization (fluorescence) detection (HPLC), liquid chromatography–mass spectrometry (LC/MS), receptor-binding assays (RBAs), surface plasmon resonance (SPR), and enzyme-linked immunosorbent assay (ELISA). Commercially available lab and field kits based on variations of ELISA are available from multiple companies.

Ecological Observations: Blooms of *Pseudo-nitzschia* occur frequently in some regions. Members of this

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genus tolerate salinities from 6 to 48, and temperatures from 5 to 48 °C. Toxigenic *Pseudo-nitzschia* grow well on multiple sources of nitrogen, and some species appear to become more toxic when grown on urea, leading to the frequent observation of enhanced blooms/toxicity with terrestrial runoff and/or anthropogenic nutrients. Bloom dynamics have also been linked to large-scale environmental changes such as El Niño. No single trigger for toxin production has been determined. Rather, *Pseudo-nitzschia* toxicity appears to be linked to slowing growth and stress conditions caused by changes in macronutrients (particularly silica and phosphorus limitation), micronutrients (including iron, copper, and lithium), irradiance, salinity, and pH. This wide range of triggers has made it difficult to predict specific conditions that would result in toxic blooms, but several lab and field studies have identified a combination of these variables as being predictors within regional studies. The biosynthetic pathway for domoic acid (and the corresponding genes) have yet to be elucidated. There are frequent reports of strains maintained in culture losing toxicity. Historical and recent work suggests that toxicity is modulated by heterotrophic bacteria, with toxin "recovered" in putatively nontoxic strains with changes in the bacterial assemblage. Allelopathic effects of domoic acid have been hypothesized, but no laboratory-based studies have definitively identified a consistent allelopathic effect of the toxin.

General Notes: *Pseudo-nitzschia* is not known to produce a resting stage, but a "quiescent phase" has been proposed, and there is some evidence for cells surviving unfavorable conditions by (e.g.) settling out to the sediment or entering a quiescent phase in association with subsurface layers within the pycnocline. The complete plastid sequence of the *P. multiseries* genome was recently (2015) published; ~80% of the transcriptome for *P. arenyensis*, *P. delicatissima*, and *P. multistriata* was also recently (2015) published; and a draft genome of *P. multiseries* has been assembled by the Joint Genome Institute.

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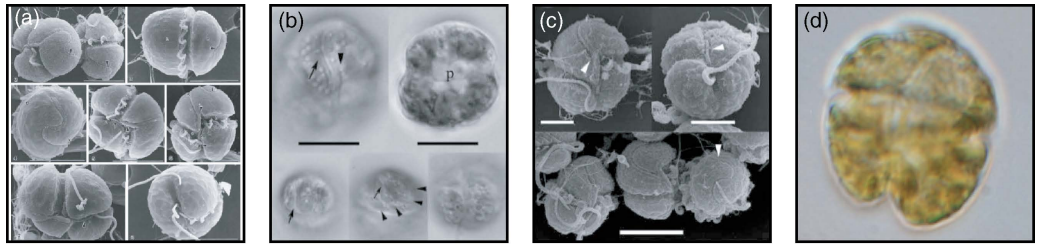

Figure 1 (a–c) Scanning electron microscopy images of *Takayama* spp., (a) *T. pulchella*, (b,c) *T. tuberculata*, (d) *T. tasmanica*.

Figure 2 Map showing general locations of potentially harmful *Takayama* spp. in and near the U.S.

Synonyms:

- *Gymnodinium pulchellum* A. Larsen 1994; also called *Takayama pulchella* (Larsen) de Salas, Bolch & Hallegraeff 2003
- *Takayama tasmanica* de Salas, Bolch & Hallegraeff 2003
- *Takayama tuberculata* de Salas 2008

Morphology: *Takayama* species are unarmored dinoflagellates with 2 flagella. *T. pulchella* is approximately 16–25 µm long and 11–16 µm wide. *T. tasmanica* is approximately 16–27 µm long, 14–26 um wide, and 10–20 µm thick. *T. tuberculata* is approximately 13–22 µm long, 10–20 µm wide, and dorsoventrally flattened.

Known Distribution: All three species are found in Florida. Blooms of *T. pulchella* have occurred in the Indian River Lagoon on the Florida east coast in 1990, 1996, and 2004. *T. tasmanica* has been observed in the Indian River Lagoon of Florida, but only in low concentrations (Phlips et al., 2009). *T. tuberculata* has bloomed annually since 2009 along the southwest coast of Florida, both along the beaches and in some coastal canals.

Cysts: None of the species has been documented to produce cysts.

Toxin: Blooms of *T. pulchella* in the Indian River Lagoon have been associated with fish kills and respiratory irritation in local boaters, suggesting a toxin that could be aerosolized. Low oxygen was not associated with these blooms. This suggests a toxin similar to the brevetoxin produced by *Karenia brevis*, a dinoflagellate that is similar to *T. pulchella*. Blooms of *T. tuberculata* in southwest Florida have

Takayama

been associated with fish kills and the death of other marine animals, but they were also associated with very low oxygen. At the present time, there is no evidence that a toxin was the cause of the deaths.

Methods for Toxin Identification: No specific toxins have been identified.

Ecological Observations: Blooms of *T. pulchella* have occurred in the summer and fall in 1990, 1996, and 2004 in the Indian River Lagoon along the east coast of Florida. *T. tasmanica* has been observed in the Indian River Lagoon of Florida, but only in low concentrations (Phlips et al., 2009). *T. tuberculata* has bloomed in the summer in canals and coastal waters of southwest Florida since 2008. Low concentrations are found year-round. That is the extent of our knowledge of the ecology of these three species.

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Appendix 1

Websites That Routinely Distribute Bulletins on the Presence of Harmful Algal Blooms (HAB) for Public Health

Area	HAB	Website
Texas and Florida, USA	*K. brevis*	https://tidesandcurrents.noaa.gov/hab/
Gulf of Maine, USA	*Alexandrium*	http://www.whoi.edu/website/northeast-psp/forecasting
Lake Erie, USA–Canada	*M. aeruginosa*	http://coastalscience.noaa.gov/research/habs/forecasting
Baltic Sea	*N. spumigena* and *A. flos-aquae*	http://www.smhi.se/en/weather/sweden-weather/ https://stw-helcom.smhi.se/
Ireland	Several dinoflagellates, *Pseudo-nitzschia*	http://www.marine.ie/Home/site-area/data-services/interactive-maps/weekly-hab-bulletin
Massachusetts, USA	*Alexandrium*	http://www.whoi.edu/groups/andersonlab/
Northwestern Atlantic shelf, USA–Canada	Multiple species	http://www.cinar.org
Worldwide	Multiple species	http://www.whoi.edu/redtide https://www.facebook.com/Harmful-Algae-210160985681846/?fref=ts
California	*Pseudo-nitzschia*	http://www.cencoos.org/data/models/habs
Puget Sound, Washington, USA	*Alexandrium*	https://catalyst.uw.edu/workspace/banasn/14943/82765

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Appendix 2

State Agencies Providing Information and Updates on Toxic and Harmful Algal Blooms and Water Quality

State	Address	Phone	Website
Alabama	Alabama Department of Public Health Bureau of Clinical Laboratories Mobile Regional Laboratory 757 Museum Drive Mobile, AL 36608	251-344-6895	http//www.issc.org/
Alaska	Alaska Department of Environmental Conservation Division of Environmental Health 555 Cordova Street Anchorage, AK 99501	907-269-7644 or 907-375-8200	http://dec.alaska.gov/eh/lab/
California	California Department of Public Health Environmental Management Branch PO Box 997377, MS 0500 Sacramento, CA 95899-7377	916-449-5693	https://www.cdph.ca.gov/programs/Pages/EMB.aspx
Connecticut	State of Connecticut Department of Agriculture 165 Capitol Avenue Hartford, CT 06106	203-874-0696 × 103	http://www.ct.gov/doag/cwp/view.asp?a=3768&Q=451508&PM=1
Delaware	DNREC – Division of Water Shellfish Safety 89 Kings Highway Dover, DE 19901	302-739-9939	http://www.dnrec.delaware.gov/swc/wa/Pages/WatershedAssessment.aspx
Florida	Fish and Wildlife Conservation Commission Fish and Wildlife Research Institute 620 South Meridian Street Tallahassee, FL 32399-1600	727-896-8626	http://myfwc.com/research/redtide/
Georgia	Georgia Department of Natural Resources Coastal Resources Division 1 Conservation Way Brunswick, GA 31520	912-264-7218	http://coastalgadnr.org/ha/wq/sf
Hawaii	State of Hawaii Department of Health Environmental Resources Office Environmental Health Analytical Services Branch 919 Ala Moana Blvd., Room 219 Honolulu, HI 96814-4920	808-453-6671	http://health.hawaii.gov/statelab/ems/
Idaho	State of Idaho Department of Environmental Quality Water Quality Division 1410 N. Hilton Boise, ID 83706	208-373-0194	http://www.deq.idaho.gov/water-quality/

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State	Address	Phone	Website
Illinois	Illinois Environmental Protection Agency Bureau of Water – Division of Water Pollution Control Surface Water Section – Lakes Unit PO Box 19276 Springfield, IL 62794-9276	217-782-3362	http://www.epa.illinois.gov/topics/water-quality/monitoring/algal-bloom/index
Indiana	Indiana Department of Environmental Management (IDEM) Office of Water Quality Watershed Assessment and Planning Branch 100 N. Senate Avenue Indianapolis, IN 46219	317-308-3173	http://www.in.gov/idem/algae/2432.htm
Iowa	Iowa Department of Public Health 321 E. 12th Street Des Moines, IA 50319-0075	515-281-8707	https://idph.iowa.gov/ehs/algal-blooms
Kansas	Kansas Department of Health and Environment 6810 SE Dwight Street Topeka, KS 66620	785-296-0801	http://www.kdheks.gov/algae-illness/
Kentucky	Kentucky Department of Environmental Protection Division of Water 200 Fair Oaks Lane, Fourth Floor Frankfort, KY 40601	502-564-3410	http://water.ky.gov/waterquality/Pages/HABS.aspx
Louisiana	Department of Health & Hospitals PO Box 629 Baton Rouge, LA 70821-0629	225-342-9500	http://dhh.louisiana.gov/index.cfm/page/629
Maine	State of Maine Department of Marine Resources 21 State House Station Augusta, ME 04333-0021	207-624-6550	http://www.maine.gov/dmr/rm/public_health/biotoxinmonitoring.htm
Maryland	Department of Natural Resources Tidewater Ecosystem Assessment Division 580 Taylor Avenue Annapolis, MD 21401	410-260-8630	http://dnr.maryland.gov/waters/bay/Pages/Algae.aspx
Massachusetts	Energy and Environmental Affairs Department of Fish and Game Marine Fisheries Division 251 Causeway Street, Suite 400 Boston, MA 02114	508-990-2860 × 122	http://www.mass.gov/eea/agencies/dfg/dmf/programs-and-projects/shellfish-sanitation-and-management.html
Michigan	Department of Environmental Quality Water Resources Division PO Box 30458 Lansing, MI 48909-7958	517-284-5567	http://www.michigan.gov/deq/0,4561,7-135-3313_3675_3691-336800--,00.html
Minnesota	Minnesota Pollution Control Agency Environmental Analysis & Outcomes Surface Water Monitoring 520 Lafayette Road N St. Paul, MN 55155-4194	651-757-2419	http://www.pca.state.mn.us/index.php/water/water-types-and-programs/surface-water/lakes/blue-green-algae-and-harmful-algal-blooms.html
Mississippi	Mississippi Department of Environmental Quality Surface Water Division PO Box 2261 Jackson, MS 39225	601-961-5155	http://www.deq.state.ms.us/mdeq.nsf/page/WQSB_Water_Quality_Standards?OpenDocument
Missouri	Missouri Department of Health and Senior Services PO Box 570 Jefferson City, MO 65102-0570	573-751-6095	http://health.mo.gov/safety/recreationalwater/naturalwaterareas.php

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State	Address	Phone	Website
Montana	Montana Department of Environmental Quality 1520 E. 6th Avenue PO Box 200901 Helena, MT 59620	406-444-6697	http://deq.mt.gov/Water/WQPB/monitoring
Nebraska	Nebraska Department of Environmental Quality (NDEQ) Surface Water Unit 1200 "N" Street, Suite 400 PO Box 98922 Lincoln, NE 68509	402-471-0096	http://deq.ne.gov/NDEQProg.nsf/OnWeb/SWMA
Nevada	Nevada Division of Environmental Protection 901 South Stewart Street, Suite 4001 Carson City, NV 89701	775-687-4670	http://ndep.nv.gov/bwqp/index.htm
New Hampshire	New Hampshire Department of Environmental Services Shellfish Program 222 International Drive, Suite 175 Pease Tradeport Portsmouth, NH 03801	603-559-1509	http://des.nh.gov/organization/divisions/water/wmb/shellfish/
New Jersey	Department of Environmental Protection Bureau of Marine Water Monitoring PO Box 402 Trenton, NJ 08625-0402	866-337-5669	http://www.nj.gov/dep/bmw/
New Mexico	New Mexico Department of Game & Fish 1 Wildlife Way Santa Fe, NM 87507	505-476-8000	http://www.wildlife.state.nm.us/fishing/fisheries-management/
New York	Bureau of Marine Resources Division of Fish, Wildlife & Marine Resources 205 North Belle Mead Road, Suite 1 East Setauket, NY 11733	631-444-0492	http://www.dec.ny.gov/outdoor/64824.html
North Carolina	N.C. Division of Marine Fisheries Shellfish Sanitation & Water Quality 3441 Arendell Street Morehead City, NC 28557	252-726-6827 × 8147	http://portal.ncdenr.org/web/mf/shellfish-sanitation-and-recreational-water-quality
Ohio	Ohio Environmental Protection Agency 8955 E. Main Street Reynoldsburg, OH 43068	614-644-4271	http://epa.ohio.gov/DivisionsandOffices/EnvironmentalServices.aspx#132754281-contacts
Oklahoma	Oklahoma State Department of Health Acute Disease Service 1000 N.E. 10th Street Oklahoma City, OK 73117	405-271-4060	http://www.ok.gov/health/Disease,_Prevention,_Preparedness/Acute_Disease_Service/Disease_Information/Blue-Green_Algae.html
Oregon	Oregon Health Authority 800 NE Oregon Street Portland, OR 97232	971-673-1222	http://public.health.oregon.gov/HealthyEnvironments/Recreation/HarmfulAlgaeBlooms/Pages/index.aspx
Pennsylvania	Department of Environmental Protection Bureau of Clean Water – Monitoring Section 2575 Interstate Drive Harrisburg, PA 17105	717-787-9637	http://www.dep.pa.gov/Business/Water/CleanWater/WaterQuality/Pages/Draft-2015-Assessment-Methodology.aspx
Rhode Island	State Health Laboratory 50 Orms Street Providence, RI 02903	401-222-5960	http://www.dem.ri.gov/programs/emergencyresponse/bart/habs.php

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State	Address	Phone	Website
South Carolina	South Carolina Department of Natural Resources Marine Resources Research Institute PO Box 12559 Charleston, SC 29422	843-953-9819	http://www.dnr.sc.gov/marine/mrri/index.html
Texas	Texas Parks & Wildlife 4200 Smith School Road Austin, TX 78744	512-389-4800	https://tpwd.texas.gov/landwater/water/environconcerns/hab/
Utah	Department of Environmental Quality 195 North 1950 West Salt Lake City, UT 84116	801-536-4400	http://www.deq.utah.gov/Pollutants/H/harmfulalgalblooms/index.htm
Vermont	Vermont Department of Health 108 Cherry Street Burlington, VT 05402	800-464-4343	http://healthvermont.gov/enviro/bg_algae/bgalgae.aspx
Virginia	The Virginia Department of Health PO Box 2448 109 Governor Street Richmond, VA 23218	757-518-2000	http://www.vdh.virginia.gov/environmental-epidemiology/harmful-algal-blooms-habs/
Washington	Washington State Department of Health Shellfish Program PO Box 47824 Olympia, WA 98504	360-236-3330	http://www.doh.wa.gov/AboutUs/ProgramsandServices/EnvironmentalPublicHealth/EnvironmentalHealthandSafety/ShellfishProgram
West Virginia	Department of Environmental Protection 601 57th Street SE Charleston, WV 25304	304-926-0440	http://www.dep.wv.gov/WWE/Programs/wqs/Pages/FilamentousAlgaeinWestVirginia.aspx
Wisconsin	Wisconsin Department of Health Services 1 West Wilson Street Madison, WI 53703	608-267-3242	https://www.dhs.wisconsin.gov/water/bg-algae/resources.htm

Appendix 3

List of General Web Resources

Center for Disease Control, "Harmful Algal Bloom (HAB) – Associated Illness":
https://www.cdc.gov/habs/index.html

Fish and Wildlife Research Institute, "Red Tide" research page:
http://myfwc.com/research/redtide/

NOAA, "Harmful Algal BloomS Observing System":
https://service.ncddc.noaa.gov/website/AGSViewers/HABSOS/maps.htm

NOAA Great Lakes Environmental Research Laboratory, "Great Lakes HABs and Hypoxia":
https://www.glerl.noaa.gov/res/HABs_and_Hypoxia/

NOAA National Centers for Coastal Ocean Science, "Harmful Algal Blooms":
https://coastalscience.noaa.gov/research/habs/

U.S. Environmental Protection Agency, "Advisory for Safe Seafood Consumption":
https://www.epa.gov/choose-fish-and-shellfish-wisely

U.S. state health departments:
https://www.cdc.gov/mmwr/international/relres.html

Woods Hole Oceanographic Institution, "National Algal Bloom":
http://www.whoi.edu/redtide/home

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