



Predicting potentially toxigenic *Pseudo-nitzschia* blooms in the Chesapeake Bay

Clarissa R. Anderson^{a,b,*}, Mathew R.P. Sapiiano^{c,d}, M. Bala Krishna Prasad^e, Wen Long^f, Peter J. Tango^g, Christopher W. Brown^h, Raghu Murtugudde^e

^a Ocean Sciences Department, University of California, Santa Cruz, 1156 High St., Santa Cruz, CA 95064, United States

^b Department of Earth and Ocean Sciences, 701 Sumter St, University of South Carolina, Columbia, SC, United States

^c Department of Atmospheric Science, Colorado State University, Fort Collins, CO, 80523, United States

^d Cooperative Institute for Climate and Satellites (CICS), Earth System Science Interdisciplinary Center (ESSIC), University of Maryland, College Park, MD, 20742, United States

^e Earth System Science Interdisciplinary Center (ESSIC), University of Maryland, College Park, MD, 20742, United States

^f Horn Point Laboratory, University of Maryland Center for Environmental Science, P.O. Box 775, Cambridge, MD 21613, United States

^g U. S. Geological Survey @ U.S. Environmental Protection Agency Chesapeake Bay Program Office, 410 Severn Ave. Suite 109, Annapolis MD 21403, United States

^h National Oceanic and Atmospheric Administration, Cooperative Institute for Climate and Satellites (CICS), M Square Office Building # 950, Suite 4001, 5825 University Research Court, College Park, MD 20740, United States

ARTICLE INFO

Article history:

Received 15 October 2009

Received in revised form 19 February 2010

Accepted 4 April 2010

Available online 24 April 2010

Keywords:

Algal blooms

Domoic acid

Amnesic shellfish poisoning

Diatoms

Prediction

Regression analysis

Chesapeake Bay

ABSTRACT

Harmful algal blooms are now recognized as a significant threat to the Chesapeake Bay as they can severely compromise the economic viability of important recreational and commercial fisheries in the largest estuary of the United States. This study describes the development of empirical models for the potentially domoic acid-producing *Pseudo-nitzschia* species complex present in the Bay, developed from a 22-year time series of cell abundance and concurrent measurements of hydrographic and chemical properties. Using a logistic Generalized Linear Model (GLM) approach, model parameters and performance were compared over a range of *Pseudo-nitzschia* bloom thresholds relevant to toxin production by different species. Small-threshold blooms (≥ 10 cells mL⁻¹) are explained by time of year, location, and variability in surface values of phosphate, temperature, nitrate plus nitrite, and freshwater discharge. Medium- (100 cells mL⁻¹) to large-threshold (1000 cells mL⁻¹) blooms are further explained by salinity, silicic acid, dissolved organic carbon, and light attenuation (Secchi) depth. These predictors are similar to other models for *Pseudo-nitzschia* blooms on the west coast, suggesting commonalities across ecosystems. Hindcasts of bloom probabilities at a 19% bloom prediction point yield a Heidke Skill Score of ~53%, a Probability of Detection ~75%, a False Alarm Ratio of ~52%, and a Probability of False Detection ~9%. The implication of possible future changes in Baywide nutrient stoichiometry on *Pseudo-nitzschia* blooms is discussed.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

The cosmopolitan diatom genus *Pseudo-nitzschia* has emerged as a major player in the global theatre of harmful algal blooms (HABs), occurring on both east and west coasts of the United States (Trainer et al., 2000; Thessen and Stoecker, 2008), the Gulf of Mexico (Dortch et al., 2000; Pan et al., 2001), and throughout Europe and parts of Asia (Bates and Trainer, 2006). In response to a coarsely understood set of environmental conditions, toxigenic *Pseudo-nitzschia* species are known to produce domoic acid (DA), a potent neurotoxin that can be devastating to aquatic life via trophic transfer in the food web (Fritz et al., 1992; Bates et al., 1998; Scholin et al., 2000). In humans, DA exposure manifests itself as amnesic shellfish poisoning (ASP) following the consumption of contaminated filter-feeding mollusks (e.g. Bates et al., 1998; Trainer et al., 2007). Samples of *Pseudo-nitzschia*

spp. collected from the largest estuary in the USA, the Chesapeake Bay, have recently tested positive for DA (Thessen and Stoecker, 2008; Thessen et al., 2009), yet, to date, there has been no reported incidence of major bird or marine mammal strandings nor ASP in the Bay. However, emerging toxicological and epidemiological research suggests that chronic, sub-acute doses of DA that do not cause any outward signs of intoxication may cause neuropathic injury to vertebrates (Levin et al., 2006; Lefebvre et al., 2007; Ramsdell and Zabka, 2008). For humans, this implies the potential for serious neurological health risks associated with repeated exposure to low levels of DA, for example in coastal communities with an established culture of shellfish consumption (Grattan et al., 2007). Furthermore, the accumulation of DA in sediments after the demise of a surface bloom may reverberate throughout the ecosystem via accumulation of toxins in benthic food webs and turbulent mixing events (Thessen et al., 2009). Clearly, there are significant benefits to extending HAB predictions from ecosystem models into the public health arena (Dyble et al., 2008).

Human-driven nutrient enrichment, or cultural eutrophication, is now considered an important factor driving the global increase in HAB

* Corresponding author. Ocean Sciences Department, University of California, Santa Cruz, 1156 High St., Santa Cruz, CA 95064, United States. Tel.: +1 831 426 5074.

E-mail addresses: clrander@ucsc.edu, clarissa@umd.edu (C.R. Anderson).

frequency and intensity (Hallegraeff, 1993; VanDolah, 2000; Anderson et al., 2002; Glibert et al., 2005; Heisler et al., 2008; Anderson et al., 2008). Some of the issues that are relevant to systems where eutrophication may exert an influence on HAB development include the expected effects of nutrient stoichiometry (reviewed in Anderson et al., 2002; Glibert et al., 2010—this issue), potential changes in ratios of dissolved organic carbon to dissolved organic nitrogen (DOC: DON; Paerl, 1988; Anderson et al., 2002), and resultant increases in the probability of toxin production by certain species, such as those within the *Pseudo-nitzschia* genus (Pan et al., 1996; Davidson and Fehling, 2006). With increased nutrient inputs from runoff come reduced Si:N and Si:P ratios (reviewed in Anderson et al., 2002), a factor shown to contribute to and/or be associated with increases of *Pseudo-nitzschia* abundance and DA production (Pan et al., 1996; Fehling et al., 2004; Parsons and Dortch, 2002; Anderson et al., 2006). Whereas most diatoms only thrive under Si-replete conditions, some *Pseudo-nitzschia* species appear to be favored by Si-limitation and certainly tend towards toxicity under extreme Si-limitation (Pan et al., 1996, 1998; Bates et al., 1998).

In the Chesapeake Bay, where HABs are common, diverse, and may be increasing in frequency, Glibert et al. (2001) documented a direct relationship between nitrogenous fertilizer use in the Bay watershed in spring and the onset of potentially toxic *Prorocentrum minimum* blooms. *Pseudo-nitzschia* spp. on the west coast generally bloom in response to surface macronutrient increases after natural, coastal upwelling events (Trainer et al., 2000; Kudela et al., 2005; Anderson et al., 2006; Garcia-Mendoza et al., 2009; Lane et al., 2009) and could respond to similar pulses from spring runoff in the Chesapeake Bay (Heisler et al., 2008). Furthermore, recent laboratory and field experiments link organic forms of nitrogen, such as glutamine and urea, to sustained bloom events in an upwelling region and even enhanced production of DA by toxigenic species of *Pseudo-nitzschia* (Howard et al., 2007; Kudela et al., 2008). In the Gulf of Mexico, time series of *Pseudo-nitzschia* spp. from sediment cores document increasing cell abundance in association with increased nutrient input from Mississippi River runoff (Parsons and Dortch, 2002). Nonetheless it remains unclear if highly eutrophied bays and estuaries experience significantly more *Pseudo-nitzschia* blooms or DA events than coastal upwelling systems and whether blooms in the Chesapeake Bay may be associated with anthropogenic nutrient enrichment (Dortch et al., 1997; Cochlan et al., 2008). Long-term hindcasts and forecasts from predictive models of HABs would be useful for understanding climate and land-use change effects on the frequency and magnitude of potentially deleterious algal blooms in the eutrophied Chesapeake Bay region (Kemp et al., 2005).

We present here our efforts to develop a threshold-based, empirical model for predicting the probability of *Pseudo-nitzschia* spp. bloom occurrence (and by extension, the potential for DA production) from a long-term monitoring dataset for the Chesapeake Bay. Currently, no such model exists for *Pseudo-nitzschia* on the east coast, despite the obvious importance of having such predictive capabilities in place. While the methods used to develop statistical models for estimating the distribution of harmful algae and pathogens are not new in scientific application, they are now more widely employed and accepted in ecological forecasting. A fully operational alert system that combines satellite imagery, an ensemble of numerical models, and a rules-based biological model is running in the Gulf of Mexico for blooms of the aerosolized toxin-producing, *Karenia brevis* (Stumpf et al., 2003; Tomlinson et al., 2004, 2009). Accompanying this trend are rapid advances in the development of dynamically-downscaled regional products for forecasting the physical and biogeochemical states of coastal ecosystems (e.g. Moore et al., 2002; Chai et al., 2002; Fennel et al., 2006; Xu and Hood, 2006; Chao et al., 2008; Allen et al., 2008). A system has been implemented that predicts the likely distribution of sea nettles (*Chrysaora quinquecirrha*), the toxic dinoflagellate *Karlodinium veneticum*, and *Vibrio*

cholerae in the Chesapeake Bay based on habitat suitability and hydrodynamic models (Decker et al., 2007; Constantin de Magny et al., in press; Brown et al., 2010). On the west coast, investigators are building upon earlier statistical models (Blum et al., 2006) for predicting the likelihood and toxicity of *Pseudo-nitzschia* blooms from a suite of hydrographic and nutrient inputs in the Monterey Bay (Lane et al., 2009) and the Santa Barbara Channel (Anderson et al., 2009). The goals of the present study are to 1) identify the physico-chemical predictors of regional *Pseudo-nitzschia* spp. blooms, 2) develop and validate a predictive habitat model based on these environmental predictors that could be employed to help predict the probability of occurrence of these blooms in the Chesapeake Bay, and 3) present spatially-explicit hindcasts of past *Pseudo-nitzschia* bloom events.

2. Materials and methods

Long-term data on surface phytoplankton abundance and surface water quality were acquired from the Maryland Department of Natural Resources (MD DNR) and US EPA Chesapeake Bay Program (US EPA-CBP) for 30 stations within the mainstem Bay and 12 tidal estuarine tributaries covering the period 1985–2007 ($N = 6989$; Fig. 1). The data subset used for model development included the monthly suite of water quality parameters collected within one day of *Pseudo-nitzschia* spp. cell abundance records at 30 sites across the broad range of salinities represented within the Chesapeake Bay (oligohaline = 0.5–5; mesohaline = 5–18; polyhaline = >18; all salinity values in psu). The majority of observations fall in the salinity range ≤ 18 ($N_{\text{non-blooms}} = 5957$; $N_{\text{blooms}} = 278$). Fewer observations are available for salinities >18 ($N_{\text{non-blooms}} = 562$, $N_{\text{blooms}} = 199$) which are typically found at the four stations in the lower Bay but can also occur seasonally in the mainstem middle Bay and in the lower reaches of tributaries (Fig. 1). Because these monitoring data were not collected with the purpose of creating predictive models, sampling biases make it a less than ideal dataset despite the long time period and sampling effort represented. Observations are heterogeneously distributed in space and time due to the often event-response nature of data collection and our requirement of daily matchups between water quality and cell count data. To help ensure taxonomic fidelity of the microscopic identifications in the available monitoring observations, we limited the majority of observations to those collected by a single taxonomist whenever possible (W. Butler, pers. comm.); this excludes data from the four lower bay sites (CB6.1, CB6.4, CB7.3E, CB7.4) that were required for full Bay coverage. Additionally, due to changes in taxonomic status for the *Pseudo-nitzschia* group over the study period and difficulties with species identification using classic light microscopy, model development (Section 3.1) is focused on *Pseudo-nitzschia* spp. with no distinction between the various individual toxigenic and non-toxigenic species recorded for the Chesapeake Bay (Marshall et al., 2005; Thessen and Stoecker, 2008).

Monthly freshwater discharge data ($\text{ft}^3 \text{s}^{-1}$) were acquired from seven of the nine sites in the USGS River Input Monitoring (RIM) Program (USGS RIMP Report, 1999) that collects water samples in the non-tidal region of major streams in the Chesapeake Bay watershed. Phytoplankton monitoring stations were grouped with the nearest, relevant RIM site for discharge matchups (Fig. 1). Negative discharge values that are reported from October 1987 to August 1988 for the Mattaponi River were eliminated from this study.

3. Modeling approach

3.1. Model development

A suite of 19 physical and chemical variables publically available through the MD DNR and US EPA-CBP long-term water quality monitoring programs was chosen to examine conditions associated

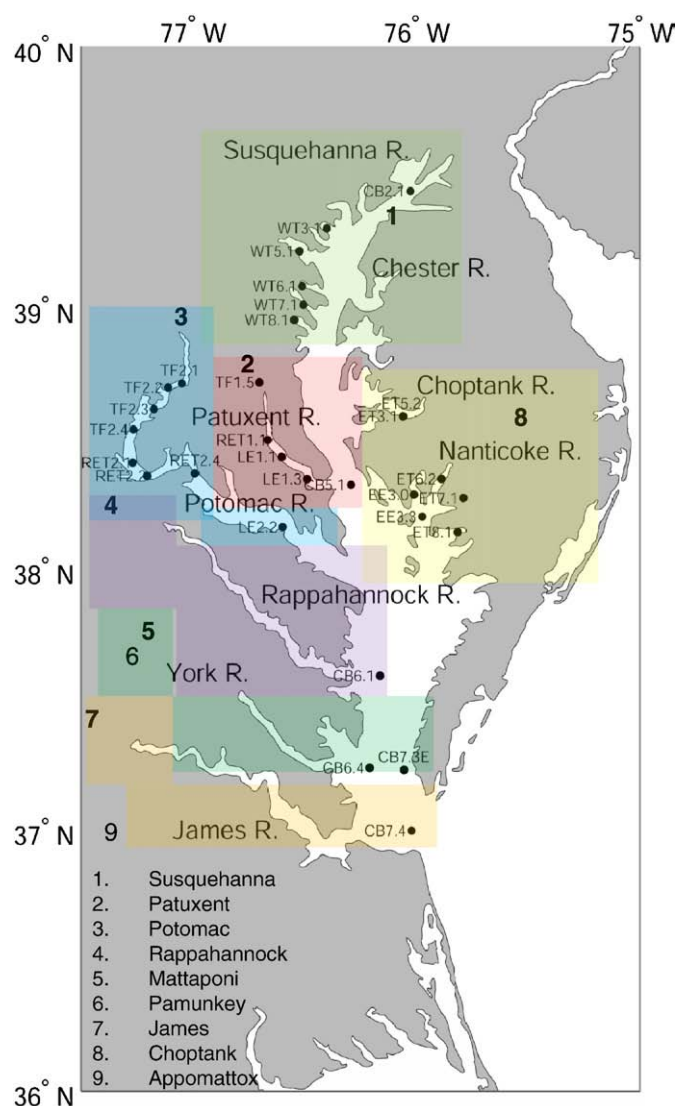


Fig. 1. Map of the Chesapeake Bay and its tributaries: dark circles represent the 30 stations for which data were retrieved from the MD DNR and EPA Chesapeake Bay Program monitoring projects from 1985–2007 ($N=6,989$). Colored, shaded regions denote monitoring station groupings for monthly freshwater discharge data collected from seven USGS River Input Monitoring (RIM) Program sites (numbers in bold correspond with listed river sites).

with regional variability in *Pseudo-nitzschia* spp. abundance (Table 1). Past studies that focused on *Pseudo-nitzschia* bloom prediction use Ordinary Least Squares (OLS) regression to model cell abundance and toxin concentration (Blum et al., 2006; Anderson et al., 2009) or logistic regression to model cell abundance (Lane et al., 2009) as a function of physico-chemical and other environmental properties. In this case, as in Lane et al. (2009), we are interested in only the presence or absence of 'bloom' levels of *Pseudo-nitzschia* spp. (hereafter referred to only as *Pseudo-nitzschia*). The response variable for cell abundance was transformed to a binary variable using several bloom values that fall within the range of threshold levels expected to trigger toxin production on the west coast for those *Pseudo-nitzschia* populations identified as frequently present in the Chesapeake Bay (Thessen and Stoecker, 2008): *P. fraudulenta* (~ 10 cells mL^{-1}), *P. multiseriata/pungens* (100 cells mL^{-1}), and *P. cuspidata/calliantha* (*P. pseudodelicatissima* complex; 1000 cells mL^{-1}) (Trainer and Suddleson, 2005). Model results for the three bloom thresholds are presented in Section 4.2.

Bloom occurrence is a binary variable which rarely follows a Gaussian (Normal) distribution and renders OLS linear regression an

Table 1

A list of the available environmental parameters tested in model development for the response variable *Pseudo-nitzschia* spp. abundance (in bold).

Parameter	Relevant abbreviations	Units
Latitude	Lat	deg
Longitude	Lon	deg
Month	Month	n/a
Chlorophyll- <i>a</i>	Chl- <i>a</i>	$\mu\text{g L}^{-1}$
Temperature	Temp	$^{\circ}\text{C}$
Salinity	Sal	psu
Freshwater discharge	Dis	$\text{ft}^3 \text{s}^{-1}$
Nitrate	NO_3	mg-N L^{-1}
Nitrite	NO_2	mg-N L^{-1}
Ammonium	NH_4	mg-N L^{-1}
Orthophosphate	PO_4	mg-P L^{-1}
Silicic acid	Si(OH)_4	mg-Si L^{-1}
Nitrate + Nitrite:Phosphate	N:P	n/a
Silicic Acid: Nitrate	Si:N	n/a
Silicic Acid: Phosphate	Si:P	n/a
Dissolved oxygen	O_2	mg L^{-1}
Dissolved organic carbon	DOC	mg-C L^{-1}
Secchi depth	Secchi	m
<i>Pseudo-nitzschia</i> spp. abundance	<i>Pseudo-nitzschia</i> abundance	cells mL^{-1}

unsuitable model. Instead, we used the Generalized Linear Model (GLM; McCullagh and Nelder, 1989; Fox, 2002; Agresti, 2007), a regression-based approach that allows for several non-Gaussian distributions in addition to OLS linear regression. The GLM for binary data is often referred to as logistic regression and assumes that the binary response follows a Bernoulli distribution or the closely-related binomial distribution. The GLM relates a transformation of the response (bloom occurrence) to a linear combination of the explanatory variables. The transformation is referred to as the logit link function for binary variables and transforms the expectation of the response to the linear predictor as:

$$\log_e[p/(1-p)] = \beta_0 + \beta_1 x_{i1} + \dots + \beta_k x_{ik}, \quad (1)$$

where the term $p/(1-p)$ represents the odds of a 'bloom' event, β_0 is the intercept, and β_i represents the regression coefficient for the k variables x . Thus, the binary GLM simply models the log probability of an event as a linear combination of the explanatory variables. Solving for p , the probability of a bloom is then described by:

$$p = P_{\text{bloom}} = e^{(\text{logit})} / [e^{(\text{logit})} + 1]. \quad (2)$$

For each bloom threshold, models were fitted by maximum likelihood using a forward stepwise approach whereby deviance residuals were tested against a Chi-square distribution for assessing the relative significance of parameters in the model (R statistical software, v. 2.7.0). Explanatory variables were added to the model individually, with the parameter leading to the largest statistically significant ($\alpha=0.05$) reduction in deviance being added at each successive step. The final degrees of freedom (DF) for a given model is a function of the particular predictors retained in the GLM since there is spatial and temporal heterogeneity in observation matchups between water quality and cell abundance data. To assess the influence of the annual cycle on environmental variables, hypothesis testing was performed twice, once with raw data values, and again with anomalies of all variables whereby monthly climatological means for each station were removed from the observations. Explanatory variables were also lagged from one to three months relative to the response variable in the GLM to test for significant lead/lag relationships.

3.2. Model validation

The resulting logistic GLM was tested using cross-validation. For each year, predictions were made from the model fitted to all years excluding the one for which data were predicted. Predictions from the model come in the form of probabilities, such that a probability threshold is required for determining bloom from non-bloom conditions. Rather than setting this probability (or prediction point as expressed in Lane et al., 2009) to an arbitrary value of 0.5 (i.e. 50%), it may be useful for extreme, episodic blooms to optimize the threshold in order to determine a relevant probability for defining a possible bloom event. Specifically, we optimized this value relative to the probability of detection (POD), the false alarm ratio (FAR), the probability of false detection (POFD), and the Heidke Skill Score (HSS; Heidke, 1926; Sohn and Park, 2008). These are defined as follows:

$$\text{POD} = (\text{correct hits}) / (\text{correct hits} + \text{false negatives}), \quad (3)$$

where correct hits are the equivalent of correct bloom predictions, and false negatives are those instances where bloom observations were predicted by the model to be non-blooms. POD is a component of the widely-used Receiver Operating Characteristic (ROC) curve which represents the relationship between specificity and sensitivity (as in Lane et al., 2009).

$$\text{FAR} = (\text{false positives}) / (\text{hits} + \text{false positives}), \quad (4)$$

where false positives are non-bloom observations that have been overpredicted as blooms by the GLM; and

$$\text{POFD} = (\text{false positives}) / (\text{correct negatives} + \text{false positives}). \quad (5)$$

where correct negatives are correctly predicted non-bloom observations. POFD is also an important aspect of the ROC curve and describes the proportion of non-bloom events that were falsely predicted to be blooms.

HSS is a generalized skill score that takes into account artificial predictability based on chance statistical relationships between parameters in the model. It is an alternative to the more general 'true skill' for describing model performance but more stringent in terms of not giving credit for predicting climatological values (Davis, 1976; Siegel and Dickey, 1986; Song and Haidvogel, 1994; Anderson et al., 2009). HSS values are functionally equivalent to Cohen's α , range from $-\infty$ to 1, and can be represented by the formula:

$$\text{HSS} = [(\text{correct hits} + \text{correct negatives}) - (\text{expected correct})_{\text{random}}] / [N - (\text{expected correct})_{\text{random}}] \quad (6)$$

where the expected number of correct predictions is expressed as:

$$\begin{aligned} (\text{expected correct})_{\text{random}} = & 1 / N [(\text{correct hits} + \text{false negatives}) \\ & \times (\text{correct hits} + \text{false positives}) \\ & + (\text{correct negatives} + \text{false negatives}) \\ & \times (\text{correct negatives} + \text{false positives})] \end{aligned} \quad (7)$$

For each year in the cross-validation, the prediction point is optimized for the POD, FAR, POFD, and HSS. Overall model performance is expressed as the mean of these iterated results in Section 4.2.1.

4. Results

4.1. Observations

Over the 22-year record of *Pseudo-nitzschia* abundance data, very large bloom events were highly episodic in the region (Fig. 2).

Mean abundance was 53 cells mL⁻¹ over the range 0–1.9 × 10⁴ cells mL⁻¹ for all 30 stations. This maximum is consistent with other *Pseudo-nitzschia* observations (Thessen and Stoecker, 2008) and is comparable to the largest blooms observed on the west coast where DA poisoning is a recurring threat to living resources and human health. The majority of blooms that exceed 100 cells mL⁻¹ in the record occur in the more meso- and poly-haline middle to lower mainstem Bay (stations CB5.1, CB6.1, CB6.4, CB7.3E, CB7.4). Notable hotspots for bloom development are located in lower tidal tributary estuarine zones (EE3.0, EE3.3, LE1.1, LE1.3, LE2.2, RET1.1; Fig. 1, Table 2). Midbay station CB5.1 experienced a period from 1993 to spring 1998 when *Pseudo-nitzschia* were absent according to this dataset (Fig. 2). Salinities over 18 can occur in these tidal estuarine zones of major rivers entering the Bay, and those intrusions are often associated with high *Pseudo-nitzschia* cell abundance. These patterns are consistent with expected salinity requirements for a marine diatom (Hasle and Syvertsen, 1996; Thessen et al., 2005) and corroborate observations associating salinities >5 with the presence of *Pseudo-nitzschia* in the Chesapeake Bay (Thessen and Stoecker, 2008). Locations with the largest variability in bloom occurrence are the middle and lower Bay stations as well as the downstream Patuxent River sites (LE1.1, LE1.3, RET1.1), the downstream Potomac River sites (LE2.2, RET2.1, RET2.2, RET2.4), the eastern Bay estuarine stations (EE3.0 and EE3.3), and the Patapsco River mouth in the north of the Bay (WT5.1; Table 2).

4.2. *Pseudo-nitzschia* bloom likelihood models

The correlation analysis of environmental variables and the response variable, *Pseudo-nitzschia* cell abundance, indicates a weakly significant relationship between previously noted correlates of *Pseudo-nitzschia* blooms: temperature ($r = -0.10$), salinity ($r = 0.12$), orthophosphate (PO₄; $r = -0.10$), nitrate (NO₃; $r = -0.07$), and silicic acid (Si(OH)₄; $r = -0.08$; $\alpha = 0.05$ level; Tables 1 and 3, Fig. 3). The correlation between *Pseudo-nitzschia* cell abundance and freshwater discharge is not significant ($r = -0.03$). However, it is expected that inorganic nutrients and salinity are biologically-relevant proxies for the effects of freshwater runoff into the Bay that incorporate the influence of precipitation, tidal fluxes, and discharge. Cell abundance is generally highest from 5 to 27 °C with an apparent temperature optimum near 10 °C and salinities from 5 to 28 with an apparent salinity optimum near 15. Cell abundance also tends to increase with a decrease in nutrient concentrations (Fig. 3). However, it should be noted that the observed range of nutrient concentrations (Fig. 3) indicate an enriched system where concentrations are only rarely limiting to phytoplankton.

These correlation values are generally consistent with the logistic GLMs that significantly associate 'blooms' of *Pseudo-nitzschia*, tested over a range of bloom thresholds, with PO₄, temperature, salinity, and variability in either NO₃ + NO₂ or NO₂ alone (Table 4). Blooms starting at 100 cells mL⁻¹ are further controlled or significantly associated with variability in the dissolved organic carbon (DOC) pool and availability of Si(OH)₄, while the largest blooms additionally are associated with reductions in light penetration in the water column (Secchi depth). Despite the low correlation between freshwater discharge and *Pseudo-nitzschia* abundance, discharge also emerges as significant for blooms below 1000 cells mL⁻¹ according to the hypothesis testing. The significant role of latitude and/or longitude (Table 4) suggests a spatially dependent factor that is not explained by any of the chemical or physical variables tested but that is clearly important for bloom formation at different thresholds. Despite the fact that climatological anomalies of all variables were tested during model development to account for seasonality in the parametric effects, the final models that best capture the environmental thresholds driving *Pseudo-nitzschia* blooms are the models built from raw data. The seasonal signal is retained with the inclusion of *month of the year* as a significant predictor of blooms

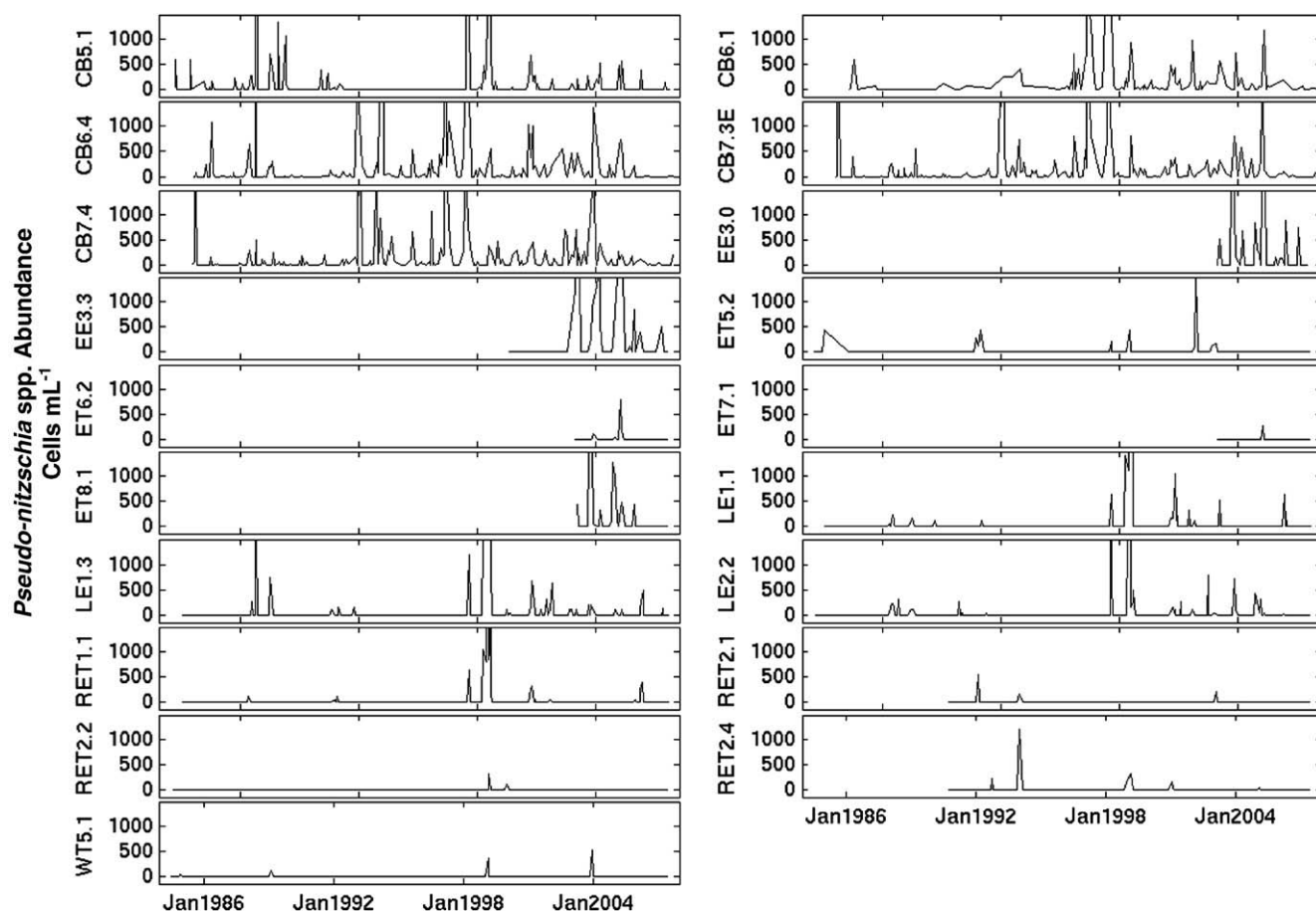


Fig. 2. Time-series plots of *Pseudo-nitzschia* spp. abundance (cells mL⁻¹) for stations where *Pseudo-nitzschia* were recorded over the study period. Maximum cell abundance is masked by the uniform x-axis range of 0–1500 cells mL⁻¹; see Table 2 for more bloom statistics. The lower Bay, polyhaline stations are CB6.1, CB6.4, CB7.3E, and CB7.4; the mesohaline mainstem station is CB5.1. Stations ET5.2, ET6.2, ET8.1, EE3.0, EE3.3, LE1.1, LE2.2, and RET2.4 fall within the meso- to oligo-haline transition zone. Stations ET7.1, RET1.1, RET2.2, and WT5.1 are more typically oligohaline.

<1000 cells mL⁻¹ (Table 4), and lag effects were not found to be significant for any of the explanatory variables tested. In agreement with a previous study (Thessen and Stoecker, 2008) and explaining the negative relationship with month of the year, the majority of bloom events above 100 cells mL⁻¹ (Table 2) occurred in winter and spring months.

4.2.1. Model selection and performance

Model fits for a logistic regression cannot be assessed using a coefficient of determination (R^2) as is often done in the case of OLS linear regression. However, 'pseudo R^2 ' measures are available such as the one suggested by Nagelkerke (1991) that can be applied to the logistic GLM and very roughly translates to the proportion of deviance explained by the model. The effect of bloom threshold on GLM performance is illustrated in Table 4 where the GLM for blooms ≥ 10 cells mL⁻¹ results in the highest maximum skill (HSS = 0.53) and Nagelkerke's R^2 ($R^2 = 0.22$), in part due to the increased predictability of the more common bloom events. While low, this threshold is currently being employed on the west coast for defining and predicting mixed-assemblage blooms of *Pseudo-nitzschia* (Lane et al., 2009; Moore et al., 2009) and may be the most likely to capture the onset of potentially deleterious blooms covering the full suite of *Pseudo-nitzschia* species present in the Chesapeake Bay. Therefore the remainder of our analyses will focus on the evaluation of the small-threshold GLM using the variety of metrics described in the Materials and methods. However, due to this model's inclusion of discharge as the final, least significant parameter (Table 4) and our current inability to grid

discharge data for spatially-explicit predictions of bloom probabilities in the Bay (Section 4.2.3), the final model will be evaluated as it is presented in Table 5, with discharge excluded, according to the algorithm:

$$\text{logit} = 128 - 1.60 \cdot \text{Lat} - 68.1 \cdot \text{PO}_4 - 0.06 \cdot \text{Temp} - 1.02 \cdot (\text{NO}_3 + \text{NO}_2) + 0.878 \cdot \text{Lon} - 0.064 \cdot \text{Month} \quad (8)$$

where the probability of a bloom (P_{bloom}) follows Eq. (2) (see Table 1 for variables). The Nagelkerke's R^2 remains effectively the same at 0.226, as does overall model performance (Table 6). Fig. 4 shows the probability of bloom occurrence as predicted by the logit in Eq. (8), split into bloom ($n = 668$; median prob = 36%) and non-bloom ($n = 6,106$; median prob = 1%) events. Points falling outside the 95th percentile whiskers in the boxplot denote outlier points ($n = 614$), or in the case of the non-bloom observations, the false positive predictions (i.e. model-predicted blooms where no blooms were observed).

Use of this logistic model to predict blooms requires definition of a threshold (i.e. prediction point) based on the probabilities generated from the GLM. If a default prediction point of 0.50 were employed to define a *Pseudo-nitzschia* bloom, the POFD would be quite low at 0.03, but the POD would also be low at 0.34 (Table 6). While a low POFD is desirable, a low POD is highly undesirable, and it is clear from this scenario that a prediction point of 0.5 would lead to an underestimation of bloom events. Since our goal is to maximize model predictive skill (in this case, HSS) and consequently maximize the POD while minimizing the FAR and the POFD, we have optimized the prediction

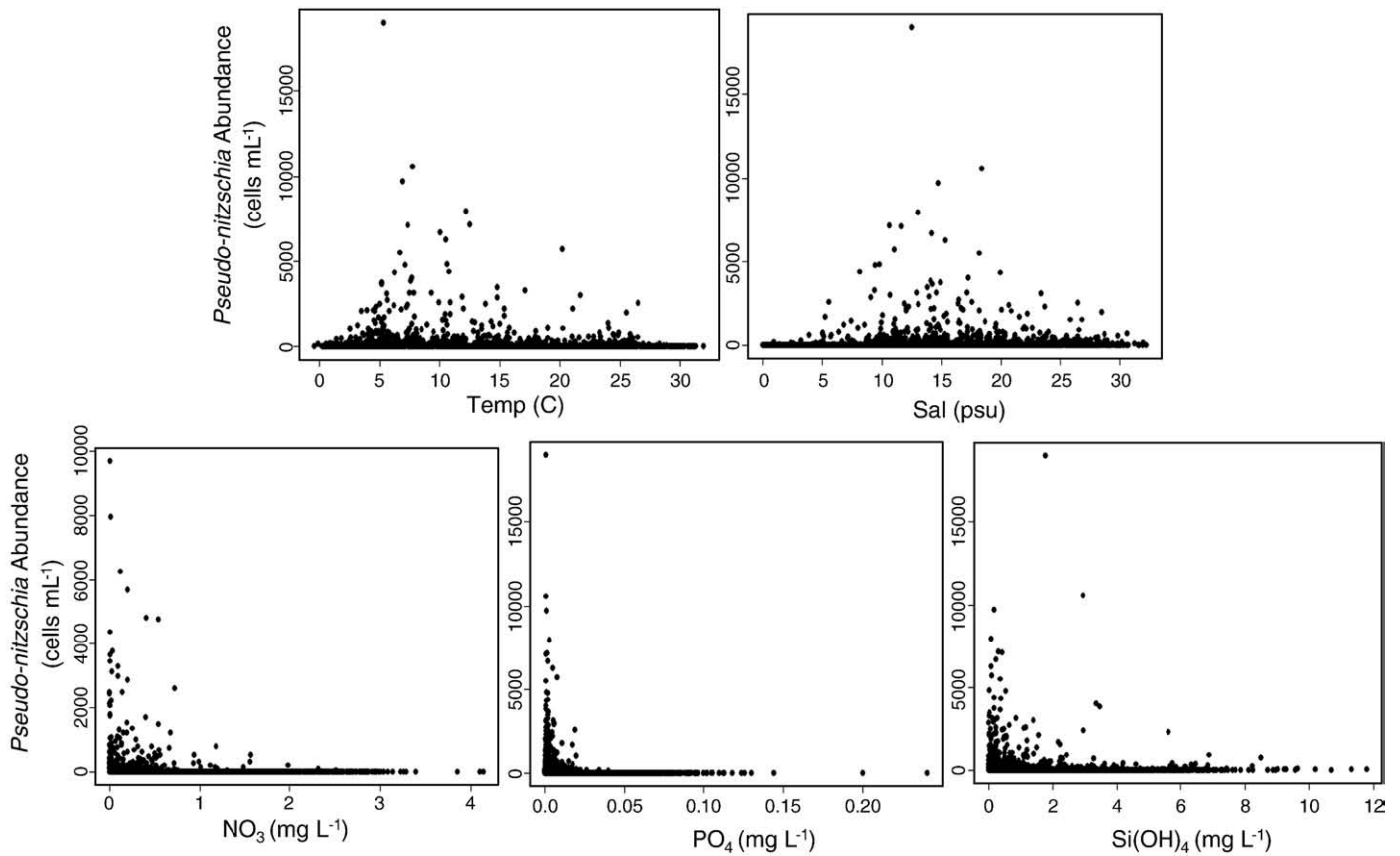


Fig. 3. Plots of the relationship between *Pseudo-nitzschia* abundance (cells mL⁻¹) and several relevant physical and chemical variables from Table 3: temperature (C), salinity, NO₃ (mg L⁻¹), PO₄ (mg L⁻¹), and Si(OH)₄ (mg L⁻¹).

interpolated according to the same grid that is used in the Chesapeake Bay Regional Ocean Modeling System (ChesROMS) model (see Section 5.2.1) over that time period (Fig. 6). Modeled hindcasts of monthly averaged surface PO₄, temperature, and NO₃ + NO₂ were paired with latitude, longitude, and month index to calculate bloom probabilities at 4.5-km² horizontal resolution according to Eqs. (8) and (2). Fig. 6 shows two of these bloom hindcast maps: a large bloom event in March 1998 (see also Figs. 2 and 3) and a non-bloom event in August 2005 when there were no *Pseudo-nitzschia* cells recorded in the phytoplankton dataset.

Although the hindcasts are generated from monthly means of the environmental parameters, the distribution of observations above the 10 cells mL⁻¹ bloom threshold in March 1998 is reasonably well-captured by the GLM which assigns probabilities between 70 and 80% to the cluster of observations at or above 1000 cells mL⁻¹ in the lower Bay (Fig. 6A). For one bloom observation in the middle mainstem region of the Bay (CB5.1 = 4770 cell mL⁻¹), the GLM assigned a probability just above the optimized 19% prediction point for designating a potentially harmful bloom (Fig. 6A), thus correctly flagging this zone yet underestimating bloom magnitude by assigning fairly low probabilities. Conversely, generally low probabilities were predicted throughout the Bay during the non-bloom event of August 2005, reflecting the absence of *Pseudo-nitzschia* in the cell abundance

records for that month (Fig. 6B). The 19% prediction point appears to cause bloom overprediction in this instance given that much of the lower Bay would have been flagged for a bloom using this threshold. However, in the absence of observations near the mouths of the James and York Rivers, it is not possible to validate the higher probabilities ~50% in that zone.

Future validation will require skill scores computed from concomitant observation-prediction matchups rather than monthly means which cannot capture the high-frequency, temporal variability of bloom dynamics. For a subset of stations, model hindcasted probabilities are compared with monthly averaged observations for the period 1990 to 2006 (Fig. 7) to roughly ascertain differences in model performance across zones. At a consistently freshwater site such as CB2.1 at the mouth of the Susquehanna River (Fig. 1) where no *Pseudo-nitzschia* are on record ($n_{\text{matchups}} = 187$), the model always correctly predicts low probabilities, far less than the 19% bloom prediction point (Fig. 7A). At a Choptank River tributary station (ET5.2) where blooms do occur but are rare, the model exhibited a tendency to underestimate blooms in this zone by assigning probabilities above the 19% prediction point to only 36% of bloom cases (>10 cells mL⁻¹) but did assign low probabilities to 89% of non-blooms (Fig. 7B). This false negative rate is consistent with the 9% POFD calculated for the GLM (Table 6). Conversely, the model

Table 4

Best-fit logistic (logit) algorithms developed over a range of bloom thresholds relevant for toxin production by different *Pseudo-nitzschia* species (see Section 3.1). Variability in model performance is given by the maximum Heidke Skill Score (HSS) and model fit by the Nagelkerke's R²; bloom probability (P_{bloom}) is a function of the GLM logit.

Bloom threshold (cells mL ⁻¹)	Best-fit logistic GLM $P_{\text{bloom}} = e^{\text{logit}} / [e^{\text{logit}} + 1]$	DF	Maximum HSS	Nagelkerke's R ²
10	logit = 125 - 1.65[Lat] - 70.4[PO ₄] - 0.059[Temp] - 0.927[(NO ₃ + NO ₂)] + 0.822[Lon] - 0.065[Month] - 0.00002[Dis]	6682	0.53	0.22
100	logit = -2.08 - 59.7[PO ₄] + 0.112[Sal] - 0.091[Temp] + 0.339[DOC] - 0.724[Si(OH) ₄] - 0.00002[Dis] - 0.088[Month] - 32.8[NO ₂]	5111	0.40	0.10
1000	logit = 75.4 - 130[PO ₄] - 0.659[Si(OH) ₄] - 0.107[Temp] - 2.78[(NO ₃ + NO ₂)] - 0.738[Secchi] - 1.93[Lat] - 0.133[Sal]	6694	0.25	0.04

Table 5

Chi-square distribution ANOVA: model coefficients and analysis of deviance/likelihood ratio test for the 10 cells mL⁻¹ threshold GLM (Table 4, $DF=6$) after removal of the discharge parameter (Nagelkerke's $R^2=0.23$). Additional performance metrics for this model are illustrated in Table 5 and Figs. 4–7.

Predictor	Coefficients	Standard error	p-val (Chi)
Intercept	128	15.6	
Lat	−1.60	0.100	<<0.001
PO ₄	−68.1	9.23	<<0.001
Temp	−0.06	0.007	<<0.001
NO ₃ + NO ₂	−1.08	0.200	<<0.001
Lon	0.878	0.223	<<0.001
Month	−0.064	0.018	<0.001

tended to overestimate blooms at the mainstem lower Bay station CB6.1: for those observations below 1000 cells mL⁻¹, the FAR is 33% with respect to the 19% prediction point (Fig. 7C). It is important to note, however, that this false positive rate is significantly lower than the overall model FAR of 52% (Table 6), reflecting the model's tendency to assign high probabilities to the largest blooms in the meso- to polyhaline transition zone.

5. Discussion

Much deserved attention has been given in recent decades to studying the potential influences of cultural nutrient enrichment (eutrophication) on HAB ecology (e.g. reviews by Anderson et al., 2002, 2008; Heisler et al., 2008; Glibert et al., 2010—this issue). While most HAB species fall within various flagellate and cyanobacterial phylogenies, the pennate diatom genus, *Pseudo-nitzschia*, is unique in being the only toxigenic genus of diatoms, the group classically used as the counterpoint in descriptions of HAB/flagellate ecophysiology (Smyda, 1997). As a result, our understanding of how a HAB-forming diatom like *Pseudo-nitzschia* will respond to land-use change and fluxes of both organic and inorganic nutrients in a complex estuarine setting is in its nascent stage. In an evaluation of Chesapeake Bay phytoplankton community composition, Marshall et al. (2005) referenced a post-European settlement shift from diatom assemblages dominated by benthic, pennate diatoms to more planktonic, centric species (Cooper and Brush, 1991), a general decrease in diatoms associated with increased anoxia and P loading (Brush and Davis, 1984), and a shift towards more lightly silicified diatom species. In contrast, the most modern surveys also document a dramatic increase in the magnitude of surface diatom abundance that coincides with an overall biomass enhancement and seasonal fluctuations of potentially toxic phytoplankton in the Bay and its tributaries (Marshall et al., 2005).

According to the time series analyzed in this study, there seems to be an increase in both the abundance and frequency of *Pseudo-nitzschia* blooms, particularly since the 1990s (Fig. 2), though sampling at many stations also increased during that decade. Although several large blooms were recorded in the lower Bay during the 1980s and 1990s, blooms at the mouths of major tributaries such as the Potomac (LE2.2), Choptank (ET5.2), and Patuxent Rivers (LE1.3) are relatively small and infrequent prior to 1997. After that time, the lower Bay and tidal

Table 6

Contingency table for the outcomes shown in Fig. 5 for the GLM in Table 5 based on a default bloom prediction point of 0.50 versus an optimized prediction point of 0.19. The Probability of Detection (POD), False Alarm Ratio (FAR), and the Probability of False Detection (POFD) are optimized relative to the upper 10% of Heidke Skill Score (HSS) values; see Section 3.2 for definitions. Performance metrics represent the mean for the ensemble of 22 cross-validation experiments.

	HSS	POD	FAR	POFD
Absent if $P_{\text{bloom}} < 0.50$	0.38	0.34	0.44	0.03
Absent if $P_{\text{bloom}} < 0.19$	0.53	0.75	0.52	0.09
Cross-validation $P_{\text{bloom}} = 0.19$	0.53	0.75	0.52	0.09

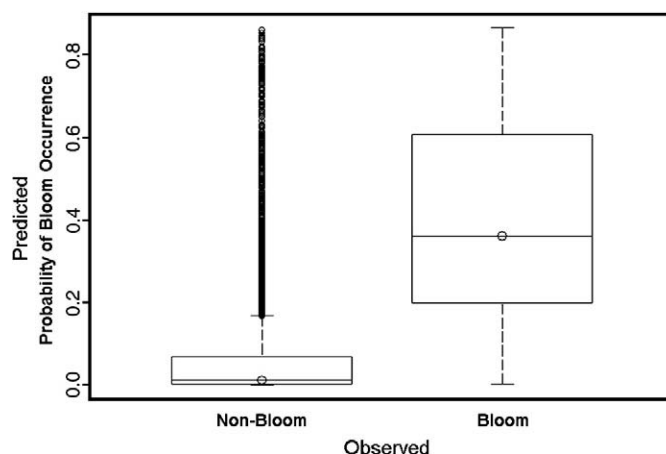


Fig. 4. Performance of the final GLM (Table 5 Eq. (8)) presented as a boxplot comparing all bloom ($n=668$) and non-bloom observations ($n=6,106$) with modeled probabilities, where the threshold for a “bloom” is 10 cells mL⁻¹. Horizontal lines are median probabilities ($\text{prob}_{\text{non-bloom}}=0.01$, $\text{prob}_{\text{bloom}}=0.36$); boxes are 25th and 75th percentiles and whiskers are 5th and 95th percentiles. Individual open circles beyond the whiskers represent probabilities outside that range, i.e. outliers ($n=614$).

estuaries appear to become synchronized in terms of bloom timing, but not necessarily intensity (Fig. 2). This potential lag between the lower Bay stations (CB6.1, CB6.4, CB7.3E, CB7.4) and the middle/upper Bay over the study period could signify the introduction of oceanic seeding populations transported through the mouth of the Bay northward into the estuary proper (Marshall et al., 2005) where they may persist at depth until an upwelling or mixing event occurs and brings them to the surface (Garrison, 1981; Mengelt, 2006). Alternatively, the observed relationship could suggest an evolution of conditions desirable for *Pseudo-nitzschia* blooms, such as eutrophication, in the mesohaline midstem portion of the Bay beginning in the late 1990s (Glibert et al., 2001; Marshall et al., 2005). Unfortunately, the discontinuous data at many stations and often event-response nature of sampling precludes a reliable trend analysis. On-going time-series analyses using spatio-temporal kriging of the *Pseudo-nitzschia* abundance data should allow for future identification of such trends and comparison with larger-scale climate indices. While further analysis of existing data is clearly required to statistically establish any increasing trend in *Pseudo-nitzschia* abundance, it is possible with the models presented in the current

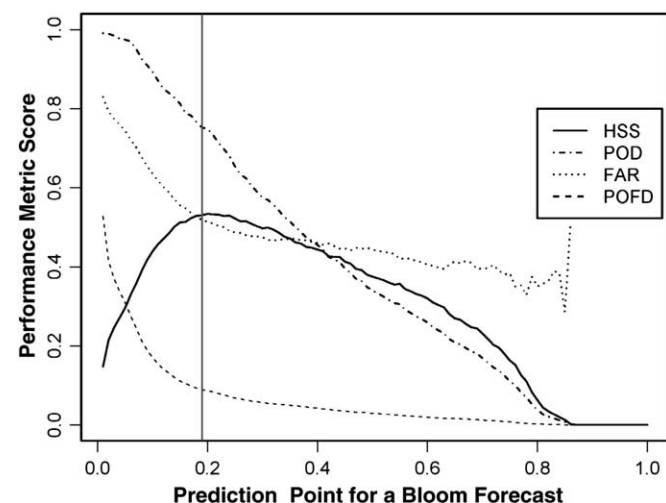


Fig. 5. Optimization of the prediction point (expressed as a decimal fraction) for determining a “bloom” forecast. The Heidke Skill Score (HSS) is optimized with respect to the Probability of Detection (POD), False Alarm Ratio (FAR), and the Probability of False Detection (POFD). The vertical line indicates the optimized prediction point = 0.19, (see Tables 5 and 6).

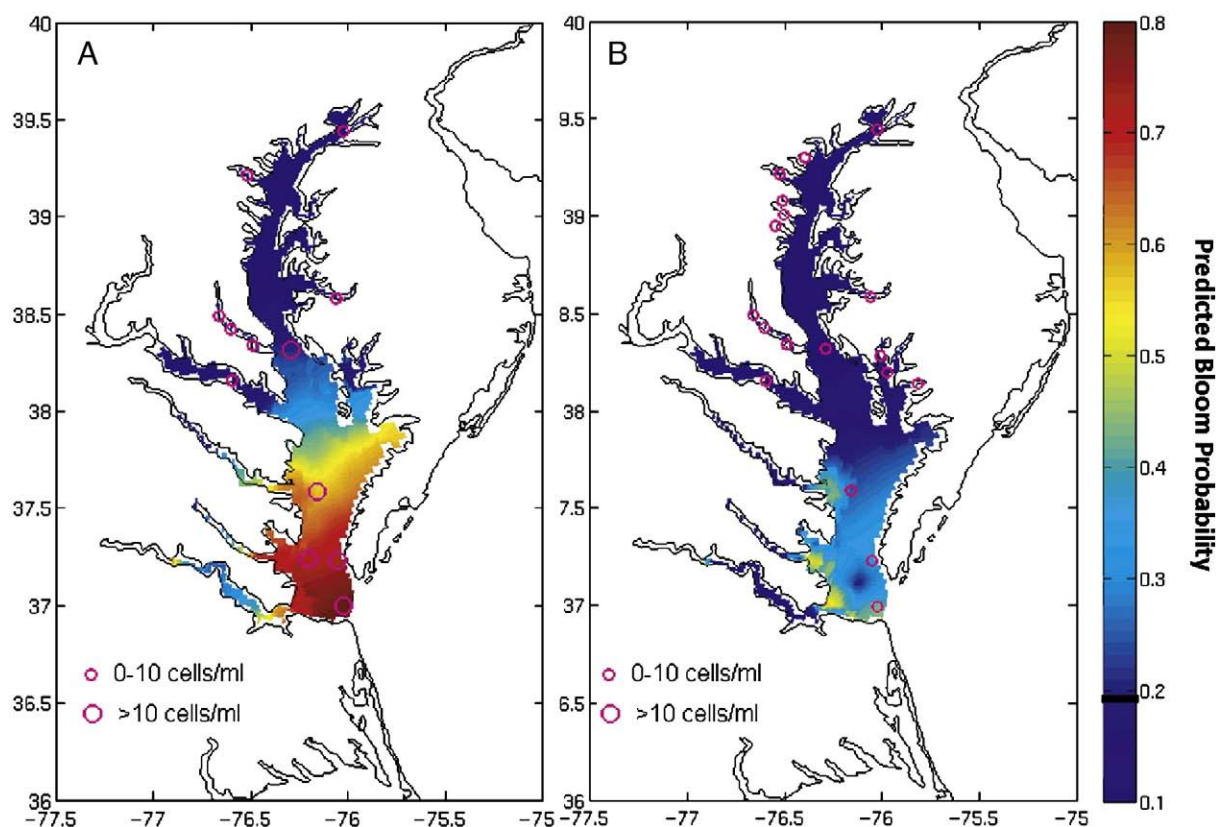


Fig. 6. Hindcast maps represent monthly means of model-predicted probabilities (colorbar) and observations (open circles) of *Pseudo-nitzschia* blooms defined by the 10 cells mL⁻¹ threshold and 19% prediction point (dark line on colorbar) for two time points in the Chesapeake Bay over the study period: A) March 1998 and B) August 2005. Predictions of bloom probabilities are generated by forcing the final GLM (Eq. (8)) with spatially interpolated estimates of observed environmental variables for the period 1990–2006 (Section 4.2.3).

study (Table 4) to identify some of the potential physico-chemical drivers of *Pseudo-nitzschia* blooms in the Bay.

5.1. Potential drivers of *Pseudo-nitzschia* blooms in the bay

In all three logistic GLMs, there exist inverse relationships between nutrient parameters and the *Pseudo-nitzschia* bloom index, invoking both concurrent surface nutrient uptake and even the potential onset of nutrient limitation as *Pseudo-nitzschia* cell abundance increases. It is important to note that these relationships to nutrient concentrations are not any more robust when lagged relative to bloom occurrence. Moreover, a meaningful lag time for correlation of the phytoplankton response to nutrient supply is expected to be less than the average monthly sampling frequency available here because of the relatively short timescales for nutrient uptake. Inorganic PO₄ is the most significant nutrient predictor in all GLMs (Table 4). In spring, when the majority of these blooms occur, the Chesapeake Bay is indeed more limited by PO₄ relative to NO₃ (Prasad et al., in press). However, in the absence of direct measurements using nutrient limitation assays, it is not possible to assess the physiological status of the phytoplankton community at the time of sampling. While *P*-limitation is certainly not an initiator of bloom formation, which of course requires sufficient *P* availability for cell division, low concentrations in tandem with high cell abundances are captured by the models, indicating surface depletion of PO₄ at the very least. We note that the onset of *P*-limitation has been shown to stimulate DA production in laboratory cultures (Pan et al., 1998). Unfortunately, the models in the current study cannot address the effects of nutrient drawdown on DA production in the Bay.

As with other *Pseudo-nitzschia* habitat models for the west coast of the U.S.A. (Blum et al., 2006; Anderson et al., 2009; Lane et al., 2009), this

nutrient limitation pattern is supported by the GLM results where a significant negative relationship between *Pseudo-nitzschia* blooms and Si(OH)₄ concentrations could indicate a lower Si-requirement for this diatom group. This may suggest a link between runoff-induced reductions in Si:N and Si:P ratios (Smayda, 1990, 1997) and the occurrence of these blooms. The inverse relationship between blooms ≥ 100 cells mL⁻¹ and Si(OH)₄ in the medium-threshold GLM (Table 4) may be related to the mere drawdown of Si(OH)₄. Alternatively, it may reflect the previously observed association between *Pseudo-nitzschia* blooms (*P. multiseriata* and *P. australis*) and the onset of Si-limitation in other regions and experiments where *Pseudo-nitzschia* may be a strong competitor in relatively Si-depleted waters following a large diatom bloom (Sommer, 1994; Marchetti et al., 2004; Anderson et al., 2006; Lane et al., 2009). Perhaps in part due to their (relatively) lightly silicified frustules, *Pseudo-nitzschia* cells seem better adapted to low-Si environments than many other diatoms, often outcompeting other groups during the late stages of a spring diatom bloom when surface waters have been fairly depleted of Si (Anderson et al., 2006; Kudela et al., 2004; Marchetti et al., 2004). However, like all diatoms, *Pseudo-nitzschia* still requires Si, N, and P for maximal growth and will bloom in response to these inputs, particularly during winter and spring months when turbulence and reduced light levels in the water column tend to favor diatoms over flagellates (Margalef, 1978; Smayda, 1997).

A negative relationship with NO₃ + NO₂ availability for the small and large bloom thresholds suggests that N-uptake by surface blooms leads to depletion of both the NO₃ and NO₂ pools, rather than to the NO₂ regeneration that is expected during more N-replete conditions (Lomas et al., 2000; Bode et al., 2002). This association with a reduced NO₂ pool is also seen in the inclusion of the NO₂ parameter in the GLM for blooms ≥ 100 cells mL⁻¹. Rapid NO₃ and NO₂ utilization may be somewhat inconsistent with evidence of a positive association

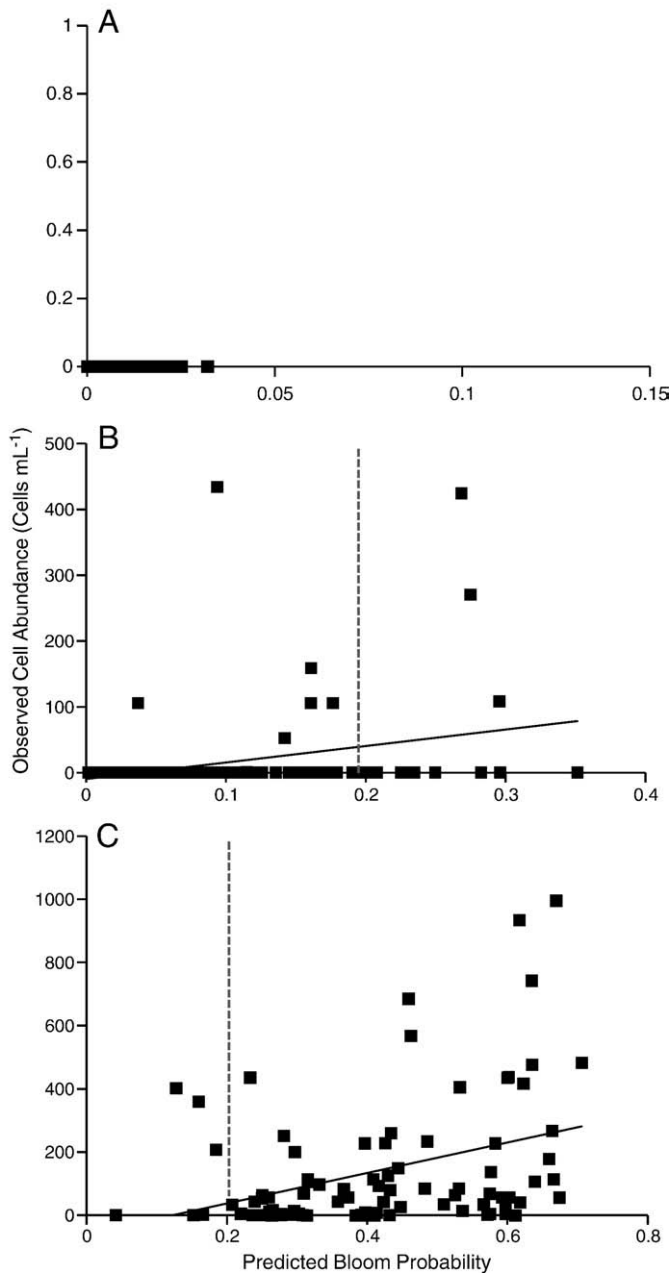


Fig. 7. Observed cell abundance vs. model-predicted probabilities for a *Pseudo-nitzschia* bloom projected over the period 1990–2006 at stations A) CB21, Susquehanna R., B) ET5.2, Choptank R. ($r^2 = 0.10$), and C) CB6, lower mainstem Bay ($r^2 = 0.14$). Trendlines (solid) were determined by linear regression, and the vertical lines (dashed) mark the 0.19 bloom prediction point.

between NO_3 , possibly from runoff, and fall *Pseudo-nitzschia* blooms in the Monterey Bay, CA (Lane et al., 2009). However, the same caveat remains that a negative relationship between a nutrient parameter and the bloom index may reflect more the effects of nutrient drawdown by actively growing phytoplankton rather than suggesting the possible onset of conditions that favor one phytoplankton group over another.

Several studies have now effectively demonstrated the uptake of regenerated forms of nitrogen — ammonium (NH_4), and urea — by *Pseudo-nitzschia* species (Howard et al., 2007; Kudela et al., 2008; Cochlan et al., 2008; Thessen et al., 2009). An increase in the potential for DA production has been noted when the organism incorporates organic N or NH_4 instead of NO_3 (Howard et al., 2007; Thessen et al., 2009). While NH_4 is not a significant explanatory variable for blooms

in the Chesapeake Bay GLMs, it is significantly associated with *Pseudo-nitzschia* abundance in this dataset (Table 3) and could be useful for predicting the future probabilities of toxic events. Because of the salinity tolerance limits for *Pseudo-nitzschia* shown in this study (Fig. 3) and elsewhere (Thessen et al., 2005; Thessen and Stoecker, 2008), blooms are expected to be extremely rare and forced predominantly by salinity anomalies (see Section 5.2) in the upper, fresh reaches (salinity < 5) of tributaries where nutrient enrichment from runoff is high. However, this enrichment is manifest in the meso- and polyhaline mainstem portions of the Bay as well (Prasad et al., in press). Given that total N from the Susquehanna River, a major tributary of the Chesapeake Bay, increased 2.5-fold from 1945 to 1990 (Kemp et al., 2005) and that urea usage in agriculture and industry is increasing worldwide (Glibert et al., 2005), the potential role of NO_3 and urban forms of N runoff in driving *Pseudo-nitzschia* blooms and perhaps even toxin production should not be ignored, particularly in light of the projected increases in precipitation and population indices for the region (Meehl et al., 2007).

The inclusion of DOC as a significant predictor in the medium-threshold GLM is particularly interesting given its connection to riverine inputs (e.g. Doering et al., 1994; McKenna, 2004) and the previously observed association between *Pseudo-nitzschia* blooms and river discharge or potential proxies of runoff on the west coast (Kudela et al., 2004; Anderson et al., 2009; Lane et al., 2009) where *P. multiseriata* is a dominant bloom former. Glibert et al. (2001) found strong correlations between DOC and DOC:DON in relation to harmful *P. minimum* populations in the Bay and underscored the difficulty in ascribing direct or indirect effects to this relationship. One possible indirect effect is the potential for covariation between DOC and DON if *Pseudo-nitzschia* cells are directly taking up organic N species during blooms as described above. There is some evidence for the uptake of organic substrates by *Pseudo-nitzschia* after dark adaptation (Mengelt, 2006), but the possibility of interference by extra-cellular or epiphytic bacteria remains (Bates et al., 2004; Stewart, 2008). Studies on the west coast also implicate some kind of allochthonous component to *Pseudo-nitzschia* blooms from either direct correlation between blooms and rain events (Trainer et al., 2000) or via the inclusion of chromophoric dissolved organic matter and precipitation/ NO_3 relationships in their habitat models (Anderson et al., 2009; Lane et al., 2009). Provided there is a causal relationship here, a positive influence of DOC on *Pseudo-nitzschia* blooms implies the potential for future changes as precipitation events over the Bay increase in frequency and/or intensity according to current IPCC projections (Meehl et al., 2007). However, the weak correlation between DOC and freshwater discharge ($r = -0.08$, Table 3) and the negative association with discharge in the small- and medium-threshold GLMs (Table 4) may indicate a more autochthonous source of DOC and/or an association between *Pseudo-nitzschia* blooms and low runoff periods or drier years. Understanding this relationship between DOC, discharge, and *Pseudo-nitzschia* blooms clearly requires more targeted studies.

The complex nature of environmental change may have opposing effects on *Pseudo-nitzschia* bloom distributions. Eutrophication is not only manifest in the bottom-up direction with respect to HABs, but is also expected to influence the entire ecosystem with the net result being more favorable conditions for HAB development in general. The timing of nutrient enrichment, for instance, may be de-coupled from predator population increases, resulting in the release of grazing pressure which can be further enhanced by a build-up in anoxia as the bloom decomposes (e.g. Buskey et al., 1997). The present study, however, found no significant relationship between chlorophyll and *Pseudo-nitzschia* abundance for the Chesapeake Bay (Table 3), suggesting a possible de-coupling of these blooms from the overall rise and fall of phytoplankton biomass. The significant positive relationship with salinity and negative relationship with temperature in the GLMs developed for small- and medium- thresholds implies physical tolerance limits and seasonality that are expected for this

diatom group and have been observed for *Pseudo-nitzschia* in the Chesapeake Bay where high cell abundances occur with colder, relatively salty waters (Table 4) (Pan et al., 1993; Thessen and Stoecker, 2008). In contrast, the weakly significant negative association with salinity in the GLM for the largest, rarest blooms is not easily explained but may be due to the fact that the majority of very large blooms fall within the 10–15 salinity range (Fig. 3). Another possibility is that since some large blooms do occur at lower salinities in the lower tidal tributary estuarine zones, two modes of bloom formation might exist in the Bay: one stemming from vertical mixing where high salinities, low temperatures, and high nutrient concentrations are in phase, and a second mode where river runoff is associated with lower salinities and high nutrients. Given the sum of these relationships, expected increases in precipitation for the region could restrict *Pseudo-nitzschia* blooms to a smaller portion of the Bay if the size of the meso- and polyhaline zones were to contract, although subsurface blooms are still probable, particularly in the lower Bay.

5.2. Performance of the *Pseudo-nitzschia* bloom GLM

Model results presented here for *Pseudo-nitzschia* blooms in the Chesapeake Bay join the growing effort to create and validate empirical habitat suitability models for species or groups of biogeochemically important or potentially deleterious pathogens, microbes, zoonotic diseases, and planktonic organisms (e.g. Iglesias-Rodriguez et al., 2002; Blauw et al., 2006; Decker et al., 2007; Patz et al., 2008; Constantin de Magny et al., 2008). An inherent constraint of these ecological forecasting models is the explanatory power of the available predictor variables, which in this study were limited to a standard array of hydrographic properties and chemical constituents from long-term water quality monitoring programs in an ecologically complex estuarine system. This complexity presents challenges for forecasting biological processes in the absence of strong physiological data to support mechanistic modeling. Great strides have been made in this direction for at least one group of HABs in the Gulf of Maine (McGillicuddy et al., 2003, 2005). However, pressing human health issues drive the need for statistical methods that take advantage of data currently available to glean process and predictive understanding as well as build models for a variety of HABs and toxic agents (Moore et al., 2008). To that end, the approach described in this paper marks the first such attempt for the *Pseudo-nitzschia* genus on the U.S. eastern seaboard.

Predictive success for the GLM is dependent on (1) the chosen threshold for defining a ‘bloom,’ and in creating the binary variable for the logistic regression (Table 4); (2) determining a relevant prediction point for forecasting blooms from model output (Fig. 5); and (3) the location along the Bay’s salinity gradient (Fig. 7). As the bloom threshold increases, bloom events decrease in frequency and model skill is reduced, as can be seen when the bloom threshold increases from 10 to 1000 cells mL⁻¹ (Table 4). Use of a single threshold for the final model (10 cells mL⁻¹ GLM; Tables 5 and 6; Figs. 5–7) then assumes that all resident species of *Pseudo-nitzschia* will bloom in response to similar ecological conditions or occupy the same niche space (Smayda, 1963; Margalef, 1978; Bidigare et al., 1990). However, since such a large proportion of resident species are potentially toxigenic and were only included in the analysis when collected from surface samples, it is reasonable to assume they are similarly adapted or that a given sample is dominated by only one or two *Pseudo-nitzschia* species most adapted to the ambient conditions (Smayda, 1963; Margalef, 1978; Bidigare et al., 1990). This implies wider utility in a model that predicts *Pseudo-nitzschia* blooms from a relatively low threshold (10 cells mL⁻¹). We note that the likelihood of DA production at or just above this density begins to increase (on the U.S. west coast) for the toxigenic *P. fraudulenta* group and for other members of the indigenous toxigenic *Pseudo-nitzschia* assemblage (*P. pungens*, *P. calliantha*, *P. cuspidata*, *P. multiseries*; Thessen and Stoecker, 2008) at even higher densities (Trainer and

Suddleson, 2005). The important caveat still remains that genetic diversity at the species and strain level can strongly regulate toxicity and cannot be accounted for in the generic GLM (Orsini et al., 2004; Evan et al., 2005; Erdner et al., 2008; Thessen et al., 2009). Any niche-space flexibility offered by such diversity introduces further complications for predicting *Pseudo-nitzschia* distributions based on habitat suitability models.

The optimization method for choosing a prediction point (Fig. 5) allows the user to adjust the operational probability according to the relative weightings of the various performance metrics. In any operational or routine monitoring context, probability maps of *Pseudo-nitzschia* blooms must be presented with annotated instructions for interpretation of these contingencies. The applicability of our optimized prediction point of 19% varies with location and date in the Bay according to our hindcasts, and without future validation and tuning, may prove to overestimate blooms in the mesohaline mainstem and underestimate the rare bloom events that tend to occur in tidal tributary zones (Figs. 6 and 7). These differences in predictability in different portions of the Bay might then be influenced by the uneven distribution of observations in the dataset with respect to salinity since much of the data used for model development fall in zones fresher than the salinity tolerance for *Pseudo-nitzschia*.

To account for any bias in model performance caused by increased skill in oligohaline regions where few blooms occur (see Fig. 7A), we tested separate models for the three different salinity zones. The results (not shown) do not display improvement in model skill for any individual zone but do, in fact, exhibit an expected decrease in skill (max HSS = 0.48) compared with our final GLM when salinities <5 are excluded from model development. It turns out to be difficult to correctly predict blooms in the oligohaline zone alone (max HSS = 0.18) because of the low frequency of bloom observations below a salinity of 5 (i.e. rare events). Salinity is indeed an important factor selecting for *Pseudo-nitzschia* blooms in the Bay, and this is accounted for in development of the final GLM (Eq. (8)) which considers salinity and location (but not an interaction term). By using all available observations, we have tuned model sensitivity at various thresholds (Table 4) to those conditions suitable for blooms as well as for the infrequent salinity anomalies in fresher zones that lead to rare bloom events in tributaries. However, future validation efforts may support the use of separate models for the different thresholds and salinity zones based on location or time of year.

Relative to *Pseudo-nitzschia*-specific predictive models for the west coast, our final 10 cells mL⁻¹ GLM in Table 5 performs comparably in its ability to correctly predict blooms for the Chesapeake Bay with a 75% probability of detection (cf. 75–89%) and a 52% false positive rate (cf. 25–62%) (Anderson et al., 2009; Lane et al., 2009). In terms of error type, minimizing the POFD over FAR is arguably more desirable in the context of protecting human health since the perceived or actual damage from missing a potential health threat may be deemed worse than a false alarm scenario. In that sense, this model performs quite well at the optimized bloom prediction point. The relatively low value of Nagelkerke’s $R^2 \sim 0.23$ (Table 5) brings into question the influence of environmental variables absent from this analysis, in particular, measures of organic nutrients and trace metals. Use of these models by resource managers will ultimately require a keen understanding of model uncertainty (see Fig. 5) along with an adaptive strategy for implementation that takes into account any model and threshold sensitivity within different zones of the Bay as well as emerging data on the risks of both acute and chronic exposure to DA toxins (Erdner et al., 2008).

5.2.1. Forecasting *Pseudo-nitzschia* blooms

Producing nowcasts or forecasts of *Pseudo-nitzschia* blooms in the Bay would be facilitated by assimilation of environmental predictions from numerical models into the diagnostic GLM. This is possible in conjunction with the Chesapeake Bay Forecast System (CBFS) which

currently uses dynamical downscaling with the Weather Research and Forecasting Model (WRF, 2010) to provide high resolution forcing of the ChesROMS ocean model and Soil and Water Assessment Tool (SWAT, 2010) river basin-scale model (Murtugudde, 2009). The forecast is generated every three days using a six to eight member ensemble forced with the National Center for Environmental Prediction – Global Ensemble System (NCEP GENS Products, 2008) and can provide accurate estimates of sea surface temperature and salinity (used only in higher threshold GLMs). The ChesROMS model is currently being tuned with an embedded Nitrogen–Phytoplankton–Zooplankton–Detritus (NPZD) ecosystem model for generating forecasts of macronutrient and chlorophyll concentrations, including a recently implemented DON component (Fennel et al., 2006). Together, the ChesROMS and NPZD models will be able to predict the six explanatory variables used in the final GLM (Table 5). However, use of higher-threshold GLMs (Table 4) will require estimates of $\text{Si}(\text{OH})_4$ and DOC which are not predicted from the ecosystem model currently coupled to ChesROMS. Present research involves diagnostic coupling of alternative ecosystem models to ChesROMS that include $\text{Si}(\text{OH})_4$ in their formulation (e.g. Moore et al., 2002; Chai et al., 2002). Unfortunately, it may not be possible to generate accurate DOC predictions from existing models which at best include a “small detritus” carbon fraction (Christian et al., 2002; Moore et al., 2002), in which case, alternative GLMs may prove more practical for making *Pseudo-nitzschia* bloom nowcasts and forecasts.

6. Concluding remarks

This modeling exercise identifies several environmental variables associated with *Pseudo-nitzschia* blooms in the Chesapeake Bay but unfortunately cannot identify environmental indicators of the presence of its associated toxic agent, DA. Indeed, defining the distinction between factors that initiate bloom development and those that trigger toxin production is a recurring theme in HAB ecology, particularly for *Pseudo-nitzschia*. The issue of toxigenesis is highly relevant in a region such as the Chesapeake Bay which, to date, has experienced no known outbreaks of DA poisoning but whose populations may still be affected by chronic exposure to moderate toxin levels (Grattan et al., 2007; Thessen and Stoecker, 2008). Forecasting endeavors that follow should not only relate these uncertainties to end-users but also continually revisit bloom thresholds and model formulation with the addition of new validation data and input from resource managers and shellfish harvesters alike. Lastly, future efforts to model HABs must look closely at the combined effects of inorganic and organic nutrient uptake, trace metal utilization (e.g. Rue and Bruland, 2001; Maldonado et al., 2002; Wells et al., 2005), allelopathic competition (e.g. Adolf et al., 2008), and grazing (e.g. Bargu et al., 2003) to move beyond statistics-based habitat models and incorporate a mechanistic understanding of the complex processes regulating HAB development and toxicity.

Acknowledgements

This study was funded by a National Research Council postdoctoral fellowship to C.R.A., sponsored by the NOAA National Environmental Satellite, Data, and Information Service (NESDIS) with additional support from NOAA MERHAB Award (PI: C.W.B.) and the Chesapeake Bay Forecasting System project (PI: R.M.). The authors gratefully acknowledge the Maryland Department of Natural Resources and USEPA Chesapeake Bay Program for contributing monitoring data, and we are especially appreciative of the technical assistance provided by J. Johnson, B. Cole, M. Naylor, W. Butler, B. Wind, M. Baith, and J. Beauchamps. J. Lane, D. McGillicuddy, and several anonymous reviewers provided comments which greatly improved the paper. We give special thanks to A. Thessen for her innovative work on *Pseudo-nitzschia* ecophysiology in the Chesapeake Bay and for significant input to this manuscript.

References

- Adolf, J.E., Bachvaroff, T., Place, A.R., 2008. Cryptophyte abundance drives blooms of mixotrophic harmful algae: a hypothesis based on *Karlodinium veneticum* as a model system. *Harmful Algae* 8, 3–13.
- Agresti, A., 2007. An introduction to categorical data analysis. Wiley-Interscience, John Wiley and Sons, Inc.
- Allen, J.I., Smyth, T.J., Siddorn, J.R., Holt, M., 2008. How well can we forecast high biomass algal events in a eutrophic coastal sea? *Harmful Algae* 8, 70–76.
- Anderson, D.M., Glibert, P.M., Burkholder, J.M., 2002. Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. *Estuaries* 25 (4B), 704–726.
- Anderson, C.R., Brzezinski, M.A., Washburn, L., Kudela, R., 2006. Circulation and environmental conditions during a toxigenic *Pseudo-nitzschia australis* bloom in the Santa Barbara Channel, California. *Mar. Ecol. Prog. Ser.* 327, 119–133.
- Anderson, D.M., Burkholder, J.M., Cochlan, W.P., Glibert, P.M., Gobler, C.J., Heil, C.A., Kudela, R.M., Parsons, M.L., Rensel, J.E.J., Townsend, D.W., Trainer, V.L., Vargo, G.A., 2008. Harmful algal blooms and eutrophication: examining linkages from selected coastal regions of the United States. *Harmful Algae* 8, 39–53.
- Anderson, C.R., Seigel, D.A., Kudela, R., Brzezinski, M.A., 2009. Empirical models of toxigenic *Pseudo-nitzschia* blooms: potential use as a remote detection tool in the Santa Barbara Channel. *Harmful Algae* 8, 478–492.
- Bargu, S., Marinovic, B., Mansergh, S., Silver, M.W., 2003. Feeding responses of krill to the toxin-producing diatom *Pseudo-nitzschia*. *J. Exp. Mar. Biol. Ecol.* 284, 87–104.
- Bates, S.S., Trainer, V.L., 2006. The ecology of harmful diatoms. In: Graneli, E., Turner, J.T. (Eds.), *Ecology of Harmful Algae*. Springer-Verlag, Berlin, p. 440.
- Bates, S.S., Garrison, D.L., Horner, R.A., 1998. Bloom dynamics and physiology of domoic acid-producing *Pseudo-nitzschia* species. In: Anderson, D.M., Cembella, A.D., Hallegraeff, G.M. (Eds.), *Physiological Ecology of Harmful Algal Blooms*. Springer-Verlag, Heidelberg, p. 267.
- Bates, S.S., Gaudet, J., Kaczmarek, I., Ehrman, J.M., 2004. Interaction between bacteria and the domoic acid producing diatom *Pseudo-nitzschia multiseries* (Hasle) Hasle: can bacteria produce DA autonomously? *Harmful Algae* 3, 11–20.
- Bidigare, R.R., Marra, J., Dickey, T.D., Iturriaga, R., Baker, K.S., Smith, R.C., Pak, H., 1990. Evidence for phytoplankton succession and chromatic adaptation in the Sargasso Sea during spring 1985. *Mar. Ecol. Prog. Ser.* 60, 113–122.
- Blaauw, A.N., Anderson, P., Estrada, M., Johansen, M., Laanemets, J., Peperzak, L., Purdie, D., Raine, R., Vahtre, E., 2006. The use of fuzzy logic models for data analysis and modelling of European harmful algal blooms: results of the HABES project. *Afr. J. Mar. Sci.* 28 (2), 365–369.
- Blum, I., Subba Rao, D.V., Pan, Y., Swaminathan, S., Adams, N.G., 2006. Development of statistical models for prediction of the neurotoxin DA levels in the pennate diatom *Pseudo-nitzschia pungens* f. *multiseries* utilizing data from cultures and natural blooms. In: Rao, D.V.S. (Ed.), *Algal Cultures: Analogues of Blooms and Applications*. Science Publishers, Enfield, NH, pp. 891–930.
- Bode, A., Castro, C.G., Doval, M.D., Varela, M., 2002. New and regenerated production and ammonium regeneration in the western Bransfield Strait region (Antarctica) during phytoplankton bloom conditions in summer. *Deep-Sea Res. Part II* 49, 787–804.
- Brown, C., Gross, T., Hood, R., Long, W., Michael, B., Murtugudde, R., Naylor, M., Ramers, D., Tango, P., Wiggert, J., Wilcox, K., Xu, J., 2010. Mapping harmful algal blooms in the Chesapeake Bay. http://155.206.18.162/cbay_hab/.
- Brush, G.A., Davis, F.W., 1984. Stratigraphic evidence of human disturbance in an estuary. *Quatern. Res.* 22, 91–108.
- Buskey, E.J., Montagna, P.A., Amos, A.F., Whitledge, T.E.Q., 1997. Disruption of grazer populations as a contributing factor to the initiation of the Texas brown tide algal bloom. *Limnol. Oceanogr.* 42 (5, part 2), 1215–1222.
- Chai, F., Dugdale, R.C., Peng, T.H., Wilkerson, F.P., Barber, R.T., 2002. One-dimensional ecosystem model of the equatorial Pacific upwelling system. Part I: model development and silicon and nitrogen cycle. *Deep-Sea Res. Part II: Top. Stud. Oceanogr.* 49 (13–14), 2713–2745.
- Chao, Y., Li, Z., Farrara, J.D., Moline, M., Schofield, O.M.E., Majumdar, S.J., 2008. Synergistic applications of autonomous underwater vehicles and regional ocean modeling system in coastal ocean forecasting. *Limnol. Oceanogr.* 53 (5), 2251–2263.
- Christian, J.M., Verschell, M.A., Murtugudde, R., Busalacchi, A., McClain, C., 2002. Biogeochemical modeling of the tropical Pacific Ocean I: seasonal and interannual variability. *Deep-Sea Res. Part II* 49, 509–543.
- Cochlan, W.P., Herndon, J., Kudela, R.M., 2008. Inorganic and organic nitrogen uptake by the toxigenic diatom *Pseudo-nitzschia australis* (Bacillariophyceae). *Harmful Algae* 8, 111–118.
- Constantin de Magny, G., Murtugudde, R., Sapiano, M.R.P., Nizam, A., Brown, C.W., Busalacchi, A.J., Yunus, M., Nair, G.B., Gil, A.I., Lanata, C.F., Calkins, J., Manna, B., Rajendran, K., Bhattacharya, M.K., Hug, A., Sack, R.B., Colwell, R.R., 2008. Environmental signatures associated with cholera epidemics. *PNAS* 105 (46), 17676–17681.
- Constantin de magny, G., Long, W., Brown, C.W., Hood, R.R., Huq, A., Murtugudde, R., Colwell, R.R., 2009. Predicting the Distribution of *Vibrio* in the Chesapeake Bay: A Case Study With *Vibrio cholerae*. *Ecohealth* 6, 378–389.
- Cooper, S.R., Brush, G.S., 1991. Long-term history of Chesapeake Bay anoxia. *Science* 251 (5034), 992–996.
- Davidson, K., Fehling, J., 2006. Modelling the influence of silicon and phosphorus limitation on the growth and toxicity of *Pseudo-nitzschia seriata*. *Afr. J. Mar. Sci.* 28 (2), 357–360.
- Davis, R.E., 1976. Predictability of sea surface temperature and sea level pressure anomalies over the North Pacific Ocean. *J. Phys. Oceanogr.* 6, 249–266.
- Decker, M.B., Brown, C.W., Hood, R.R., Purcell, J.E., Gross, T.F., Matanoski, J.C., Bannon, R.O., Setzler-Hamilton, E.M., 2007. Predicting the distribution of the scyphomedusa *Chrysaora quinquecirrha* in Chesapeake Bay. *Mar. Ecol. Prog. Ser.* 329, 99–113.

- Doering, P.H., Oviatt, C.A., McKenna, J.H., Reed, L.W., 1994. Mixing behavior of dissolved organic carbon and its potential biological significance in the Pawcatuck River Estuary. *Estuaries* 17 (3), 521–536.
- Dortch, Q., Robichaux, R., Pool, S., Milsted, D., Mire, G., Rabalais, N.N., Soniat, T.M., Fryxell, G.A., Turner, J.E., Parsons, M.L., 1997. Abundance and vertical flux of *Pseudo-nitzschia* in the northern Gulf of Mexico. *Mar. Ecol. Prog. Ser.* 146, 249–264.
- Dortch, Q., Parsons, M.L., Doucette, G.J., Fryxell, G.A., Maier, A., Thessen, A., Powell, C.L., Soniat, T.M., 2000. *Pseudo-nitzschia* spp. in the northern Gulf of Mexico: overview and response to increasing eutrophication. Symposium on Harmful Marine Algae, Marine Biological Laboratory, Woods Hole, Massachusetts, p. 27.
- Dyble, J., Bienfang, P., Dusek, E., Hitchcock, G., Holland, F., Laws, E., Lerczak, J., McGillicuddy, D., Minnett, P., Moore, S., O'Kelly, C., Solo-Gabriele, H., Wang, J., 2008. Environmental controls, oceanography, and population dynamics of pathogens and harmful algal blooms: connecting sources to human exposure. *Environ. Health* 7 (Suppl 2), S5.
- Erdner, D.L., Dyble, J., Parsons, M.L., Stevens, R.C., Hubbard, K.A., Wrabel, M.L., Moore, S.K., Lefebvre, K., Anderson, D.M., Bienfang, P., Bidigare, R.R., Parker, M.S., Moeller, P.D., Brand, L.E., Trainer, V.L., 2008. Centers for oceans and human health: a unified approach to the challenge of harmful algal blooms. *Environ. Health* 7 (Suppl. 2).
- Evan, K.M., Kuhn, S.F., Hayes, P.K., 2005. High levels of genetic diversity and low levels of genetic differentiation in North Sea *Pseudo-nitzschia pungens* (Bacillariophyceae) populations. *J. Phycol.* 41 (3), 506–514.
- Fehling, J., Davidson, K., Bolch, C.J., Bates, S.S., 2004. Growth and DA production by *Pseudo-nitzschia seriata* (Bacillariophyceae) under phosphate and silicate limitation. *J. Phycol.* 40 (4), 674–683.
- Fennel, K., Wilkin, J., Levin, J., Moisan, J., O'Reilly, J., Haidvogel, D., 2006. Nitrogen cycling in the Middle Atlantic Bight: results from a three-dimensional model and implications for the North Atlantic nitrogen budget. *Glob. Biogeochem. Cycles* 20, GB3007.
- Fox, J., 2002. An R and S-Plus companion to applied regression. Sage Publications, Inc., Thousand Oaks, London, New Delhi.
- Fritz, L., Quilliam, M.A., Wright, J.L.C., Beale, A.M., Work, T.M., 1992. An outbreak of DA poisoning attributed to the pennate diatom *Pseudonitzschia australis*. *J. Phycol.* 28, 439–442.
- García-Mendoza, E., Rivas, D., Olivares-Ortiz, A., Almazán-Becerril, A., Castaneda-Vega, C., Pena-Manjarrez, J.L., 2009. A toxic *Pseudo-nitzschia* bloom in Todos Santos Bay, northwestern Baja California, Mexico. *Harmful Algae* 8, 493–503.
- Garrison, D.L., 1981. Monterey Bay Phytoplankton II: Resting spore cycles in coastal diatom populations. *Journal of Plankton Research* 3, 137–156.
- Glibert, P.M., Magnien, R., Lomas, M.W., Alexander, J., Fan, C., Haramoto, E., Trice, M., Kana, T.M., 2001. Harmful algal blooms in the Chesapeake and Coastal Bays of Maryland, USA: comparisons of 1997, 1998, and 1999 events. *Estuaries* 24, 875–883.
- Glibert, P.M., Seitzinger, S., Heil, C.A., Burkholder, J.M., Parrow, M.W., Codispoti, L.A., Kelly, V., 2005. The role of eutrophication in the global proliferation of harmful algal blooms: new perspectives and new approaches. *Oceanography* 18 (2), 198–209.
- Glibert, P.M., Icarus Allen, J., Bouwman, A.F., Brown, C.W., Flynn, K.J., Lewitus, A.J., Madden, C.J., 2010. Modeling of HABs and Eutrophication: Status, Advances, Challenges. *Journal of Marine Systems* 83, 262–275.
- Grattan, L.M., Roberts, S., Trainer, V., Boushey, C., Burbacher, T., Grant, K., Tracy, K., Morris, J.G., 2007. DA neurotoxicity in native americans in the Pacific Northwest: human health project methods and update. Fourth Symposium on Harmful Algae in the U.S., Woods Hole, MA.
- Hallegraeff, G.M., 1993. A review of harmful algal blooms and their apparent global increase. *Phycologia* 32 (2), 79–99.
- Hasle, G.R., Syvertsen, E.E., 1996. Marine Diatoms. In: Tomas, C.R. (Ed.), Identifying marine phytoplankton. Academic Press, Inc., New York, pp. 5–385.
- Heidke, P., 1926. Berechnung des Erfolges und der Gote der Windstarkevorhersagen im Sturmwarnungsdienst. *Geogr. Ann.* 8, 301349.
- Heisler, J., Glibert, P.M., Burkholder, J.M., Anderson, D.M., Cochlan, W., Dennison, W.C., Dortch, Q., Gobler, C.J., Heil, C.A., Humphries, E., Lewitus, A., Magnien, R., Marshall, H.G., Sellner, K., Stockwell, D.A., Stoecker, D.K., Suddleson, M., 2008. Eutrophication and harmful algal blooms: a scientific consensus. *Harmful Algae* 8, 3–13.
- Howard, M.D.A., Cochlan, W.P., Ladizinsky, N., Kudela, R.M., 2007. Nitrogenous preference of toxigenic *Pseudo-nitzschia australis* (Bacillariophyceae) from field and laboratory experiments. *Harmful Algae* 6, 206–217.
- Iglesias-Rodríguez, M.D., Brown, C.W., Doney, S.C., Kleypas, J., Kolber, D., Kolber, Z., Hayes, P.K., Falkowski, P.G., 2002. Representing key phytoplankton functional groups in ocean carbon cycle models: coccolithophorids. *Glob. Biogeochem. Cycles* 16 (4), 1100.
- Kemp, W.M., Boynton, W.R., Adolf, J.E., Boesch, D.F., Boicourt, W.C., Brush, G., Cornwell, J.C., Fisher, T.R., Glibert, P.M., Hagy, J.D., Harding, L.W., Houde, E.D., Kimmel, D.G., Miller, W.D., Newell, R.L.E., Roman, M.R., Smith, E.M., Stevenson, J.C., 2005. Eutrophication of Chesapeake Bay: historical trends and ecological interactions. *Mar. Ecol. Prog. Ser.* 303, 1–29.
- Kudela, R., Cochlan, W., Roberts, A., 2004. Spatial and temporal patterns of *Pseudo nitzschia* spp. in central California related regional oceanography. In: Steidinger, K.A., Landsberg, J.H., Tomas, C.R., Vargo, G.A. (Eds.), *Harmful Algae 2002*. Florida and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO.
- Kudela, R., Pitcher, G., Probyn, T., Figueiras, F., Moita, T., Trainer, V.L., 2005. Harmful algal blooms in coastal upwelling systems. *Oceanography* 18 (2), 185–197.
- Kudela, R.M., Lane, J.Q., Cochlan, W.P., 2008. The potential role of anthropogenically derived nitrogen in the growth of harmful algae in California, USA. *Harmful Algae* 8, 103–110.
- Lane, J.Q., Raimondi, P.T., Kudela, R.M., 2009. Development of a logistic regression model for the prediction of toxigenic *Pseudo-nitzschia* blooms in Monterey Bay, California. *Mar. Ecol. Prog. Ser.* 383, 37–51.
- Lefebvre, K.A., Tilton, S.C., Bammler, T.K., Beyer, R.P., Srinouanprachan, S., Stapleton, P.L., Farin, F.M., Gallagher, E.P., 2007. Gene expression profiles in zebrafish brain after acute exposure to domoic acid at symptomatic and asymptomatic doses. *Toxicol. Sci.* 107 (1), 65–77.
- Levin, E.D., Pang, W.G., Harrison, J., Williams, P., Petro, A., Ramsdell, J.S., 2006. Persistent neurobehavioral effects of early postnatal DA exposure in rats. *Neurotoxicol. Teratol.* 28, 673–680.
- Lomas, M.W., Rumbley, C.J., Glibert, P.M., 2000. Ammonium release by nitrogen sufficient diatoms in response to rapid increases in irradiance. *J. Plankton Res.* 22 (12), 2351–2366.
- Maldonado, M.T., Hughes, M.P., Rue, E.L., Wells, M.L., 2002. The effect of Fe and Cu on growth and DA production by *Pseudo-nitzschia multiseries* and *Pseudo-nitzschia australis*. *Limnol. Oceanogr.* 47 (2), 515–526.
- Marchetti, A., Trainer, V.L., Harrison, P.J., 2004. Environmental conditions and phytoplankton dynamics associated with *Pseudo-nitzschia* abundance and DA in the Juan de Fuca eddy. *Mar. Ecol. Prog. Ser.* 281, 1–12.
- Margalef, R., 1978. Life-forms of phytoplankton as survival alternatives in an unstable environment. *Oceanol. Acta* 1, 493–509.
- Marshall, H.G., Burchardt, L., Lacouture, R., 2005. A review of phytoplankton composition within Chesapeake Bay and its tidal estuaries. *J. Plankton Res.* 27 (11), 1083–1102.
- McCullagh, P., Nelder, J.A., 1989. Generalized Linear Models. Chapman and Hall, London.
- McGillicuddy, D.J., Stock, C.A., Anderson, D.M., Signell, R.P., 2003. Hindcasting blooms of the toxic dinoflagellate *Alexandrium* spp. in the western Gulf of Maine. In: Valette Silver, S.A. (Ed.), *Ecological Forecasting Report*, pp. 49–54.
- McGillicuddy, D.J., Anderson, D.M., Lynch, D.R., Townsend, D.W., 2005. Mechanisms regulating large-scale seasonal fluctuations in *Alexandrium fundyense* populations in the Gulf of Maine: results from a physical-biological model. *Deep-Sea Res. Part II* 52, 2698–2714.
- McKenna, J.H., 2004. DOC dynamics in a small temperate estuary: simultaneous addition and removal processes and implications on observed nonconservative behavior. *Estuaries* 27 (4), 604–616.
- Meehl, G.A., Stocker, T.F., Collins, W.D., Friedlingstein, P., Gaye, A.T., Gregory, J.M., Kitoh, A., Knutti, R., Murphy, J.M., Noda, A., Raper, S.C.B., Watterson, I.G., Weaver, A.J., Zhao, Z.C., 2007. Global Climate Projections. In: Solomon, S., Quin, D., Manning, M., Marquis, M., Avery, K., Tignor, M.M.B., Miller, H.L.J., Chen, Z. (Eds.), *Climate change 2007: the physical science basis*. Cambridge Univ. Press, Cambridge, UK and New York.
- Mengelt, C., 2006. How two species of the diatom genus *Pseudo-nitzschia* respond to adverse conditions: *P. australis* and *P. multiseries* UV-photocology, dark survival, and seasonal abundance at two coastal sites in Central California. Ph.D., University of California, Santa Barbara, Santa Barbara.
- Moore, J.K., Doney, S.C., Kleypas, J.A., Glover, D.M., Fung, I.Y., 2002. An intermediate complexity marine ecosystem model for the global domain. *Deep-Sea Res. Part II* 49, 403–462.
- Moore, S.K., Trainer, V.L., Mantua, N.J., Parker, M.S., Laws, E., Backer, L.C., Fleming, L.E., 2008. Impacts of climate variability and future climate change on harmful algal blooms and human health. *Environ. Health* 7 (Suppl. 2).
- Moore, S.K., Trainer, V.L., Hickey, B., 2009. *Pseudo-nitzschia* blooms on the Washington coast: an analysis of long-term monitoring data from the ORHAB Program. Fifth Symposium on Harmful Algae in the U.S., Ocean Shores, WA.
- Murtugudde, R., 2009. Regional Earth System prediction: a decision-making tool for sustainability? *Curr. Opin. Environ. Sustain.* 1, 37–45. doi:10.1016/j.cosust.2009.07.004.
- Nagelkerke, N.J.D., 1991. A note on a general definition of the coefficient of determination. *Biometrika* 78, 691–692.
- National Centers for Environmental Prediction, 2008. NCEP Products Inventory, GENS Products. <http://www.nco.ncep.noaa.gov/pmb/products/gens/>.
- Orsini, L., Procaccini, G., Sarno, D., Montresor, M., 2004. Multiple rDNA ITS-types within the diatom *Pseudo-nitzschia delicatissima* (Bacillariophyceae) and their relative abundances across a spring bloom in the Gulf of Naples. *Mar. Ecol. Prog. Ser.* 271, 87–98.
- Paerl, H.W., 1988. Nuisance phytoplankton blooms in coastal, estuarine, and inland waters. *Limnol. Oceanogr.* 33 (4, part 2), 823–847.
- Pan, Y., Subba Rao, D.V., Mann, K.H., Li, W.K.W., Warnock, R.E., 1993. Temperature dependence of growth and carbon assimilation in *Nitzschia pungens* f. *multiseries*, the causative diatom of DA poisoning. In: Smayda, T.J., Shimizu, Y. (Eds.), *Toxic Phytoplankton Blooms in the Sea*. Elsevier Science Publishers B.V., pp. 619–624.
- Pan, Y., Subba Rao, D.V., Mann, K.H., Brown, R.G., Pocklington, R., 1996. Effects of silicate limitation on production of DA, a neurotoxin, by the diatom *Pseudo-nitzschia multiseries*. I. Batch culture studies. *Mar. Ecol. Prog. Ser.* 131 (2–3), 225–233.
- Pan, Y., Bates, S.S., Cembella, A.D., 1998. Environmental stress and DA production by *Pseudo-nitzschia*: a physiological perspective. *Nat. Toxins* 6, 127–135.
- Pan, Y., Parsons, M.L., Busman, M., Moeller, P.D., Dortch, Q., Powell, C.L., Doucette, G.J., 2001. *Pseudo-nitzschia* sp. cf. *pseudodelicatissima* – a confirmed producer of DA from the northern Gulf of Mexico. *Mar. Ecol. Prog. Ser.* 220, 83–92.
- Parsons, M.L., Dortch, Q., 2002. Sedimentological evidence of an increase in *Pseudo nitzschia* (Bacillariophyceae) abundance in response to coastal eutrophication. *Limnol. Oceanogr.* 47 (2), 551–558.
- Patz, J.A., Vavrus, S.J., Uejio, C.K., McLellan, S.L., 2008. Climate change and waterborne disease risk in the Great Lakes Region of the U.S. *Am. J. Prev. Med.* 35 (5), 451–458.
- Prasad, M.B.K., Sapiano, M.R.P., Long, W., Anderson, C.R., Murtugudde, R., in press. Long-term variability of nutrients in the Chesapeake Bay: A Retrospective Analysis, 1990–2006. *Estuaries and Coasts* (accepted with revision).
- Ramsdell, J.S., Zabka, T.S., 2008. In utero DA toxicity: a fetal basis to adult disease in the California sea lion (*Zalophus californianus*). *Mar. Drugs* 6 (2), 262–290.
- Rue, E.L., Bruland, K.W., 2001. DA binds iron and copper: a possible role for the toxin produced by the marine diatom *Pseudo-nitzschia*. *Mar. Chem.* 76, 127–134.
- Scholín, A., Gulland, F., Doucette, G.J., Benson, S., Busman, M., Chavez, F.P., Cordaro, J., DeLong, R., De Vogelaere, A., Harvey, J., Haulena, M., Lefebvre, K.A., Lipscomb, T.,

- Loscutoff, S., Lowenstine, L.J., Marin III, R., Miller, P.E., McLellan, W.A., Moeller, P.D.R., Powell, C.L., Rowles, T., Silvagni, P., Silver, M.W., Spraker, T., Trainer, V.L., Van Dolah, F.M., 2000. Mortality of sea lions along the central California coast linked to a toxic diatom bloom. *Nature* 403, 80–83.
- Siegel, D.A., Dickey, T.D., 1986. Variability of net longwave radiation over the Eastern North Pacific Ocean. *J. Geophys. Res.* C 91 (C6), 7657–7666.
- Smayda, T.J., 1963. Succession of phytoplankton, and the ocean as an holocoenotic environment. In: Oppenheimer, C.H. (Ed.), *Symposium on marine microbiology*. Thomas, Springfield, pp. 260–274.
- Smayda, T.J., 1990. Novel and nuisance phytoplankton blooms in the sea: evidence for a global epidemic. In: Graneli, E.E.A. (Ed.), *Toxic Marine Phytoplankton: Fourth International Conference*, Lund, Sweden, June 26–30, 1989. Elsevier Science Publishing Co., Inc., New York, New York, pp. 29–40.
- Smayda, T.J., 1997. Harmful algal blooms: their ecophysiology and general relevance to phytoplankton blooms in the sea. *Limnol. Oceanogr.* 42 (5, part 2), 1137–1153.
- Sohn, K.T., Park, S.M., 2008. Guidance on the choice of threshold for binary forecast modeling. *Adv. Atmos. Sci.* 25 (1), 8388.
- Soil and Water Assessment Tool, 2010. <http://swatmodel.tamu.edu/>.
- Sommer, U., 1994. Are marine diatoms favoured by high Si:N ratios? *Mar. Ecol. Prog. Ser.* 115, 309–315.
- Song, Y., Haidvogel, D.B., 1994. A semi-implicit ocean circulation model using a generalized topography-following coordinate system. *J. Comput. Phys.* 115 (1), 228–244.
- Stewart, J.E., 2008. Bacterial involvement in determining DA levels in *Pseudo nitzschia multiseries* cultures. *Aquat. Microb. Ecol.* 50 (2), 135–144.
- Stumpf, R.P., Culver, M.E., Tester, P.A., Tomlinson, M., Kirkpatrick, G.J., Pederson, B.A., Truby, E., Ransibrahmanakul, V., Soracco, M., 2003. Monitoring *Karenia brevis* blooms in the Gulf of Mexico using satellite ocean color imagery and other data. *Harmful Algae* 2, 147–160.
- Thessen, A.E., Stoecker, D.K., 2008. Distribution, abundance, and DA analysis of the toxic diatom genus *Pseudo-nitzschia* in the Chesapeake Bay. *Estuar. Coast.* 31 (4), 664–672.
- Thessen, A.E., Dortch, Q., Parsons, M.L., Morrison, W., 2005. Effect of salinity on *Pseudo nitzschia* species (Bacillariophyceae) growth distribution. *J. Phycol.* 41, 21–29.
- Thessen, A.E., Bowers, H.A., Stoecker, D.K., 2009. Intra- and interspecies differences in growth and toxicity of *Pseudo-nitzschia* while using different nitrogen sources. *Harmful Algae* 8, 792–810.
- Tomlinson, M.C., Stumpf, R.P., Ransibrahmanakul, V., Truby, E.W., Kirkpatrick, G.J., Pederson, B.A., Vargo, G.A., Heil, C.A., 2004. Evaluation of the use of SeaWiFS imagery for detecting *Karenia brevis* harmful algal blooms in the eastern Gulf of Mexico. *Remote Sens. Environ.* 91 (3–4), 293–303.
- Tomlinson, M.C., Wynne, T.T., Stumpf, R.P., 2009. An evaluation of remote sensing techniques for enhanced detection of the toxic dinoflagellate, *Karenia brevis*. *Remote Sens. Environ.* 113, 598–609.
- Trainer, V.L., Suddleson, 2005. Monitoring approaches for early warning of DA Events in Washington State. *Oceanography* 18 (2), 228–237.
- Trainer, V.L., Adams, N.G., Bill, B.D., Stehr, C.M., Wekell, J.C., Moeller, P.D.R., Busman, M., Woodruff, 2000. DA production near California coastal upwelling zones, June 1998. *Limnol. Oceanogr.* 45 (8), 1818–1833.
- Trainer, V.L., Cochlan, W.P., Erickson, A., Bill, B.D., Cox, F.H., Borchert, J.A., Lefebvre, K.A., 2007. Recent DA closures of shellfish harvest areas in Washington State inland waterways. *Harmful Algae* 6, 449–459.
- United States Department of the Interior- U.S. Geological Survey, 1999. Monitoring nutrients in the major rivers draining to Chesapeake Bay. *Water Resource Investigations Report* 99-4238, November 1999.
- VanDolah, F.M., 2000. Marine algal toxins: origins, health effects, and their increased occurrence. *Environ. Health Perspect.* 108 (Suppl 1), 133–141.
- Weather Research and Forecasting Model, 2010. <http://www.wrf-model.org/index.php>.
- Wells, M.L., Trick, C.Q., Cochlan, W.P., Hughes, M.P., Trainer, V.L., 2005. DA the synergy of iron, copper, and the toxicity of diatoms. *Limnol. Oceanogr.* 50 (6), 1908–1917.
- Xu, J., Hood, R.R., 2006. Modeling biogeochemical cycles in Chesapeake Bay with a coupled physical-biological model. *Estuar. Coast. Shelf Sci.* 69 (1–2), 19–46.