Journal of Marine Systems 83 (2010) 170–180

Contents lists available at ScienceDirect

Journal of Marine Systems

journal homepage: www.elsevier.com/locate/jmarsys

Do external resource ratios matter? Implications for modelling eutrophication events and controlling harmful algal blooms

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ARTICLE INFO

Article history:
Received 15 September 2009
Received in revised form 31 March 2010
Accepted 6 April 2010
Available online 24 April 2010

Keywords:
Phytoplankton
N:P resource ratio
Competition
P-stress
Limiting resources
Light limitation

ABSTRACT

Relationships between nutrient N:P ratio and P-limitation in phytoplankton are explored using a multi-nutrient photoacclimative quota-based model. The relationship depends on concentrations of input and residual nutrients, and also on variable phytoplankton C:N:P stoichiometry. In reality, usually only the residual nutrient concentrations and their ratios are known. However, the total amount of nutrient present in the system affects biomass growth potential through self-shading, and thence the potential for variation in organismal N:P. The critical external N:P resource ratio above which P becomes limiting increases as residual concentrations of nutrients increase to saturate transport kinetics; oligotrophic waters require a lower nutrient N:P to avoid P-limitation than do eutrophic waters. In eutrophic systems, which may support harmful algal blooms (HABs), and/or in systems in which light is rapidly attenuated (sediment loading, gelbstoff), P-limitation may not develop even in high resource N:P situations due to light limitation. This is more likely in high washout systems, where phytoplankton growth rates must remain elevated. The only diagnostics for nutrient stress are cellular functions (C-fixation, C:N:P), and the only nutrient parameters of consequence are concentrations and not ratios of them. Control of resource ratios alone should not be considered as a tool for mitigating HABs.

1. Introduction

The ratio of nutrients (N:P, P:Si, and N:Si) supporting phytoplankton growth has long interested aquatic science, primarily as factors affecting succession. There is a rich history of practical and theoretical research on the topic, much building from work in the 1970's. Two lines of research were explored at that time, the one principally ascribed to the work of Tilman (Tilman, 1977, 1982) considered the importance of the external resource ratio (e.g., Si:P) as a factor affecting competition and succession. The second originated from the work of Droop (1974), and developed by others (e.g., Mykelstad, 1977; Rhee, 1978; Rhee and Gotham, 1980; Turpin, 1986), considered the role in competition of the internal resource ratio (more often referred to as nutrient quota ratios, e.g., N:P). In many of these works, for both internal and external resources, deviations of the ratio around that described by the Redfield ratio is considered significant; this is because that ratio is typically considered to be the "optimal" ratio for phytoplankton growth. In fact there appears no physiological basis upon which to assume the importance of such a fixed ratio (Geider and La Roche, 2002).

For both of these subject lines (external and internal ratios) the resource ratio at which growth is equally limited by two nutrients is identified as having particular significance as a switch point, of particular importance in defining the competitive advantage of one species over another when growing in an environment with different resource availabilities. Throughout this work this critical ratio will be identified as ${}^{ext}R_{crit}$ or ${}^{int}R_{crit}$ for external or internal resources respectively. Interest in the topic has been expanded with the realisation that the ratio as, and if, reflected in internal cellular N:P also affects the value of phytoplankton as food organisms by virtue of the stoichiometric disparity between predators and their prey (e.g., Urabe, 1993). The impact of this disparity can be exacerbated by other processes, such as the accumulation of noxious compounds (Mitra and Flynn, 2005; Pohnert et al., 2007).

The concepts of ${}^{ext}R_{crit}$ or ${}^{int}R_{crit}$ have driven extensive theoretical discussion, supported by modelling. In the context of phytoplankton, Tilman (1977) used both a Monod and an internal-stores (Droop-quota) type of model, reporting that they gave similar results. The work considered P and Si limitations; co-existence and competition between organisms could be explained across a gradient of nutrient ratios. To date this work has been cited approximately 500 times, generating a mass of observational, experimental and theoretical studies. The work was developed by Tilman in various outputs, perhaps most notably in Tilman (1982). Although the original theory,

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doi:10.1016/j.jmarsys.2010.04.007

variously termed resource ratio or resource-competition theory, was described for phytoplankton, it has been extended to other microbes (Smith, 1993), higher plants (Craine et al., 2005) and to predators (Fox and Vasseur, 2008). The implications of the ideas have not been without criticism (e.g. Reynolds, 1999; Craine, 2005; Miller et al., 2005; Tilman 2007), and interpretations of field data in the context of the theory have not always been straightforward (e.g., Sommer, 1993).

Emphasis in earlier works on external resource ratios was placed upon the ratios of Si and P in controlling freshwater diatoms and non-diatoms (e.g. Makulla and Sommer, 1993; Ferris and Lehman, 2007). However, nutrient ratios of N:P have also been widely related to species or algal-group composition in other systems (e.g. freshwater — Bulgakov and Levich, 1999; estuary — Domingues et al., 2005; and marine oligotrophic — Charles et al., 2005). The ratio of inorganic N:P has been related to species composition in harmful algal bloom (HAB) events and the N:P of organic forms has also been implicated (Anderson et al., 2002). The importance for HABs is not just for the selection of one species or another (e.g., Glasgow et al., 2001), but linked to the stimulation of toxicity (Granéli and Flynn, 2006). The external N:P ratio has accordingly been the subject of manipulation in laboratory experiments with studies of half saturation constants for DIN and DIP acquisition (e.g., John and Flynn, 2000; Ignatiades et al., 2007).

Work on the significance of ${}^{\mathrm{int}}R_{\mathrm{crit}}$ for phytoplankton was reviewed by Flynn (2002). It was argued that the internal resource ratio alone had limited impact on selective advantage because of the pre-eminent role of nutrient transporters. This line of argument is not dissimilar in some ways to the criticism of the significance of ${}^{\mathrm{ext}}R_{\mathrm{crit}}$ for higher plants made by Craine et al. (2005); for terrestrial plants, controlling the availability of water around the roots affects nutrient availability for transport.

Where the two concepts, ${}^{\mathrm{ext}}R_{\mathrm{crit}}$ and ${}^{\mathrm{int}}R_{\mathrm{crit}}$, coincide is over the importance of model formulation and the role of internally accumulated nutrients. Although Tilman (1977) decided that there was little advantage to using internal-stores (quota) models, Revilla and Weissing (2008) questioned the use of Monod models in theoretical explorations of resource-competition theory, noting the potential role for nutrient storage which is described in internal-stores/quota-style models. This may be expected to be of particular importance in areas in which conditions fluctuate widely, as they will in coastal waters. This was explored earlier by Roelke et al. (1999) who specifically argued the need for models able to simulate so-called luxury consumption. Flynn (2010) has subsequently argued that Monod–Redfield models of phytoplankton are dysfunctional and should not be deployed under any circumstance.

Phytoplankton photosynthesis is restricted by light and thence (in addition to any other water-borne light absorption) by self-shading. As the concentration of nutrient increases, so does the restriction on nutrient consumption due to self-shading. In consequence, one may expect low nutrient systems to show a closer relationship between cellular N:P and nutrient input N:P. In contrast, in highly eutrophic systems cellular N:P may be expected to more closely match physiological optima (as often considered to be reflected by the Redfield ratio). However, nutrient concentrations measured in the environment or media are residual levels. Such values, and their ratios, need not necessarily reflect input values. Further, light limitation is expected to result in a decrease in the half saturation constant for nutrient-limited growth (K_g; Flynn, 2003), affecting the kinetics of resource acquisition, and hence affecting competition between species.

The potential significance of external nutrient ratios in affecting phytoplankton succession is a key topic in many water management activities. It is especially important for our understanding of the impacts of eutrophication on HAB development, because P-stress is often associated with toxicity (Granéli and Flynn, 2006) and waters entering marine systems tend to show an enhanced N:P. This is because of the emphasis, and ease, of removing DIP in comparison with DIN during water (sewage) treatment. To consider this topic, here a model describing N–P limitations coupled with photoacclimation is operated under conditions in which light (i.e., C) limitation interacts with the nutrient load. The aim is to describe the types of systems in which the input N:P may actually be of importance in setting organismal N:P, and thence to allow a questioning of the utility of nutrient N:P values.

2. Methods

The model of Flynn (2001) was used for this work; the values of constants used in this model are given in Table 1. In brief, the model employs normalised quotas of N and P to describe nutrient limitation, coupled with an active depression of uptake of the non-limiting nutrient (Flynn 2008a,b). A Liebig approach is then used to tie whichever is the most limiting nutrient (N or P) to photosynthesis using a photoacclimative description of chlorophyll dynamics (Flynn, 2001). The basis of the model has been demonstrated over many years, against various data series (e.g., John and Flynn, 2002; Flynn, 2008b; Flynn, 2010). The following explains the relationships between the kinetics of the control of growth from the internal nutrient resource, and how this relates to nutrient transport kinetics.

2.1. Nutrient quota control

For a single nutrient limitation, growth is limited as a function of internal nutrient availability using a normalised quota description (quota XC, as N:C or P:C) according to Eq. (1). XC varies between $XC_{\min}$ and $XC_{\max}$, and (assuming nothing else is limiting) the growth rate μ varies between 0 (at $XC = XC_{\min}$) and $\mu_{\max}$ (at $XC = XC_{\max}$). Constant KQX controls the shape of the relationship between XC and μ. KQN tends to be high (ca. 10), giving a linear relationship, while KQP tends to be low (ca. 0.1), giving a distinct curvi–linear relationship — see Flynn (2008a,b). This relationship for the model employed here is shown by Fig. 1.

$$\mu = \mu_{\max} \cdot \underbrace{\frac{(1 + KQX)\cdot(XC - XC_{\min})}{(XC - XC_{\min}) + KQX\cdot(XC_{\max} - XC_{\min})}}_{A} \quad (1)$$

Part A in Eq. (1) can be used to describe a quotient for nutrient stress. For N that quotient is termed NCu, for P as PCu. If the nutrients are equally co-limiting then NCu = PCu.

The value of XC at a given value of μ, assuming light is not limiting, is given by Eq. (2). For a model configuration in which $\mu = \mu_{\max} \cdot \mathrm{MIN}$ (NCu, PCu), Eq. (2) can be used to obtain the values of XC (as N:C and

Table 1
Model constants applied to the model as described in Flynn (2001).

Constant	Description and unit	Value
Alpha	Initial slope of photosynthesis–irradiance curve ($m^2\ g^{-1}$ chl)·(gC μmol^{-1} photon)	$7 \cdot 10^{-6}$
$ChlC_{max}$	Maximum Chl:C ($g\ g^{-1}$)	0.06
KQN	Shape factor for N-quota–μ curve (dimensionless)	10
KQP	Shape factor for P-quota–μ curve (dimensionless)	0.1
NC_{abs}	Absolute maximum cellular N:C ($g\ g^{-1}$)	0.25
NC_{max}	Maximum N:C affecting μ ($g\ g^{-1}$)	0.16
NC_{min}	Minimum cellular N:C ($g\ g^{-1}$)	0.05
${}^{N}K_t$	Half saturation constant for DIN transport ($\mu g\ N\ L^{-1}$)	14
PC_{abs}	Absolute maximum cellular P:C ($g\ g^{-1}$)	0.04
PC_{max}	Maximum P:C affecting μ ($g\ g^{-1}$)	0.02
PC_{min}	Minimum cellular P:C ($g\ g^{-1}$)	0.003
${}^{P}K_t$	Half saturation constant for DIP transport ($\mu g\ P\ L^{-1}$)	31
Surge	Constant controlling surge capacity for DIP transport	1–4
μ_{max}	Maximum growth rate (d^{-1})	1

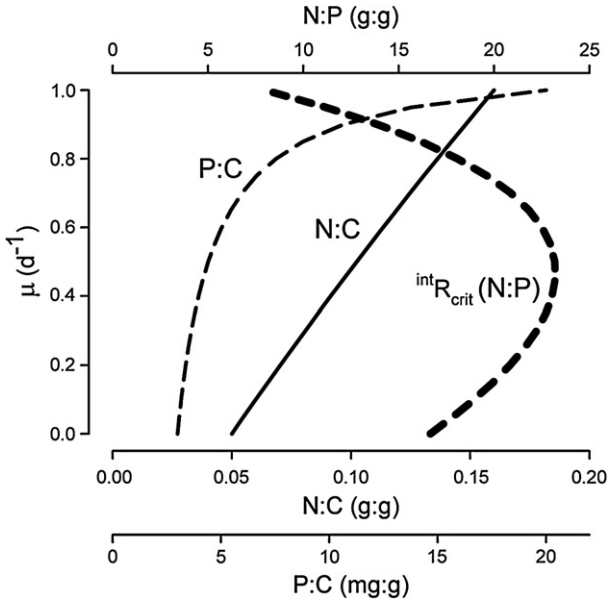

Fig. 1. Variation in cellular N:C and P:C with growth rate (μ) when either N or P is limiting. All ratios are by mass. The N:P ratio plotted ($^{int}R_{crit}$) is that given when N and P are both equally limiting; under single nutrient limitation cellular N:P can vary over a much wider range than shown here. As here $\mu_{max} = 1\ d^{-1}$, the μ-axis could equally be scaled as the relative growth rate (μ/μ_{max}).

P:C) at a given value of μ. This then can be used to obtain the value of $^{int}R_{crit}$ (as cellular N:P) when NCu = PCu at any specified μ.

$$XC = \left(\frac{KQX \cdot (XC_{max} - XC_{min})}{\left(\mu_{max} \cdot (1 + KQX) \Big/ \mu \right) - 1} + XC_{min} \right) \quad (2)$$

Fig. 1 shows that as cell growth rate deteriorates in consequence of N- and/or P-stress, the N:C and/or P:C ratio changes in a characteristic fashion for the limiting element. If both N and P are limiting growth by the same degree (i.e., NCu = PCu), then the cellular N:P, $^{int}R_{crit}$, alters as indicated in Fig. 1. It is noteworthy that cellular N:P is only as low as the Redfield ratio (mole ratio of 16N:1P, mass ratio 7.23:1) when growth is not limited by either nutrient.

2.2. Nutrient transport

Nutrient enters phytoplankton through a transporter with half saturation constant K_t. The transport rate required to match nutrient demand when $\mu = \mu_{max}$ (and hence when $XC = XC_{max}$) is given by $\mu_{max} \cdot XC_{max}$. However, it is common for nutrient transporters in phytoplankton to show a surge capacity of several times that rate. This enables cells to rapidly accumulate nutrients beyond their immediate needs. The half saturation constant for growth on element X is given by Eq. (3) (from Flynn, 2002); parameter "*surge*" takes a value of ≥1. The half saturation constant for growth (K_g) is always less than that for transport (K_t).

$$^{X}K_g = \frac{^{X}K_t}{\left(\frac{XC_{max} \cdot surge}{XC_{min} + \frac{0.5 \cdot KQX \cdot (XC_{max} - XC_{min})}{(0.5 + KQX)}} - 1 \right)} \quad (3)$$

For reasons explored by Flynn (1998), there are serious logistical problems in measuring values of K_g and especially K_t. Here it is assumed that the value of K_t for both dissolved inorganic N and P (DIN and DIP) is 1 μM. However, because the need for P is less than that for N, and because of the different shape of the quota curve defining the kinetics of internal nutrient usage (Fig. 1), even with values of $^{P}K_t$ and $^{N}K_t$ set at the same value, $^{P}K_g$ is much lower than $^{N}K_g$ (Fig. 2). This means that to support a given growth rate, and for that growth to be equally nutrient limited by N and P, a much lower concentration of DIP is required than for DIN. Depending on the relative values of *surge*, there may be orders of magnitude difference in the required concentration of N vs P to attain K_g. In addition, if light is limiting growth, then de facto the over capacity (*surge*) for transport increases, giving an expected increase in affinity (decrease in K_g) as light limitation increases (Flynn, 2003). Flynn (2002) showed the great potential for variation in $^{int}R_{crit}$ as the values of XC_{min}, *surge* and KQX are altered. Although both N and P transport exhibits surge transport kinetics, here, to simplify matters, only the value for P transport is considered as >1. Values for *surge* for P transport were considered over the range 1 to 4. This range was considered sufficient for the tests conducted here as they amply demonstrate the point being considered — the greater the difference between surge transport kinetics for N and P, the greater the potential disparity between resource N:P and cellular N:P.

2.3. Model operation

The model was operated within a system of a fixed optical depth (1, 5 or 10 m), assuming homogeneity of cells within the water column. Accordingly, depth-integrated photosynthesis was computed. Growth of phytoplankton in this system was either considered under a dynamic stretch-batch scenario (which shows batch dynamics while excess nutrients are consumed, then enters steady state growth at a low dilution rate), or in a steady state system in which the growth rate (μ) matched the dilution rate.

For steady state operation, the model was constructed so that the input concentration of dissolved inorganic P, [DIP], was varied during the simulation run until the level of phytoplankton N- and P-stress was equal (i.e., NCu = PCu). Using this approach, the value of the critical N:P ratio for internal ($^{int}R_{crit}$) and external nutrients ($^{ext}R_{crit}$) was computed at a given concentration of DIN.

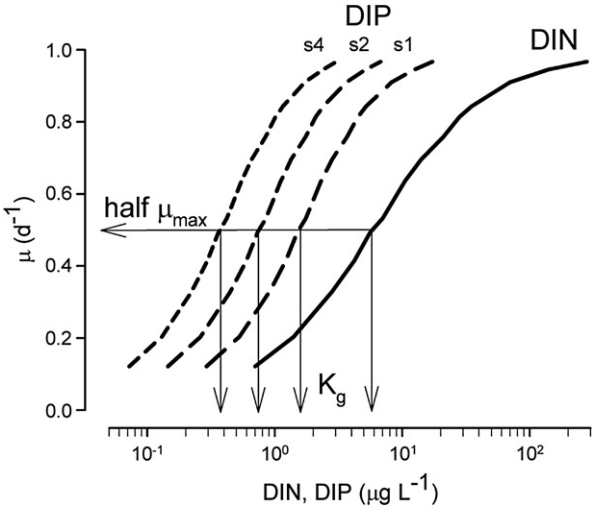

Fig. 2. Relationship between external nutrient concentration and growth rate (μ), for dissolved inorganic N (DIN) or P (DIP). The vertical arrows indicate the nutrient concentrations required to support $\mu_{max}/2$ (i.e., the values of K_g). For DIP, the consequences of having a surge transport capacity (*surge* in Eq. (3)) enabling transport to exceed that required to support maximum growth by a factor of 2 (s2) or 4 (s4) is shown with comparison to the default *surge* = 1 (s1). Even though the value of K_t (half saturation for nutrient transport was the same for DIN and DIP (1 μM = 14 μg N L^{-1} or 31 μg P L^{-1}), K_g for DIP is much lower than for DIN because of the difference in cellular requirements (see Fig. 1).

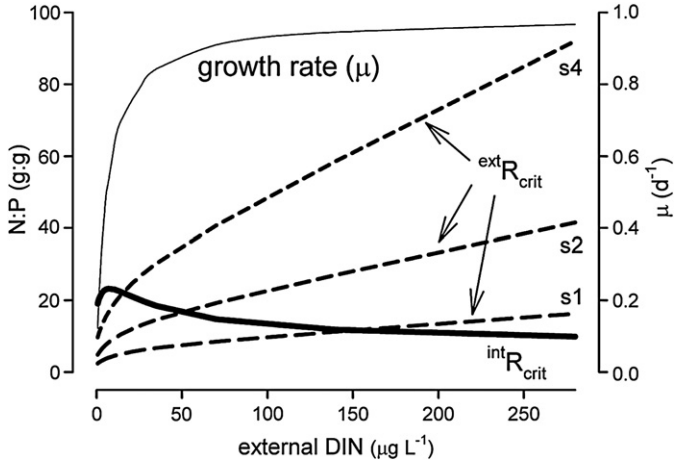

Fig. 3. Steady state growth at different concentrations of external DIN under which N and P co-limit growth equally. Changes in algal cellular N:P for $^{int}R_{crit}$ vary with growth rate in accordance with Fig. 1. The ratio of external N:P supporting co-limitation, $^{ext}R_{crit}$, also varies with growth rate, and hence with external DIN. It also varies with the value of *surge* for P transport as this affects the value of K_g for DIP (see also Fig. 2).

Models were constructed and operated within Powersim Constructor 2.51 (Isdalstø, Norway), running under a 4th order, variable step size, Runge–Kutta algorithm.

3. Results

3.1. Steady state simulations

The model was run to steady state at different external DIN concentrations such that N and P limitations (NCu = PCu) were equal. This allows the determination of the value of $^{int}R_{crit}$ at different values of DIN (Fig. 3). This shows the same relationship between DIN and μ as seen in Fig. 2, and the same algal N:P relationship ($^{int}R_{crit}$) with μ as shown in Fig. 1. Depending on the value of *surge* (Eq. (3)) the N:P value of the external nutrients that enable $^{int}R_{crit}$ (i.e., the value of $^{ext}R_{crit}$) varies over a wide range. However, a common relationship is seen in which the need for P relative to N decreases (i.e., $^{ext}R_{crit}$ increases) as the availability of external DIN increases. The reason for the lower $^{ext}R_{crit}$ at low external DIN is because external DIP becomes increasingly limiting for transport, and this is not compensated by the lower cellular need for N:P at low growth rates. In contrast, at high DIN, the lower K_g for DIP relative to that for DIN (cf. Fig. 2) results in a lessening of the need for a high external DIP, and hence a higher $^{ext}R_{crit}$ is attained.

When the model (with *surge* = 4) is run in a simulated system with a mixing depth of 1 m or 10 m, at different input concentrations of DIN, and at different dilution rates, the steady state relationships are as shown in Fig. 4. These systems are akin to chemostat systems, but could also be likened to estuary systems with different output flows. At steady state, dilution rates equate to growth rates (μ). There are no values given at low input DIN and high dilution rates because of washout (i.e., μ<dilution rate). As with the simulations shown in Fig. 3, the availability of DIP in the input stream was adjusted to achieve an equal balance of N and P limitation. It is important to recall, however, that the important feature affecting organism physiology is, as always, the residual nutrient concentrations relative to demand. The plots show the residual DIN and the resultant residual nutrient N:P (=$^{ext}R_{crit}$) and algal N:P (=$^{int}R_{crit}$).

In the system simulated in Fig. 4 the conversion of input N to biomass results in a progressive increase in self-shading as phytoplankton grow. The quotient NPCu (=MIN(NCu,PCu), which in this instance is where NCu = PCu) shows the degree of nutrient sufficiency. A value for NPCu of 1 indicates limitation either by the maximum growth potential of the organism (here $\mu_{max} = 1\ d^{-1}$), or by light. Light limitation occurs at lower values of input DIN with

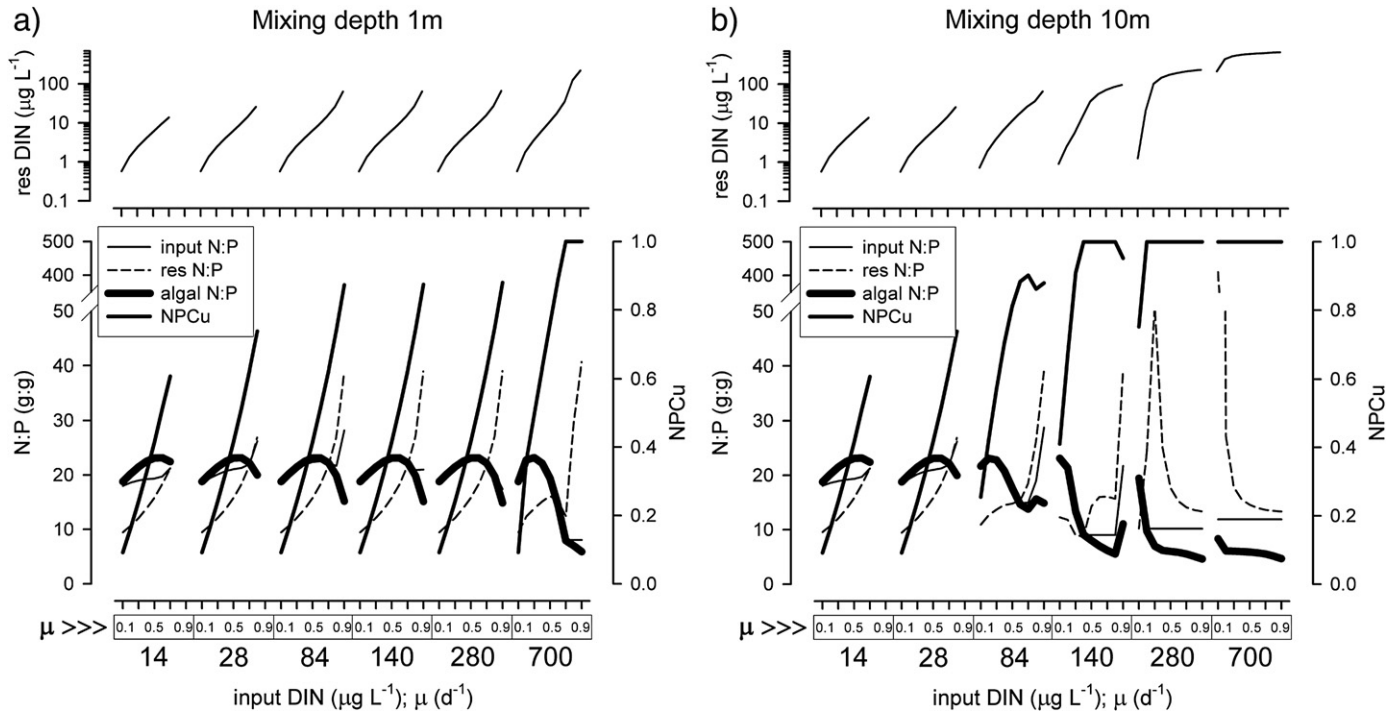

Fig. 4. Steady state growth at six different input DIN concentrations, at different growth rates (μ = 0.1, 0.3, 0.5, 0.7 and 0.9 d^{-1}) and in systems with mixing depths of either 1 m (a) or 10 m (b). Systems were run under chemostat-like conditions, in which the growth rate equates to the dilution rate of the system; the input DIP was adjusted so as to provide equal N–P co-limitation for phytoplankton growth. The upper plot in each panel shows the residual concentration of DIN (res DIN) increasing as the growth rate (μ) increases. The main plot shows how the input N:P, residual nutrient N:P (res N:P = $^{ext}R_{crit}$), and algal N:P (=$^{int}R_{crit}$) varies; note the difference between the residual and algal N:P values. Under a proportion of conditions the input N:P matches (i.e., co-plots with) the algal N:P. At lower values of input DIN, μ cannot attain the dilution rate; the simulated organisms are washed out and hence no values are plotted for these scenarios. A colour version of this plot is available online.

deeper mixing depth systems (Fig. 4a vs. b) because of the accumulated effect of attenuation through self-shading.

The relationship between algal N:P and dilution rate follows the form seen in Fig. 1, noting that the dilution rate equates to the growth rate at steady state. Generally the input N:P matches the algal N:P (Fig. 4); the occasions when this is not so are when most of the input nutrient is not converted to algal biomass. This happens at high dilution (= high growth) rates; note the values of the residual DIN in the upper sub-panels of Fig. 4a and b. The value of the residual nutrient N:P ($=^{ext}R_{crit}$) increases with dilution (growth) rate, and also with the value of the input DIN. This result is consistent with the form of the values of $^{ext}R_{crit}$ shown in Fig. 3.

However, an important deviation from this pattern is seen at the interface between nutrient and light limitation. Here the residual N:P ($^{ext}R_{crit}$) does not follow the same trend as noted above; this is seen in the 1 m mixing depth scenario (Fig. 4a) at DIN = 700 μg N L^{-1} and $\mu = 0.7\ d^{-1}$, and in the 10 m scenario (Fig. 4b) at 140 μg N L^{-1}. Depending on the growth rate (which affects algal N:P at $^{int}R_{crit}$; Figs. 1 and 3) and the degree of light limitation, $^{ext}R_{crit}$ can vary over a range exceeding an order of magnitude. This is seen at high input DIN in the 10 m depth scenario (Fig. 4b).

3.2. Dynamic simulations

The simulations run for Fig. 4 require many days to attain steady state. In nature conditions are continuously varying, not least under the influence of the diurnal light–dark cycle and for coastal regions with strong tides. An understanding of responses under a dynamic setting is required. Simulations were run to give a range of contrasting conditions with different mixing depths, different input DIN and input N:P (either Redfield, or 10 times Redfield N:P), and also at different dilution rates (either 0.05 d^{-1}, or 0.2 d^{-1}).

Fig. 5 shows results for systems with mixing depth of 1 or 5 m, with attenuation only from the phytoplankton themselves. In low DIN systems (5 μM) there is relatively little difference between residual nutrient N:P and algal N:P. Thus a high N:P input leads to elevated algal N:P and thence P-limited growth (grey arrow in Fig. 5b). In high DIN systems (250 μM), however, strongly contrasting relationships between residual nutrient N:P and algal N:P can be seen. Thus, Fig. 5c shows in the 1 m simulation a spike of very high external nutrient N:P with no algal P-stress, while its 5 m mixing depth equivalent shows much slower growth over the simulation period with close alignment of external and cellular N:P (Fig. 5g). The high DIN, high N:P input

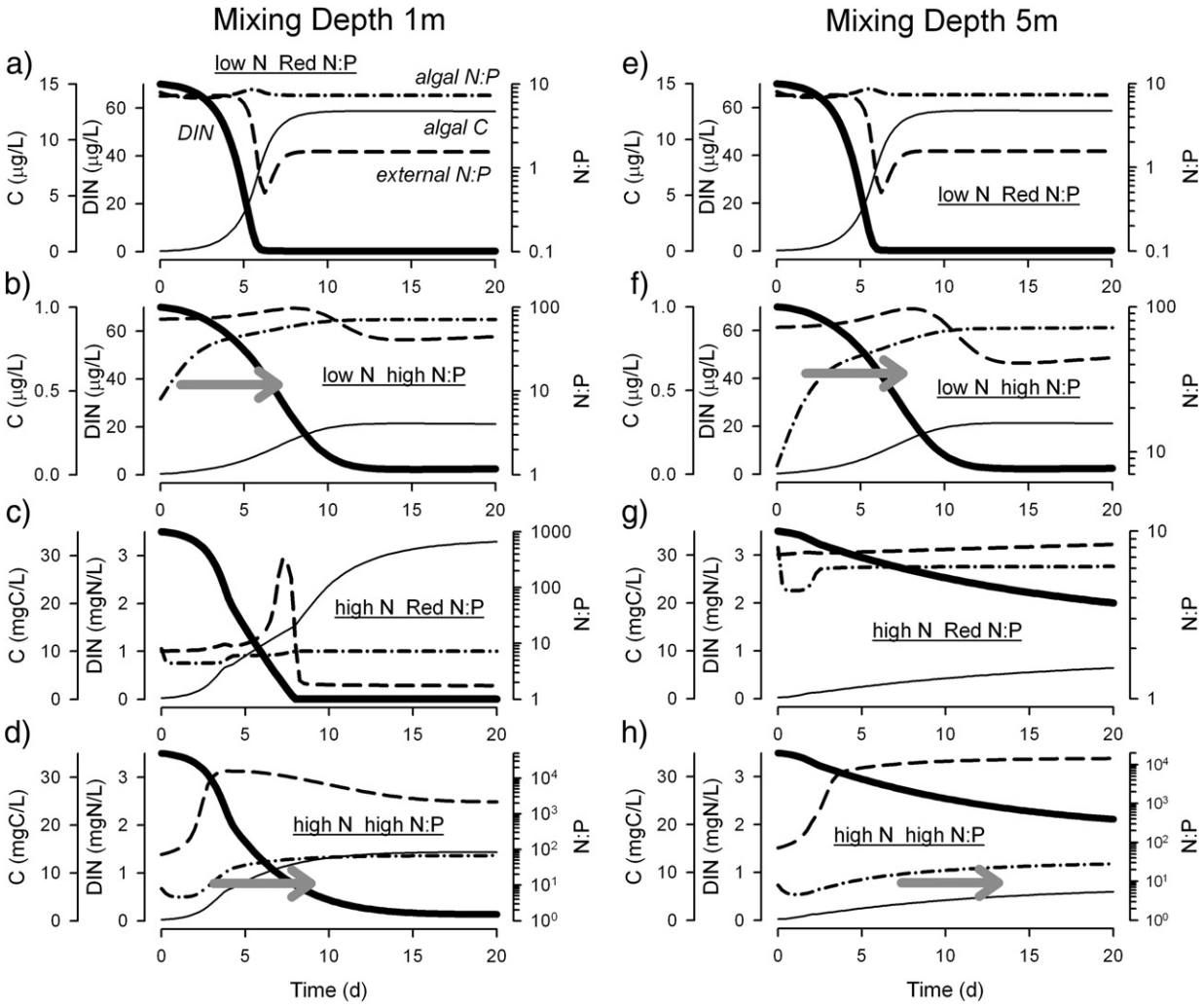

Fig. 5. Dynamic simulations in which nutrients are supplied with DIN at either low (low N) or high (high N) values, and with the DIN:DIP either at Redfield (Red N:P) or high N:P (10 times Redfield N:P) values. The systems were subjected (chemostat-style) to a dilution rate of 0.05 d^{-1}, though initial growth was under batch-like conditions. Water attenuation was assumed to be 0 m^{-1}, but with growth in mixed depths of either 1 m (panels a–d) or 5 m (panels e–h); light attenuation developed as algal growth progressed, being more important with high N and at greater mixing depth. Lines show the remaining external inorganic N (DIN), the external (residual) nutrient N:P, algal biomass (algal C), and algal N:P. All ratios are by mass. P-stress developed from the point indicated from the base of the arrows. Note the different scales used for C-biomass and DIN, and the log scales for N:P.

systems (Fig. 5d, h) show the development of extremely high residual nutrient N:P, and consequent P-limited growth.

In systems in which light attenuation is more important, very high nutrient N:P can develop with no consequent P-stress; this is shown in Fig. 6 for 10 m depth systems either with only phytoplankton-generated attenuation (Fig. 6d) or with additional water attenuation of 0.92 m^{-1}; (Fig. 6h). An attenuation of 0.92 m^{-1} cuts surface light to 1% at 5 m depth.

The rate of system dilution is also important, as may be expected given the steady state results shown in Fig. 4. Thus Fig. 7 shows high dilution rate systems with mixing depths of 1 m or 10 m depending solely on phytoplankton-derived light attenuation. High residual N:P can again occur in a system supplied with nutrients at Redfield N:P (Fig. 7c); this can happen because light limitation results in an accumulation of P within the cells raising cellular P:C above that expected from Redfield. A high input N:P in high DIN systems with light limitation (Fig. 7h) need not result in elevated algal N:P and development of P-stress.

To test for a robust relationship between the external residual N:P and algal nutrient status, data collected from the dynamic simulations shown in Figs. 5–7 were co-plotted (Fig. 8). It can be seen, with reference to the line of equality between nutrient and cellular N:P (dashed lines in Fig. 8), that there is great variability in the relationship; only rarely is there actually an equality between nutrient and cellular N:P, with many high residual nutrient N:P values being associated with values of cellular N:P orders of magnitude lower. There are also instances where the external N:P is lower than the cellular value. The series from lower dilution rate simulations (Fig. 8a) show a wider range of external N:P on account of the wider growth rate distribution possible in those scenarios. At a higher dilution rate, in which growth rate cannot fall so low, higher residual nutrient concentrations are required and these are associated with lower internal and external N:P values.

4. Discussion

The simulations presented here indicate the problems of trying to identify the limiting nutrient by reference to nutrient availability and nutrient ratios. There are various facets to this issue, some of which are related to algal physiology, and some to the dynamics of the chemico-physical environment in which the phytoplankton grow. The matter is important because of the implications of nutrient stress for trophic dynamics and toxicity in harmful algal species, and of eutrophication for ecosystem management.

The physiology of phytoplankton is directly linked to their ability to acquire nutrients. Determinations of the half saturation constants for nutrient transport (K_t) and growth (K_g) are fraught with logistic complications due to a combination of measurement sensitivities and feedback processes within the organisms that trans-inhibit transport within seconds or minutes of nutrient addition (Flynn, 1998). There are additional issues that further complicate the matter. These include the roles of organic nutrients (e.g., urea and organic-P compounds),

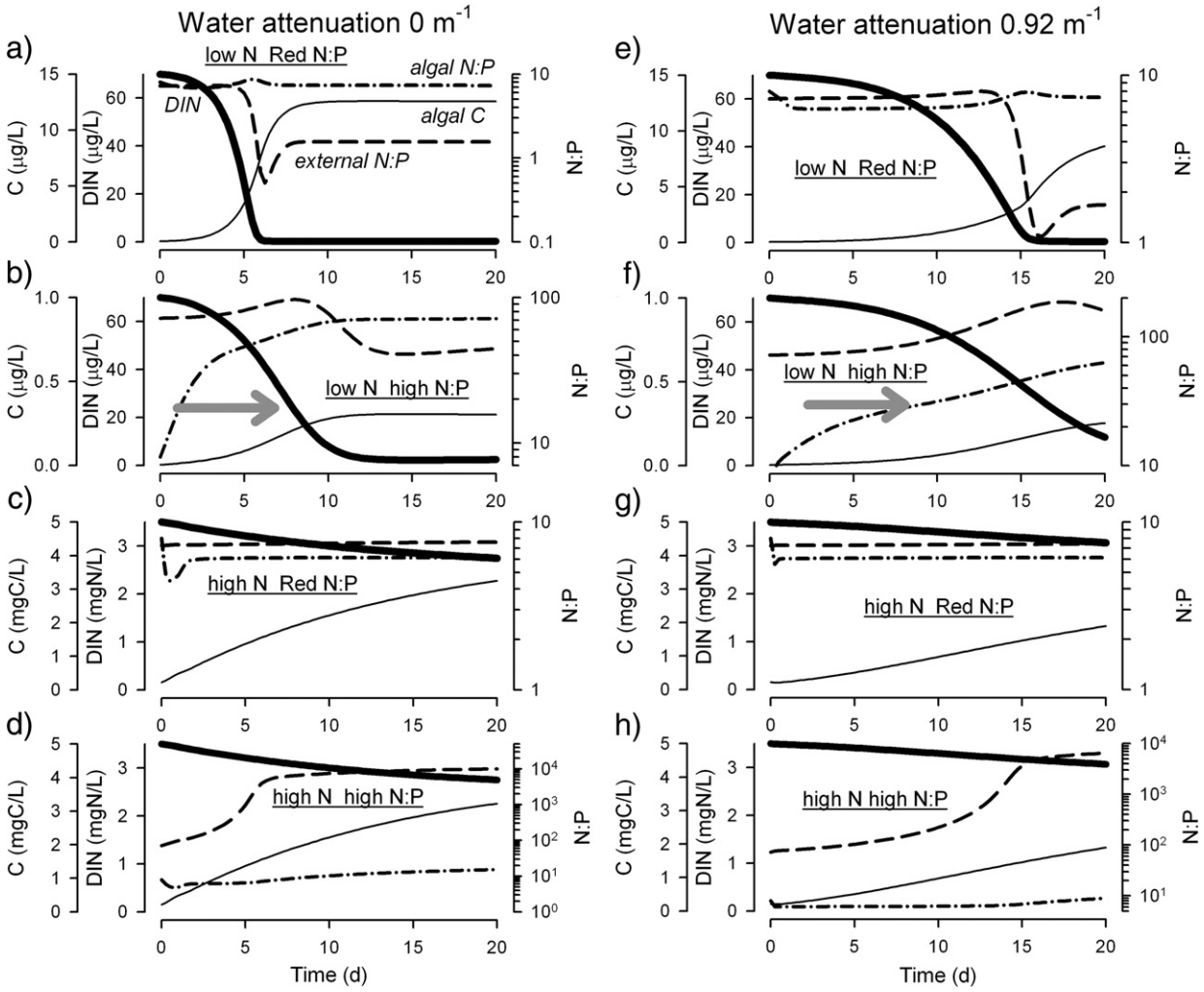

Fig. 6. As for Fig. 5, but all using a mixing depth of 10 m, and with either water attenuation at 0 m^{-1} (panels a–d) or 0.92 m^{-1} (e–h). P-stress developed from the point indicated from the base of the arrows. Note the different scales used for C-biomass and DIN, and log scales for N:P.

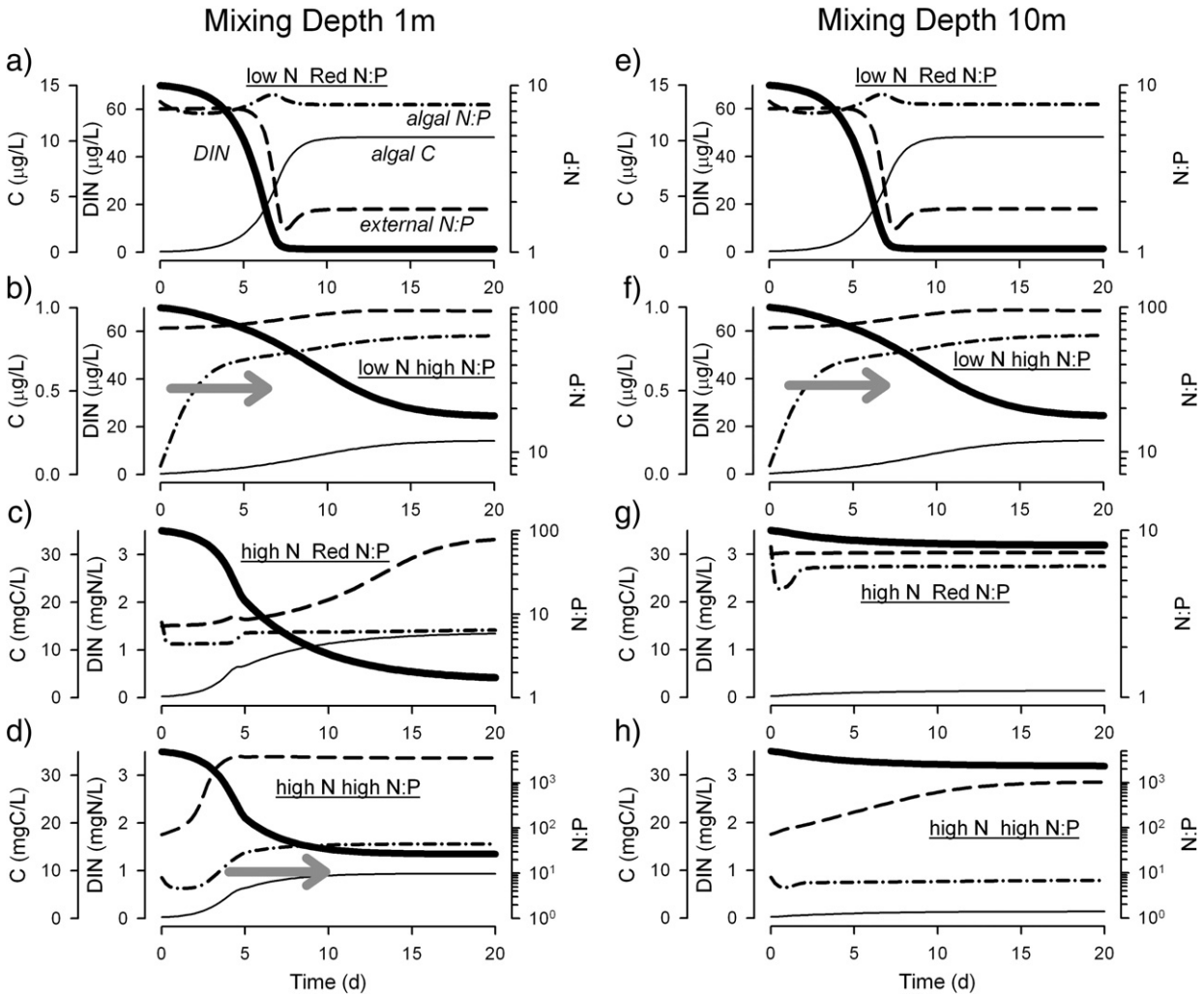

Fig. 7. As Fig. 5, with water attenuation of 0 m^{-1}, except with a mixing depth of 1 m (panels a–d) or 10 m (e–h), and running at a higher dilution rate, of 0.2 d^{-1}. P-stress developed from the point indicated from the base of the arrows. Note the different scales used for C-biomass and DIN, and log scales for N:P.

that the bioavailability of P within so-called soluble reactive phosphate is unknown, and the usual problems of determining the importance of nutrient fluxes versus measured concentrations of nutrients in bulk waters. Other factors associated with nutrient ratios include changes in uptake kinetics in consequence of phenotypic changes; for example, affinity for DIP in *Phaeocystis* is increased in the colonial form (Veldhuis et al., 1991). Further, short-term measurements of nutrient use may mask whole day integrated uptake (Veldhuis et al., 1991; Clark et al., 2002). Against an appreciation of such complications we proceed with a consideration of the results from this work.

What becomes evident immediately from the simulations presented here is that the Redfield ratio of nutrient N:P cannot be used to provide a robust indicator of the internal nutrient status of the phytoplankton, and neither can it be safely used alone to predict whether one or the other nutrient will ultimately limit growth. Aside from the caveats mentioned in the preceding paragraph, potentially the external (residual) N:P can be several orders of magnitude greater than the internal value (Figs. 3–7). Further, even when the supply N:P is in Redfield proportions, the residual N:P can become strongly elevated (Figs. 5c and 7c). Indeed, the direction of that change is worth noting; it may be suggestive of P-stress during growth under so-called optimum initial nutrient conditions.

N-stress develops much more rapidly than does P-stress as phytoplankton C:N:P deviates from the optimal internal ratio (Flynn, 2008a,b). Any laboratory researcher who has attempted to grow batch cultures of phytoplankton to P-limitation in media containing ca. 50–100 µM DIN will appreciate the difficulty in preventing DIN exhaustion. Using the cell-quota configuration employed here, to attain a minimum P:C of 3 mgP/gC while retaining the maximum N:C of 0.16 gN/gC requires a mass N:P of 53.33. This equates to a N:P nutrient supply mole ratio of nearly 118, which is far from the Redfield value of 16. In batch-type systems, or in stretch-batch (these are systems that initially show batch culture like dynamics, but which change to steady state with a low residual dilution rate), wide deviation between N:P ratios are to be expected during the dynamics of growth. In a chemostat system, where the input concentrations are usually high, and the residual levels very low, the input N:P and organismal N:P will inevitably be closely matched. In such systems a balance point can be located, the so-called critical N:P ($^{int}R_{crit}$), at which N-stress and P-stress are equal. Determining $^{ext}R_{crit}$ in a chemostat is non-trivial because the residual nutrient concentrations are typically below the limits of detection. In nature things are rather different to those experienced in typical laboratory situations. The input nutrient concentrations and their ratio are unknown, there is a variable and unknown level of nutrient regeneration, and there is no specific reason why external supply and internal nutrient ratios should match as they would in a chemostat.

Flynn (2002) showed that the internal cellular critical N:P, $^{int}R_{crit}$, need not necessarily be a critical determinant for competition between species. Growth rate (μ) is affected by other factors, especially the capacity for surge nutrient transport, affecting the

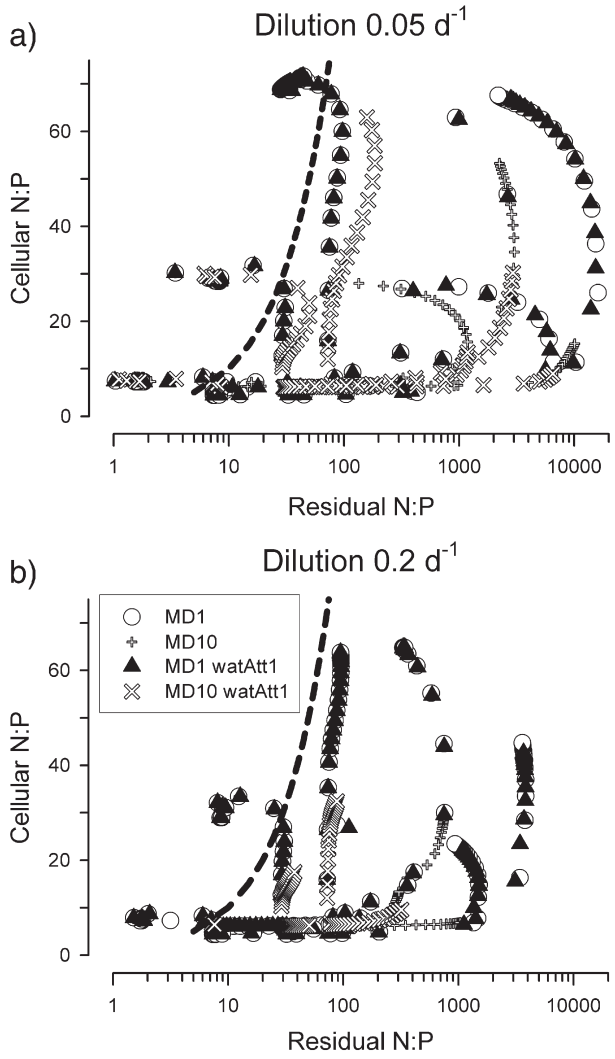

Fig. 8. Scatter plots of external residual nutrient N:P against cellular N:P. Data are for runs with mixing depths of either 1 m (MD1) or 10 m (MD10), and with water attenuation at 0 m^{-1}, or at 0.92 m^{-1} (watAtt1). All ratios are by mass. Plot (a) for slow dilution systems (0.05 d^{-1}), plot (b) at faster dilution (0.2 d^{-1}). The dashed line indicates equality between nutrient and cellular N:P. The presence of points to the right of the line, and especially with a cellular N:P of ca. 10, shows instances where the apparent P-impoverished state of the nutrient regime bears no relationship at all to the level of P-stress in the phytoplankton. The actual level of cellular N:P that is indicative of growth being limited by P-stress (i.e. N:P>$^{int}R_{crit}$) varies with growth rate, and hence with nutrient and light availability (see Figs. 1–7).

kinetics of nutrient acquisition, and these could be at least as important as factors affecting the kinetics of internal nutrient utilisation. The latter interactions, as reflected by nutrient quota type relationships, may be expected to be non-trivial because of the difference in the form of the N:C quota–μ curve (which is typically linear) and the P:C quota–μ curve (typically strongly curved) (Flynn, 2008a,b; Fig. 1). In reality, it is complicated further by the interactions between one nutrient stress upon another (about which little is known), and by the dynamics of internal nutrient redistribution on external nutrient exhaustion under dynamic conditions. The important link between nutrients and algal growth is between internal and external nutrient availabilities. It is not to their respective resource ratios.

4.1. What limits growth?

Identifying the limiting factor for phytoplankton growth is one of the "Holy Grails" of limnological and oceanographic research. Determining the nutrient status of phytoplankton in a simulation is easy. Attempting to determine the same in laboratory cultures, let alone in field populations of unknown nutrient history is non-trivial, if not very difficult. Traditional approaches have often involved biomanipulations, the addition of one or more test nutrients; in essence these are manipulations of nutrient supply ratios. At sea experiments involving additions of macronutrients such as N and P are in bottles or at most mesocosms (Lagus et al., 2004), but in freshwater systems whole-system experiments can be conducted (Hough and Thompson, 1996), for example to consider the control of toxic cyanobacteria (Kim et al., 2007), or stream periphyton (Stelzer and Lamberti, 2001). From such works, Roelke et al. (1999) went so far as to question whether HABs could be regulated by controlled release of nutrients from sewage treatment works. While short-term bioassays (Flynn, 1990), together with molecular biology, provide additional tools, the extent to which these indicate stress rather than growth limitation may not be clear. For example, the presence of phosphatase activity, while indicative of a level of P-stress (sufficient to de-repress phosphatase synthesis), need not indicate P-limitation of growth.

In contrast to these assays, measuring nutrient concentrations is relatively quick, cheap and easy; it may also be automated. A desire to relate phytoplankton well-being to such parameters is wholly understandable. Likewise, simplifying nutrient availability as a ratio in comparison with a conceptual "optimum" Redfield value is also understandable. The problem is that these concepts are made upon flawed assumptions. Ultimately, the N:P supply ratio is, at best, of secondary importance. The factors that matter are the absolute concentrations of nutrient N and P, the light field, and hence the C–N–P physiological status of the organisms. The weakness (absence) of a relationship between nutrient and cellular N:P is clear in Fig. 8, with very few instances of equality.

There are various reasons to reject a simple interpretation of the importance of either internal or external N:P ratios in phytoplankton physiology. The range of phytoplankton mole N:P is <5 to >100, with values under conditions conducive to optimal growth between 5 and 19 (Geider and La Roche, 2002). The biochemical analysis conducted by Geider and La Roche (2002) suggests that the value of $^{int}R_{crit}$ may be expected to be mole N:P 15–30 (ca. 6.8–13.5 gN:gP). To this one can then add the analysis of Flynn (2002) which showed that the value of $^{int}R_{crit}$ need not in any case be a critical determinant of competitive success because of the importance of transport kinetics. Only at the extreme where internal resources are down at the level of the minimum quotas (i.e. N:C$\approx$NC$_{min}$ and P:C$\approx$PC$_{min}$) may $^{int}R_{crit}$ be important, as this affects the C-biomass achievable for a given amount of nutrient. In nature, and certainly in marine systems, growth of phytoplankton to attain μ approaching zero is unlikely except in extreme conditions. To attain dominance under such conditions, almost certainly an individual phytoplankton species will have needed to escape predation (Irigoien et al., 2005), quite likely by engaging some other strategy to enhance its competitive strength (such as anti-grazing or allelopathy; Pohnert et al., 2007; Flynn, 2008c).

The bottom line is that cellular physiology is not a function of cellular N:P; it is a function (or at least a much closer function) of cellular N:C and P:C, the critical common element here being C. Unsurprisingly, therefore, the interaction between N and P in cell physiology cannot be separated from C-assimilation, and thence from photosynthesis. Even setting aside the matter of C-assimilation (which will be explored further below), in practice N vs P nutrient stress is not simply a function of the ratio of nutrient availability but is also a function of absolute concentration of the residual nutrient in the medium. At low nutrient concentrations the likelihood of limitation will be affected by diffusion and hence by water turbulence and/or cell motility. From Fig. 3 it can be seen that $^{ext}R_{crit}$ increases as the residual concentration of DIN increases; initially it is actually less than $^{int}R_{crit}$.

The lower the value of K_g for DIP, the higher the values of ${}^{ext}R_{crit}$. The implication is that, assuming all else is equal, P needs to be relatively more abundant in oligotrophic waters than in eutrophic waters if it is not to limit more than does N (Fig. 3). Eutrophic waters are also far more likely to represent light-limiting regimes.

4.2. Nutrient loading, ratios and light limitation

Light, as another "nutrient", has also been subjected to a Tilman'esque resource-competition treatment (e.g. Passarge et al., 2006; Caputo et al., 2008). Light-P as a resource pair have been found not to follow standard resource-competition expectations (Passarge et al., 2006), though given the role of P in cellular energetics (Flynn et al., 2010) this is perhaps not unexpected. Unsurprisingly, combinations of nutrient (N, P, and Si) and light limitation generate additional interactions when linked to the light–dark cycle. Thus diel periodicity affects not only (obviously) photosynthesis, but also the acquisition of N (Clark et al., 2002; Flynn et al., 2002) and P (Ahn et al., 2002). Variation in the ability to assimilate nutrients in darkness, especially in a tidal environment with its changing periodicity overlain upon the light–dark cycle, may be expected to affect competitive advantage. Leonardos and Geider (2005), studying a cryptophyte, report that the chemostat N:P (and hence, with effectively total nutrient consumption, cellular N:P) at which N and P co-limited was unaffected by irradiance. This assumption is made by the model used in this work.

In eutrophic waters there is an increasing likelihood of self-shading by the high phytoplankton biomass. Further, in estuarine systems sediment loading and the presence of coloured dissolved organics are often important as factors affecting light availability, especially in highly mixed systems. In such waters light limitation can override the expected implications of eutrophication (Colijn and Cadee, 2003). Combinations of these factors act to impart a level of C-limitation upon the phytoplankton which will limit the ability of the organisms to draw down nutrients. The N:P value of the inorganic nutrients becomes increasingly irrelevant in such waters as the capacity to assimilate even the nutrient present at the lowest concentration becomes restricted. The nitrate:phosphate drawdown ratio can vary greatly from Redfield, and increases at low light (Leonardos and Geider, 2004). The implication is that estuarine waters, which are often high nutrient low-light environments, require a significant increase of input N:P before P-stress even becomes likely, let alone limiting. The river and/or tidal streams present an additional physiological stress; growth rate must exceed the dilution rate for population growth. The washout of phytoplankton will further decrease the demand for nutrients, while affecting the internal nutrient balance. All of these expectations are borne out by the simulations (Figs. 5, 6 and 7).

Eutrophication is often associated with harmful algal bloom (HAB) events (Heisler et al., 2008), with emphasis placed not only upon species monitoring but also upon that of nutrient levels (Glibert et al., 2008). Understanding the linkage between nutrient input to coastal areas, the promotion of eutrophication and HABs is important not only from a scientific angle but because of the governmental policy developments that spring from it (e.g., Maier et al., 2009; but see Colijn and Cadee, 2003). Inevitably the nutrient concentrations measured in natural waters will be residual values, rather akin to the concentrations within a chemostat culture vessel. Further, much (most) nutrient in coastal waters is of terrestrial origin entering through a myriad of dispersed routes (Heisler et al., 2008; Maier et al., 2009). Recycling of nutrients, especially P, in sediments complicates matters further (Pasternak et al., 2009).

While some invoke recycling as an explanation for why growth continues when nutrient ratios are different from Redfield values (e.g. Carlsson and Granéli, 1999), others (e.g. Bulgakov and Levich, 1999) identify plasticity in cellular C:N:P as an important explanatory factor. The work presented here shows additional routes for such deviation. Either way, nutrient limitation of phytoplankton affects their quality for zooplankton and other consumers (including the benthos), and thence limits nutrient regeneration to support the next generation of phytoplankton (Urabe, 1993; Mitra and Flynn, 2005). To add to this is the role of organic nutrition that can overturn the implications of inorganic N:P for HAB growth, especially if mixotrophy is involved (Lagus et al., 2004). The involvement of mixotrophy and of toxicity as defence (anti-grazer) mechanisms is one which is ripe for modelling and theoretical studies (Mitra and Flynn, 2006; Flynn and Mitra, 2009) but one for which more field and experimental data are required.

4.3. The importance of model structure in the analysis

Models provide an obvious route for exploring the implications of various water management strategies. However, as with all modelling work, the use of an appropriate modelling strategy is critical. The simplest approach is to assume fixed Redfield ratios of N:P within the phytoplankton, with clear (and simple) implications for comparing nutrient N:P to that same Redfield value. This is the assumption made in the types of work conducted by Tilman (1977). However, this is clearly a flawed approach, as can be seen from Fig. 1 (see also Geider and La Roche, 2002; Flynn, 2008a,b, 2010). The implications of variable (i.e. non-Redfield) stoichiometry in the phytoplankton are also great for trophic dynamics and for our understanding of the impacts of eutrophication.

Of significance, much of the classic research on the subject of nutrient resource ratios and algal succession was conducted with Si as one of the nutrients (limiting diatom growth). Si is a nutrient that cannot be redistributed within cells upon exhaustion of external supply, and must not be modelled using quota-style techniques (Flynn, 2003). In consequence the external rather than the internal (cellular) concentrations of Si are most important and indeed the relationship between Si and growth rate is best described using a Monod approach (Flynn and Martin-Jézéquel, 2000). However, while that is so for Si, it is not for N or P; the cellular ratios of N:C and P:C and not external concentrations are the critical determinants of cellular N and P status.

For N and P the Monod model is dysfunctional (Flynn, 2010), and should not be used. Diatoms become increasingly dominant at high Si:N (Sommer et al., 2004) and Dortch et al. (2001) suggest that the Si:N ratio is as important as the absolute nutrient concentrations in affecting the likelihood of hypoxia due to diatoms. Popovich et al. (2008) argue that low P as much as low Si may terminate diatom blooms. Getting the model description of diatoms correct is clearly important; one may question whether the appropriate tools and data types were employed in so much of the work supporting the resource-ratio theory. While Revilla and Weissing (2008) are correct to question the use of Monod rather than internal-stores (quota) models in resource-ratio studies (Flynn, 2010), the form of the quota model itself may also be significant (Flynn, 2008b). The way in which light and nutrient limitations interact in models is also important; if done incorrectly then K_g is unaltered while it is expected to decrease as light limitation develops (Flynn, 2003).

4.4. Which is important — resource ratio or concentrations?

In much work the importance of concentration versus nutrient ratio has become blurred. Thus Lampert and Sommer (2007; p134) state "Note that it is the *ratio of the limiting resources* and not the absolute amount of resources that defines the boundaries of co-existence and exclusion in the model" (their italics). Koiv and Kangro (2005) question whether the absolute concentrations rather than ratios are most important, while Interlandi and Kilham (2001) stress the importance of the number of nutrients available at physiologically limiting levels for co-existence. The work of Stelzer and Lamberti

(2001) on stream periphyton (which may be compared to benthic algae growing in estuaries) also stresses the importance of concentration, and not just N:P supply ratios. Reynolds (1999) makes a much stronger statement in his argument for the importance of nutrient concentration rather than nutrient ratios; he concludes that "… {at no point} does the ratio of one nutrient to another determine the dynamic performance of the contenders." Sommer (1999) says that while one may draw analogies in the correlation between nutrient ratios and phytoplankton succession in chemostat and field conditions, the underlying explanations may differ. Both Reynolds (1999) and Sommer (1999) identify the importance of the behaviour of early-season species in setting the environmental conditions for later species. One can also see why flexibility in C:N:P stoichiometry, coupled with the ability to display "luxury consumption" is also important (Roelke et al., 1999). However, it should be noted that the concept of luxury consumption is only really applicable to P and micro-nutrients; it is not an appropriate concept for N, and certainly not for Si (Flynn, 2003, 2005b).

That the work here shows N:P values of both ${}^{\text{int}}R_{\text{crit}}$ and ${}^{\text{ext}}R_{\text{crit}}$ ranging so far either side of the Redfield molar ratio of 16 could be considered as the final nail in the proverbial coffin for external resource-ratio arguments for phytoplankton. External resource ratios, alone, do not matter.

Translating all the above into a modelling exercise, one can see why it is so important to ensure that models describing phytoplankton are not dysfunctional (Flynn, 2005a,b, 2010). It is worth noting again that much theoretical work has been conducted using models that may be criticised on this ground, including the work by Tilman (1977) using Monod descriptors.

5. Conclusions

Three issues arise from this work. The first is that nutrient concentrations as experienced by phytoplankton, and not ratios of those nutrients, are the critical determinants of resource acquisition. The link from external conditions to internal cellular conditions that control growth is complex. The external conditions as perceived by organisms in nature are not really known and in any case the link from external to internal is via transport systems subject to feedback processes, with resultant kinetics that are far from constant. Ratios can be dangerous tools; they may be elevated because the denominator is low or the numerator is high. They are outputs, or consequences, of real processes; they are not primary drivers of them. A key problem with eutrophication is not knowing the real input amounts (or indeed ratios) of nutrients. In a biological system in which growth is self-limiting, here by self-shading at high biomass levels, the most important thing is to limit the anthropogenic release of nutrients into water bodies.

The second issue concerns the role of resource ratios in shaping plankton ecology. More so than at any time in the past we are aware of the interactions between bottom-up and top-down processes. While the implications of stoichiometry in trophic dynamics and nutrient regeneration do provide an indirect link to resource ratios (at least to internal resource ratios), resource-ratio theory primarily affects bottom-up control. Different organisms have evolved different mechanisms to resolve resource issues and these may be expected to endow organisms with different growth capacities (different $\mu_{\max}$) with advantages over others under different external resource availabilities. Some autecologcal issues are yet to be resolved, such as the benefits of having a low $\mu_{\max}$ (Flynn, 2009), but there are clearly other factors that can readily overturn predictions that one may make from considering only resource acquisition (Mitra and Flynn, 2006; Pohnert et al., 2007). There is a rich vein of potential research here that warrants investigation and will help in understanding the factors that affect development of harmful algal blooms.

The final conclusion concerns the importance of using appropriate model constructs not only for predictive simulation work but also for theoretical studies. There is no logic, and arguably great risk, in using inappropriate (if not flawed) constructs in theoretical studies. An argument for the use of (over) simplified models on the grounds that these are mathematically more tractable is dangerous. Our ecological understanding, and our data series, are typically not powerful enough for us to discriminate between model fits for the correct or incorrect reason (e.g., Mitra et al., 2007). The least we can, and should, do is not to employ models with known conceptual flaws. This, then, provides us with a route forward, to involve the vast wealth of non-parametric information collected by biologists and ecologists in model validation.

References

Ahn, C.Y., Chung, A.S., Oh, H.M., 2002. Diel rhythm of algal phosphate uptake rates in P-limited cyclostats and simulation of its effect on growth and competition. J. Phycol. 38, 695–704.

Anderson, D.M., Glibert, P.M., Burkholder, J.M., 2002. Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. Estuaries 25, 704–726.

Bulgakov, N.G., Levich, A.P., 1999. The nitrogen:phosphorus ratio as a factor regulating phytoplankton community structure. Arch. Hydrobiol. 146, 3–22.

Caputo, L., Naselli-Flores, L., Ordonez, J., Armengol, J., 2008. Phytoplankton distribution along trophic gradients within and among reservoirs in Catalonia (Spain). Freshw. Biol. 53, 2543–2556.

Carlsson, P., Granéli, E., 1999. Effects of N:P:Si ratios and zooplankton grazing on phytoplankton communities in the northern Adriatic Sea. II. Phytoplankton species composition. Aquat. Microb. Ecol. 18, 55–65.

Charles, F., Lantoine, F., Brugel, S., Chretiennot-Dinet, M.J., Quiroga, I., Riviere, A., 2005. Seasonal survey of the phytoplankton biomass, composition and production in a littoral NW Mediterranean site, with special emphasis on the picoplanktonic contribution. Estuar. Coast. Shelf Sci. 65, 199–212.

Clark, D.R., Flynn, K.J., Owens, N.J.P., 2002. The large capacity for dark nitrate-assimilation in diatoms may overcome nitrate limitation of growth. New Phytol. 155, 101–108.

Colijn, F., Cadee, G.C., 2003. Is phytoplankton growth in the Wadden Sea light or nitrogen limited? J. Sea Res. 49, 83–93.

Craine, J.M., 2005. Reconciling plant strategy theories of Grime and Tilman. J. Ecol. 93, 1041–1052.

Craine, J.M., Fargione, J., Sugita, S., 2005. Supply pre-emption, not concentration reduction, is the mechanism of competition for nutrients. New Phytol. 166, 933–940.

Domingues, R.B., Barbosa, A., Galvao, H., 2005. Nutrients, light and phytoplankton succession in a temperate estuary (the Guadiana, south-western Iberia). Estuar. Coast. Shelf Sci. 64, 249–260.

Dortch, Q., Rabalais, N.N., Turner, R.E., Qureshi, N.A., 2001. Impacts of changing Si/N ratios and phytoplankton species composition. Coast. Estuar. Sci. 58, 37–48.

Droop, M.R., 1974. The nutrient status of algal cells in continuous culture. J. Mar. Biol. Assoc. UK 54, 825–855.

Ferris, J.A., Lehman, J.T., 2007. Interannual variation in diatom bloom dynamics: roles of hydrology, nutrient limitation, sinking, and whole lake manipulation. Water Res. 41, 2551–2562.

Flynn, K.J., 1990. The determination of nitrogen status in microalgae. Mar. Ecol. Prog. Ser. 61, 297–307.

Flynn, K.J., 1998. Estimation of kinetic parameters for the transport of nitrate and ammonium into marine phytoplankton. Mar. Ecol. Prog. Ser. 169, 13–28.

Flynn, K.J., 2001. A mechanistic model for describing dynamic multi-nutrient, light, temperature interactions in phytoplankton. J. Plankton Res. 23, 977–997.

Flynn, K.J., 2002. How critical is the critical N:P ratio? J. Phycological 38, 961–970.

Flynn, K.J., 2003. Modelling multi-nutrient interactions in phytoplankton; balancing simplicity and realism. Prog. Oceanogr. 56, 249–279.

Flynn, K.J., 2005a. Castles built on sand; dysfunctional plankton models and the failure of the biology–modelling interface. J. Plankton Res. 27, 1205–1210.

Flynn, K.J., 2005b. Modelling marine phytoplankton growth under eutrophic conditions. J. Sea Res. 54, 92–103.

Flynn, K.J., 2008a. Use, abuse, misconceptions and insights from quota models: the Droop cell-quota model 40 years on. Ocean Mar. Biol. Ann. Rev. 46, 1–23.

Flynn, K.J., 2008b. The importance of the form of the quota curve and control of non-limiting nutrient transport in phytoplankton models. J. Plankton Res. 30, 423–438.

Flynn, K.J., 2008c. Attack is not the best form of defense; lessons from harmful algal bloom dynamics. Harmful Algae 8, 129–139.

Flynn, K.J., 2009. Going for the slow burn: why should possession of a low maximum growth rate be advantageous for microalgae? Plant Ecology & Diversity 2, 179–189.

Flynn, K.J., 2010. Ecological modelling in a sea of variable stoichiometry; dysfunctionality and the legacy of Redfield and Monod. Progress in Oceanography 84, 52–65.

Flynn, K.J., Martin-Jézéquel, V., 2000. Modelling Si–N limited growth of diatoms. J. Plankton Res. 22, 447–472.

Flynn, K.J., Mitra, A., 2009. Building the "perfect beast": modelling mixotrophic plankton. J. Plankton Res. 31, 965–992.

Flynn, K.J., Clark, D.R., Owens, N.J.P., 2002. Modelling suggests that optimization of dark nitrogen-assimilation need not be a critical selective feature in phytoplankton. New Phytol. 155, 109–119.

Flynn, K.J., Raven, J.A., Rees, T.A.V., Finkel, Z., Quigg, A., Beardall, J., 2010. Is the growth rate hypothesis applicable to microalgae? Journal of Phycology 46, 1–12.
Fox, J.W., Vasseur, D.A., 2008. Character convergence under competition for nutritionally essential resources. Am. Nat. 172, 667–680.
Geider, R.J., LaRoche, J., 2002. Redfield revisited: variability of C:N:P in marine microalgae and its biochemical basis. Eur. J. Phycol. 37, 1–17.
Glasgow, H.B., Burkholder, J.M., Mallin, M.A., Deamer-Melia, N.J., Reed, R.E., 2001. Field ecology of toxic *Pfiesteria* complex species and a conservative analysis of their role in estuarine fish kills. Environ. Health Perspect. 109, 715–730.
Glibert, P.M., Kelly, V., Alexander, J., Codispoti, L.A., Boicourt, W.C., Trice, T.M., Michael, B., 2008. In situ nutrient monitoring: a tool for capturing nutrient variability and the antecedent conditions that support algal blooms. Harmful Algae 8, 175–181.
Granéli, E., Flynn, K.J., 2006. Chemical and physical factors influencing toxin production. In: Granéli, E., Turner, J.T. (Eds.), Ecology of Harmful Algae, Ecological Studies, Vol. 189. Springer-Verlag, Berlin, pp. 229–241.
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.
Hough, R.A., Thompson, R.L., 1996. The influence of a dissolved inorganic nitrogen gradient on phytoplankton community dynamics in a chain of lakes. Hydrobiologie 319, 225–235.
Ignatiades, L., Gotsis-Skretas, O., Metaxatos, A., 2007. Field and culture studies on the ecophysiology of the toxic dinoflagellate *Alexandrium minutum* (Halim) present in Greek coastal waters. Harmful Algae 6, 153–165.
Interlandi, S.J., Kilham, S.S., 2001. Limiting resources and the regulation of diversity in phytoplankton communities. Ecology 82, 1270–1282.
Irigoien, X., Flynn, K.J., Harris, R.P., 2005. Phytoplankton blooms: a "loophole" in microzooplankton grazing impact? J. Plankton Res. 27, 313–321.
John, E.H., Flynn, K.J., 2000. Growth dynamics and toxicity of *Alexandrium fundyense* (Dinophyceae): the effect of changing N:P supply ratios on internal toxin and nutrient levels. Eur. J. Phycol. 35, 11–23.
John, E.H., Flynn, K.J., 2002. Modelling changes in paralytic shellfish toxin content of dinoflagellates in response to nitrogen and phosphorus supply. Mar. Ecol. Prog. Ser. 225, 147–160.
Kim, H.S., Hwang, S.J., Shin, J.K., An, K.G., Yoon, C.G., 2007. Effects of limiting nutrients and N: P ratios on the phytoplankton growth in a shallow hypertrophic reservoir. Hydrobiologie 581, 255–267.
Koiv, T., Kangro, K., 2005. Resource ratios and phytoplankton species composition in a strongly stratified lake. Hydrobiologie 547, 123–135.
Lagus, A., Suomela, J., Weithoff, G., Heikkila, K., Helminen, H., Sipura, J., 2004. Species-specific differences in phytoplankton responses to N and P enrichments and the N:P ratio in the Archipelago Sea, northern Baltic Sea. J. Plankton Res. 26, 779–798.
Lampert, W., Sommer, U., 2007. Limnoecology, 2nd ed. Oxford University Press, U.K.
Leonardos, N., Geider, R.J., 2004. Effects of nitrate: phosphate supply ratio and irradiance on the C:N:P stoichiometry of *Chaetoceros muelleri*. Eur. J. Phycol. 39, 173–180.
Leonardos, N., Geider, R.J., 2005. Elemental and biochemical composition of *Rhinomonas reticulata* (Cryptophyta) in relation to light and nitrate-to-phosphate supply ratios. J. Phycol. 41, 567–576.
Maier, G., Nimmo-Smith, R.J., Glegg, G.A., Tappin, A.D., Worsfold, P.J., 2009. Estuarine eutrophication in the UK: current incidence and future trends. Aquat.Cons. Mar. Freshw. Ecosystems 19, 43–56.
Makulla, A., Sommer, U., 1993. Relationships between resource ratios and phytoplankton species composition during spring in 5 North German lakes. Limnol. Oceanogr. 38, 846–856.
Miller, T.E., Burns, J.H., Munguia, P., Walters, E.L., Kneitel, J.M., Richards, P.M., Mouquet, N., Buckley, H.L., 2005. A critical review of twenty years' use of the resource-ratio theory. Am. Nat. 165, 439–448.
Mitra, A., Flynn, K.J., 2005. Predator–prey interactions: is "ecological stoichiometry" sufficient when good food goes bad? J. Plankton Res. 27, 393–399.
Mitra, A., Flynn, K.J., 2006. Promotion of harmful algal blooms by zooplankton predatory activity. Biol. Lett. 2, 194–197.
Mitra, A., Flynn, K.J., Fasham, M.J.R., 2007. Accounting correctly for grazing dynamics in Nutrient–Phytoplankton–Zooplankton models. Limnol. Oceanogr. 52, 649–661.
Mykelstad, S., 1977. Production of carbohydrates by marine planktonic diatoms. II. Influence of the N/P ratio in the growth medium on the assimilation ratio, growth rate, and production of cellular and extracellular carbohydrates by *Chaetoceros affinis* va. *Willei* (Gran.) Hustedt and *Skeletonema costatum* (Grev.) Cleve. J. Exp. Mar. Biol. Ecol. 29, 161–179.
Passarge, J., Hol, S., Escher, M., Huisman, J., 2006. Competition for nutrients and light: stable coexistence, alternative stable states, or competitive exclusion? Ecol. Monograph 76, 57–72.
Pasternak, A., Hillebrand, H., Floder, S., 2009. Competition between benthic and pelagic microalgae for phosphorus and light — long-term experiments using artificial substrates. Aquat. Sci. 71, 238–249.
Pohnert, G., Steinke, M., Tollrian, R., 2007. Chemical cues, defence metabolites and the shaping of pelagic interspecific interactions. TREE 22, 198–204.
Popovich, C.A., Spetter, C.V., Marcovecchio, J.E., Freije, R.H., 2008. Dissolved nutrient availability during winter diatom bloom in a turbid and shallow estuary (Bahl'a Blanca, Argentina). J.Coast. Res. 24, 95–102.
Revilla, T., Weissing, F.J., 2008. Nonequilibrium coexistence in a competition model with nutrient storage. Ecology 89, 865–877.
Reynolds, C.S., 1999. Non-determinism to probability, or N:P in the community ecology of phytoplankton. Arch. Hydrobiol. 146, 23–35.
Rhee, G.-Y., 1978. Effects of N:P atomic ratios and nitrate limitation on algal growth, cell composition and nitrate uptake. Limnol. Oceanogr. 23, 10–25.
Rhee, G.-Y., Gotham, I.J., 1980. Optimum N:P ratios and coexistence of planktonic algae. J. Phycol. 16, 486–489.
Roelke, D.L., Eldridge, P.M., Cifuentes, L.A., 1999. A model of phytoplankton competition for limiting and nonlimiting nutrients: implications for development of estuarine and nearshore management schemes. Estuaries 22, 92–104.
Smith, V.H., 1993. Applicability of resource-ratio theory to microbial ecology. Limnol. Oceanogr. 38, 239–249.
Sommer, U., 1993. Phytoplankton competition in Pluβsee — a field-test of the resource-ratio hypothesis. Limnol. Oceanogr. 38, 838–845.
Sommer, U., 1999. A comment on the proper use of nutrient ratios in microalgal ecology. Arch. Hydrobiol. 146, 55–64.
Sommer, U., Hansen, T., Stibor, H., Vadstein, O., 2004. Persistence of phytoplankton responses to different Si:N ratios under mesozooplankton grazing pressure: a mesocosm study with NE Atlantic plankton. Mar. Ecol. Prog. Ser. 278, 67–75.
Stelzer, R.S., Lamberti, G.A., 2001. Effects of N:P ratio and total nutrient concentration on stream periphyton community structure, biomass, and elemental composition. Limnol. Oceanogr. 46, 356–367.
Tilman, D., 1977. Resource competition between planktonic algae — experimental and theoretical approach. Ecology 58, 338–348.
Tilman, D., 1982. Resource Competition and Community Structure. Princeton Univeristy Press, Princeton NJ, U.S.A.
Tilman, D., 2007. Resource competition and plant traits: a response to Craine et al. 2005. J. Ecol. 95, 231–234.
Turpin, D.H., 1986. Growth rate dependent optimum ratios in *Selenastrum minutum* (Chlorophyta): implications for competition, coexistence and stability in phytoplankton communities. J. Phycol. 22, 94–102.
Urabe, J., 1993. N-cycling and P-cycling coupled by grazers activities — food quality and nutrient release by zooplankton. Ecology 74, 2337–2350.
Veldhuis, M.J.W., Colijn, F., Admiraal, W., 1991. Phosphate utilization in *Phaeocystis pouchetii* (haptophyceae). Mar. Ecol. Pub. Stat. Zoo Napoli 12, 53–62.