

Harmful algal blooms and eutrophication: “strategies” for nutrient uptake and growth outside the Redfield comfort zone

Patricia M. GLIBERT^{1,*}, JoAnn M. BURKHOLDER²

¹ University of Maryland Center for Environmental Science, Horn Point Laboratory, PO Box 775, Cambridge, MD 21613, USA

² Center for Applied Aquatic Ecology, North Carolina State University, Raleigh, NC 27606, USA

Received Nov. 12, 2010; revision accepted Mar. 05, 2011

© Chinese Society for Oceanology and Limnology, Science Press, and Springer-Verlag Berlin Heidelberg 2011

Abstract While many harmful algal blooms have been associated with increasing eutrophication, not all species respond similarly and the increasing challenge, especially for resource managers, is to determine which blooms are related to eutrophication and to understand why particular species proliferate under specific nutrient conditions. The overall goal of this brief review is to describe why nutrient loads are not changing in stoichiometric proportion to the “Redfield ratio”, and why this has important consequences for algal growth. Many types of harmful algae appear to be able to thrive, and/or increase their production of toxins, when nutrient loads are not in proportion classically identified as Redfield ratios. Here we also describe some of the physiological mechanisms of different species to take up nutrients and to thrive under conditions of nutrient imbalance.

Keyword: HABs; mixotrophy; nitrogen; nutrient loading; nutrient ratios; nutrient stoichiometry; organic nutrients; phosphorus

1 INTRODUCTION

Harmful algae (HA) are considered here as species that produce toxins which result in shellfish toxicity, cause fish kills due to direct toxicity and indirect effects or alter ecosystem function in a manner that adversely affects trophic transfer (Granéli and Turner, 2006). Some HA are toxic and cause harm even when present at low levels in an algal assemblage; others cause harmful algal blooms (HABs), which, in the extreme can attain near monospecific proportions.

All blooms require nutrients to be sustained. There, is a general growing awareness that eutrophication is one of the major causes of many blooms (Anderson et al., 1989, 2002, 2008; Glibert et al., 2005a, b, 2010; Glibert and Burkholder, 2006; Heisler et al., 2008), although not all blooms are the result of anthropogenic changes in nutrient loadings. Eutrophication and nutrient pollution are occurring due to increasing human population, increasing demands on energy, increases in nitrogen (N) and phosphorus (P) fertilizer use for agriculture, changes

in diet leading to more meat production and animal waste, and expanding aquaculture industries (Smil, 2001; Galloway and Cowling, 2002; Galloway et al., 2002; Howarth et al., 2002; Wassmann, 2005; Howarth, 2008; Glibert et al., 2010; Fig.1). While increased total algal biomass is often an obvious response to nutrient loading (Cloern, 2001; Anderson et al., 2002), the overall species-specific response will depend upon the physiology of the mix of organisms present, the environmental conditions, and the form of nutrient supplied (Glibert and Burkholder, 2006; Burkholder et al., 2008). Relationships have been shown between P loads and, secondarily, N loads and harmful cyanobacteria blooms (Schindler, 1977; Burkholder, 2002), and increasing linkages between nutrient loading (N, P) and estuarine/coastal marine HABs have more recently been recognized (Smayda, 1990, 1997; Anderson et al., 2002; Trainer et al., 2003; Glibert et al., 2005a, b; Glibert and Burkholder, 2006).

* Corresponding author: glibert@umces.edu

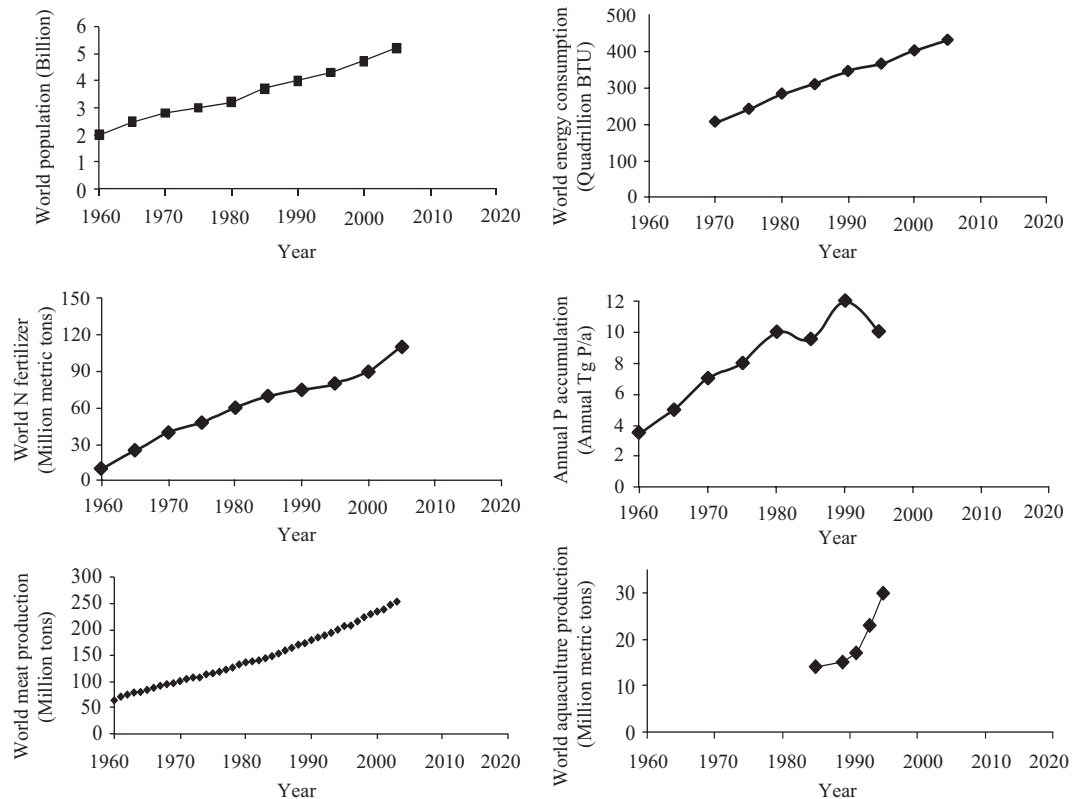


Fig.1 General trends in population, energy consumption, fertilizer use and accumulation, meat and aquaculture production from 1960 to present

Data compiled from www.census.gov/ipc/www/img/worldpop.gif, the Global Fertilizer Industry, www.fertilizer.org, and the Food and Agriculture Organization (FAO) of the United Nations (2007). Figure reproduced from Glibert et al. (2010), with permission of the publisher.

These recent examples include not only species that form high-biomass blooms, but also some that are toxic but not necessarily in high abundance. High-biomass algal blooms often result in reduced transfer of energy to higher trophic levels, as many HAB species are not efficiently grazed, resulting in a decreased transfer of carbon and other nutrients to fish stocks when HAB species replace more readily consumed algal species (Irigoien et al., 2005; Mitra and Flynn, 2006).

The increasing challenge is to determine which blooms are related to eutrophication, and to understand why particular species proliferate under specific nutrient conditions. Nutrients are not the only factors that define species success, but if the relationships between HABs and anthropogenic nutrient loading can be better understood, there may be opportunities to identify management strategies for prevention or reduction of such blooms. The relationships between nutrient loading and HAB proliferation are complex, and numerous efforts are ongoing to identify the loads and forms of nutrients derived from land-based sources, the biogeochemical

pathways of nutrient transformation leading to HABs, and the nutritional “strategies” of HABs in comparison to non-HAB species, as well as the development of models that capture these complexities (Glibert and Burkholder, 2006; Rothenberger et al., 2009; Glibert et al., 2010; Flynn, 2010).

Although eutrophication is occurring globally, nutrient export from coastal watersheds is not evenly distributed, leading to highly varying N and P loads to coastal waters (Seitzinger et al., 2002, 2005; Howarth et al., 2005; Burkholder et al., 2006; Glibert et al., 2006a). Global inorganic N export to coastal waters is estimated to be highest from European and Asian watersheds, although significant discharges also are contributed from the United States and other parts of the world (Seitzinger and Kroeze, 1998; Dumont et al., 2005; Harrison et al., 2005a, b; Van Drecht et al., 2005; Bouwman et al., 2009; Seitzinger et al., 2009). Rates of nutrient consumption have increased dramatically in recent years in some parts of the world; moreover, the relative consumption of N and P is not proportional across geographic regions (Seitzinger et al., 2005; Bouwman et al., 2009). For

example, Asia now consumes 59% of world N fertilizer and 54% of world P fertilizer, while Latin America consumes only 6.4% of world N fertilizer and 15% of world P fertilizer (FAO, 2005).

In addition to the global increase in nutrient loading and the alteration of N:P in land-based nutrient loads, there has also been a worldwide change in the quality of N applied as land-based fertilizers. In particular, use of urea as a N fertilizer and feed additive increased more than 100-fold in the past four decades, and doubled in the decade from 1990–2000 alone (Glibert et al., 2006a; Fig.2). There is mounting evidence that urea differentially stimulates the growth of some types of phytoplankton in coastal waters and that it may, under some conditions, promote a shift in phytoplankton species to organisms that are more noxious to the ecosystem and to human health (Berg et al., 1997, 2003; Gobler et al., 2002; Glibert et al., 2001, 2004, 2005b). For example, for exponentially-growing cultures of *Pseudo-nitzschia australis*, nitrate (NO_3^-) and ammonium (NH_4^+)-grown cells produce equivalent amounts of dissolved and particulate domoic acid (DA), whereas DA production is enhanced in cultures grown solely on urea (Cochlan et al., 2005; Armstrong-Howard et al., 2007). Urea is rapidly hydrolyzed to NH_4^+ in the environment, but the rate of hydrolysis depends on pH, ambient temperature, whether the urea fertilizers were chemically treated with other additives, and other factors (Glibert et al., 2006a).

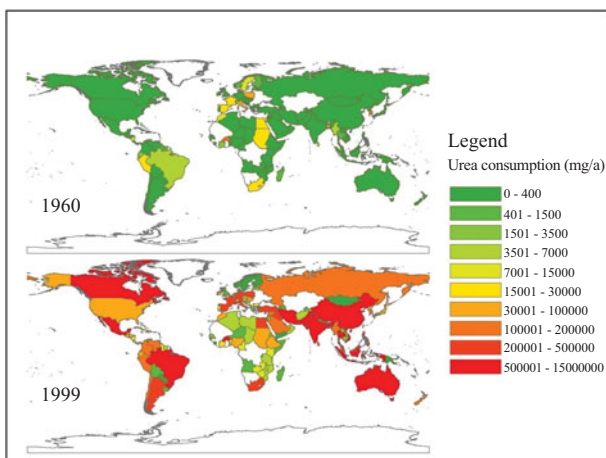


Fig.2 Global distribution of the consumption of urea fertilizer, in tonnes per year by country, in 1960 (upper panel) and in 1999 (lower panel), based on data from the Global Fertilizer Industry data base (FAO 2001)

Figure reproduced from Glibert et al. (2006), *Biogeochem.*, with permission of the publisher.

Other factors also contribute to the increase in reduced, as compared to oxidized, forms of N. One of these is the discharge of sewage effluent. In the U.S., many treatment plants provide secondary treatment and discharge substantial NH_4^+ . For example, one treatment plant in the San Francisco Bay Delta system has increased the discharge of NH_4^+ from 5 to 14 tonnes per day into the Sacramento River over the period from 1982 (when the plant first came on line) to the present (Van Nieuwenhuysse, 2007; Glibert, 2010; Fig.3), which, in turn, has altered the ratio of NO_3^- to NH_4^+ in the receiving waters (Dudgale et al., 2007). Other land-based nutrient sources that contribute to the discharge of NH_4^+ are confined animal feed operations (CAFOs), for which there is little waste treatment (Burkholder et al., 1997 and references therein; Mallin, 2000). Intensification of industrialized animal agriculture is considered a major reason why NH_4^+ concentrations have increased over the past decade in the Neuse River Estuary, a major tributary of the second largest estuary on the U.S. mainland, the Albemarle-Pamlico Estuarine System, and the largest contributor of nutrients to that system (Burkholder et al., 2006, and references therein). Atmospheric deposition of NH_4^+ is yet another important source of reduced N in many coastal environments, contributing as much as 50% of the N load in some areas (National Research Council, 2000; Galloway and Cowling, 2002; Costanza et al., 2008). Of interest with respect to HABs is the fact that many types of HA can thrive and/or increase toxin production when nutrient loads are not in Redfieldian proportion and are not dominated by inorganic nutrient forms. The end result is that reduced forms of N are increasing in many coastal ecosystems.

The consequence of these alterations in global N and P is that many receiving waters are now not

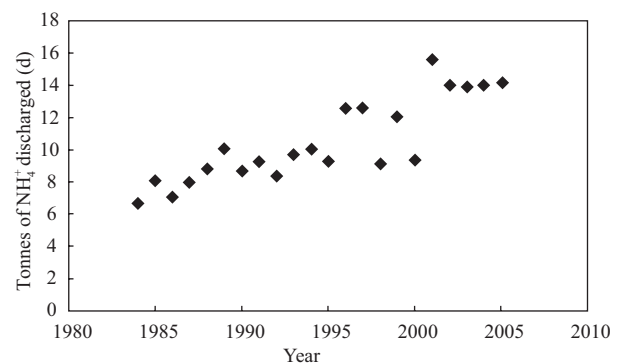


Fig.3 Change in loading of ammonium (tonnes/d) from the effluent of the Sacramento Regional Wastewater Treatment Plant, located on the Sacramento River, California, USA

only enriched with nutrients, but also these nutrients are in a different stoichiometric proportion, and the chemical forms of N are different from those of decades past. The relative proportion of N and P in nutrient loads to many aquatic environments also diverge considerably from the nutrient ratios that have long been associated with phytoplankton growth: the Redfield proportions of 16:1 for N:P on a molar basis (Redfield, 1934, 1956; Harris, 1986). Various surveys of the “optimal” N:P molar ratios in a broad range of phytoplankton found that, while the data clustered around the Redfield ratio, there were numerous examples at both the high and low ends of the spectrum (Hecky and Kilham, 1988; Klausmeier et al., 2004). Note that the “optimum” N:P is the ratio of the values where the cell maintains the minimum N and P cell quotas (Klausmeier et al., 2004).

The N:P ratio provides a simple, easily measurable index, and total, particulate and/or dissolved N:P ratios are frequently used to evaluate nutrient status (Fisher et al., 1992; Glibert et al., 1995, 2004, 2006b). Changes in this ratio have been compared to shifts in phytoplankton composition, yielding insights about the dynamics of nutrient regulation of plankton assemblages (Tilman, 1977; Smayda, 1990; Hodgkiss and Ho, 1997; Hodgkiss, 2001; Heil et al., 2007).

N:P ratios can be useful in a relative sense, the same ratio can be obtained from vastly different numerators and denominators, as long as their proportions remain the same. Thus, an elevated N:P ratio, suggestive of P limitation, can be obtained by a depletion in P (true P limitation), or by an increase in N without a corresponding depletion in P. Moreover, depending on how the ratio is calculated, it may not fully reflect the full availability of nutrients available to cells. Most applications of N:P ratios consider only inorganic forms of N and P. Different ratios may be obtained depending on which form(s) is (are) included in the ratio (Dodds, 2003). Thus, inferences about whether a system (or a cell) is N- or P-limited may differ substantially depending upon whether the N:P ratio is calculated solely with inorganic forms of N and P, or with both inorganic and organic forms. The case is made here that while inorganic Redfield ratios can yield useful information, they do not provide a complete perspective of the nutrients available. Variable nutrient forms, accessible to some but not all phytoplankton, is one reason why some species can thrive under non-Redfieldian conditions. The goal

here is to describe some nutritional “strategies” that may contribute to the success of HABs in nutrient environments where the N:P ratio is not in Redfieldian proportion.

2 HABs AND NON-REDFIELD NUTRIENT RATIOS

Many phytoplankton have a biomass composition that generally reflects the nutrient composition of their external environment, but under transient conditions, some phytoplankton may have highly variable N:P ratios, depending on their ability to take up and store nutrients (Flynn, 2002). An example of steady-state conditions that led to variable N:P in biomass is that of the chlorophyte *Scenedesmus* when grown over a range of N:P ratios in culture (Rhee, 1978). HABs also occur under a wide range of nutrient conditions, often when DIN:DIP ratios are altered and deviate from the Redfieldian proportion. For example, decreases in N:P ratios due to P loading have sometimes been related to increased abundance of certain harmful dinoflagellate species. In Tolo Harbor, Hong Kong, where P loading increased due to human population growth in the late 1980s, a shift from diatoms to dinoflagellates was observed, coincident with a decrease in the ambient N:P ratio from ca. 20:1 to <10:1 (Hodgkiss and Ho, 1997; Hodgkiss, 2001). On shorter time scales, in Tunisian aquaculture lagoons, blooms of toxic dinoflagellates developed when the N:P ratio decreased in autumn (Romdhane et al., 1998). Blooms of the toxic dinoflagellate *Karenia brevis* on the western Florida shelf occur in waters with lower dissolved inorganic N:P ratios than in water directly to the south with higher N:P ratios, where diatoms tend to be more prevalent (Heil et al., 2007). The following sections highlight several key physiological strategies that contribute to the success of these types of organisms under non-Redfieldian conditions.

2.1 High N:P environments

There are numerous mechanisms or “strategies” for coping successfully with an imbalance in N:P ratios wherein P available is low relative to that of N: 1) Make do with less P relative to the needs of competing organisms; 2) Use an alternate form of P compared to competing organisms; 3) Use mixotrophy to obtain the requisite nutrient through consuming other organisms or dissolved organic substrates; 4) Acquire the required nutrient and

release what is not needed; 5) Maintain defense strategies to maintain viability at low growth rates; and 6) Use metabolism to create a favorable microenvironment. Each of these is described below.

Different phytoplankton taxa differ in cell size by up to orders of magnitude, and cell size is an important determinant of elemental composition (Harris, 1986; Finkel et al., 2010). Small cells have a lower requirement for P due to the smaller need for structural components in the cell (Finkel et al., 2010, Fig.4). In comparison to diatoms, cyanobacteria such as *Synechococcus* have a much larger cellular C:P ratio, on average (Finkel et al., 2010). It is, then, not surprising that small cells, such as *Synechococcus*, have been found to thrive in waters that are comparatively P-poor, such as Florida Bay (Glibert et al., 2004). A second mechanism for “making do with less” is physiological substitution of a P-requiring cellular compound with a non-P-requiring compound. Such substitutions have been found, for example, in various lipids (Van Mooney et al., 2009; Fig.5). It should be noted, however, that while many cyanobacteria can substitute P-containing lipids with non-P-containing lipids, bacteria and many eukaryotes do not appear to have this ability (Van Mooney et al., 2009).

Another mechanism for thriving in relatively P-poor environments is to make use of P sources that are not available to competitors. The use of dissolved organic P (DOP), for example, is common among many phytoplankton species, including HA. The bioavailability of a given DOP compound depends upon a range of factors, from its composition to the physiology of the organisms present (reviewed by Dyhrman and Ruttenberg, 2006). The most labile DOP forms are generally considered to be phosphomonoesters, although some cyanobacteria may be able to use phosphonates, and there is recent evidence that some species may also be able to take up phosphines (Karl and Björkman, 2001; Palenik et al., 2003; Dyhrman and Ruttenberg, 2006). Multiple enzyme systems are involved in the use of these different DOP substrates (Skelton et al., 2006), among which the alkaline phosphatases (APs) are the best known (Chróst, 1991; Uchida, 1992; Dyhrman and Ruttenberg, 2006). Dinoflagellates and cyanobacteria include harmful representatives that use APs to cleave phosphate from phosphomonoesters. In the East China Sea, where large-scale blooms of the dinoflagellate *Prorocentrum*

donghaiense have occurred virtually every year in the past decade, the mean AP activity (APA) in the bloom area was significantly higher than in the non-HAB area (Zhou et al., 2003; Huang et al., 2007; Li et al., 2009). Furthermore, species-specific APAs measured in natural assemblages during blooms showed that diatoms and chrysophytes had low APA, while dinoflagellates had the highest APA, although significant differences also existed among individual species within a given algal phylum (Huang et al., 2007). Florida Bay and the mouth of the Caloosahatchee River, systems both dominated by cyanobacteria, similarly have been reported to have high APA (Glibert et al., 2004; Heil et al., 2007, 2009). Higher APA was observed in the south-central region of Florida Bay compared to the northwest and north-central regions (Cotner et al., 2000), and long-term median APA (on a volumetric basis) was higher in the central region than in the eastern and western basin (Boyer et al., 1999). In another P-limited system, the Northern Gulf of Aqaba, APA was significantly correlated with abundance of *Synechococcus* sp. (Li et al., 1998).

Mixotrophy provides yet another mechanism for some algal species to acquire a needed resource even when it is not available in dissolved substrate form, and this form of nutrition is widespread among algae in both nutrient-poor and nutrient-rich habitats. Many flagellate species, including various harmful dinoflagellates, are mixotrophic or heterotrophic phagotrophs that consume predominantly particulate rather than dissolved nutrients (Nygaard and Tobiesen, 1993; Stoecker, 1999; Parrow and Burkholder, 2003; Jeong et al., 2005a, b, c, 2010). As reviewed by Burkholder et al. (2008), many authors have suggested that mixotrophy is operable and advantageous in nutrient-poor habitats as a mechanism to supplement nutrient supplies (Granéli et al., 1999; Stibor and Sommer, 2003; Stoecker et al., 2006). Stibor and Sommer (2003) showed that the simultaneous uptake of P by the harmful haptophyte, *Chrysochromulina polylepis*, from dissolved inorganic and particulate (radiolabeled bacteria) sources followed basic predictions of optimal foraging theory (Stephens and Krebs, 1986). The onset of mixotrophy depended upon the dissolved inorganic P concentration: At low concentrations of dissolved inorganic P (DIP), *C. polylepis* took up P from both bacterial and dissolved sources, whereas the major source was DIP under more water column-enriched conditions. Experiments on phagotrophy with fluorescently

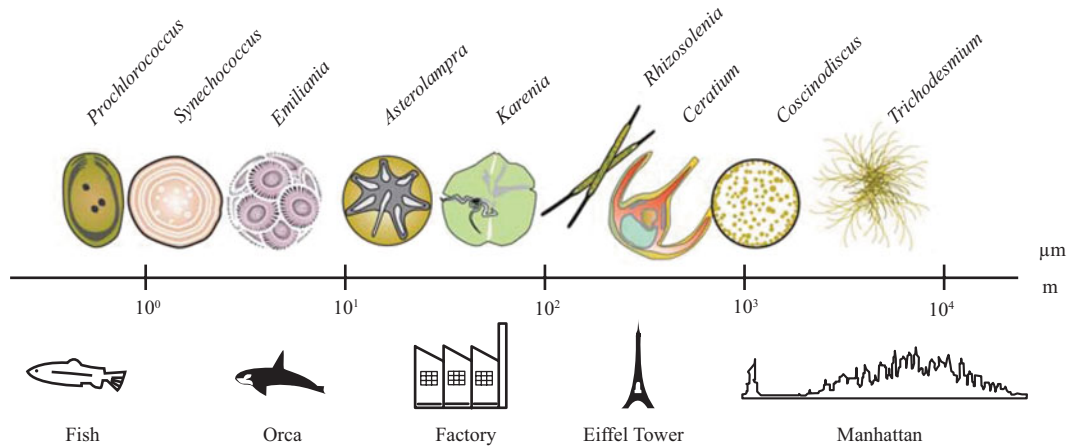


Fig.4 Schematic depiction of the range in cell size of various phytoplankton in relation to well recognized objects
 Reproduced from Finkel et al. (2010), with permission of the publisher.

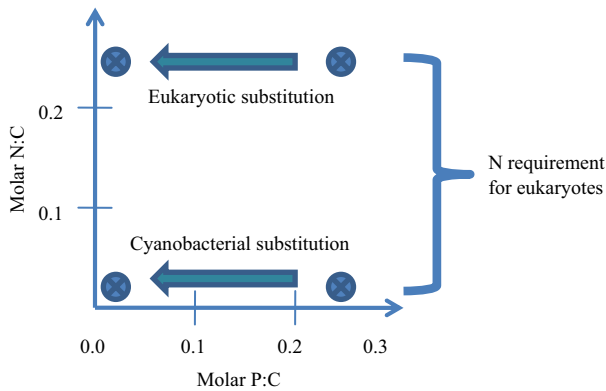


Fig.5 Schematic depiction of the C:N:P stoichiometric changes that can occur in eukaryotes and prokaryotes due to different types of lipids
 The 4 circles represent various lipids. While lipids with low P:C can be substituted by some species for lipids with high P:C, similar substitutions do not occur for lipids ranging in N:C. Redrawn from Van Mooy et al. (2009).

labeled bacteria suggested that bacterivory can be an important source of P for the *C. polylepis* during blooms in coastal Norwegian waters. (Nygaard and Tobiesen, 1993). Ingestion of P-rich bacteria by *C. polylepis* (~6 cell/h) in the natural phytoplankton assemblage from surface waters comprised about 60% of the total bacterial grazing. Supporting laboratory experiments on several harmful species fed fluorescently labeled bacteria or radiolabeled bacteria (¹⁴C- amino acids) indicated that bacterivory increased under P limitation, and provided significantly more P than was needed to maintain equilibrium population growth rates (*k*=0.3/d). On the other hand, many harmful algal species in nutrient-rich environments have also been found to

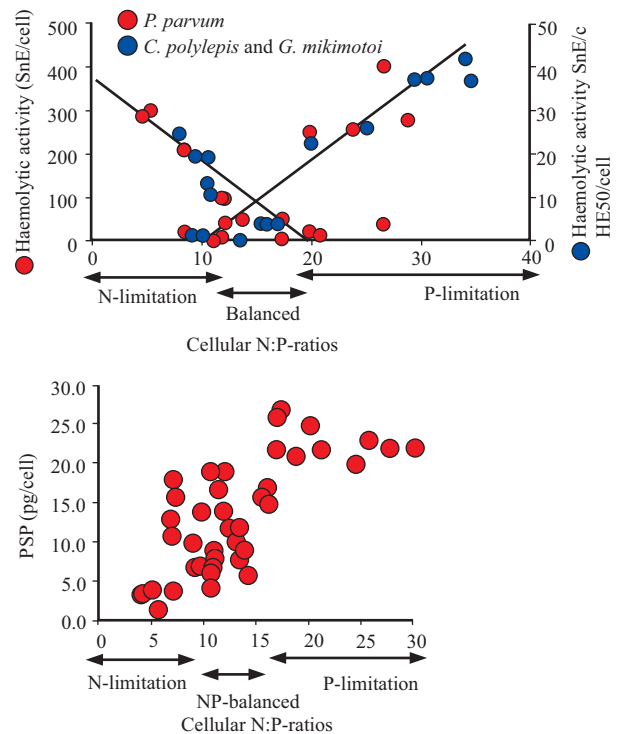


Fig.6 Upper panel: Relation between toxicity and nitrogen/phosphorus for ichthyotoxic species. Lower panel: Relation between toxicity and nitrogen to phosphorus ratio for the paralytic shellfish toxin-producing species *Alexandrium tamarense*

Reproduced from Granéli et al., www.ut.ee/~olli/eutr/, upper panel redrawn from Johansson and Granéli (1999a, b) with permission of the author.

be mixotrophic, phagocitizing other microorganisms that are more abundant in the eutrophic conditions (Burkholder et al., 2008).
 High N:P conditions are more likely to promote species that have slow growth rates. This is due, in

part, to a high P demand for ribosomes that are required to maintain high growth rates (Sterner and Elser, 2002). Many HA are not necessarily rapidly growing cells, but have defense mechanisms that allow them to maintain viability even when they cannot outgrow their competitors. For example, many phytoplankton, including harmful cyanobacteria, dinoflagellates, haptophytes and raphidophytes, produce allelopathic chemicals that negatively affect the growth of other organisms (Legrand et al., 2003; Granéli and Hansen, 2006). In fact, allelopathy, here including toxin production, sometimes has increased when cells that first are in nutrient-replete conditions become N- or P-limited (Granéli and Hansen, 2006). Examples are the haptophytes *C. polylepis* and *Prymnesium parvum*, both of which have strong negative effects on other protists when in a nutrient-deficient state following an enriched condition (Johansen and Granéli, 1999a, b). The toxins of these two species lyse their competitors within minutes, thereby not only reducing competition but also releasing nutrients from the lysed cells to the water column (Hansen, 1998; Uchida, 2001; Fistarol et al., 2003). Allelopathic interactions between *Prorocentrum micans* and *Karenia mikimotoi* have also been observed (Ji et al., 2011).

Toxin production by numerous other HA has been shown to increase when the cells are not grown under nutrient-balanced conditions, as well as when they sustain a change in N or P availability or depletion (Flynn et al., 1994, 1995; Johansson and Granéli, 1999a, b; Granéli and Flynn, 2006). Production of toxins rich in N might be regarded as a dissipatory mechanism, whereby cells acquire the nutrient(s) they need but release (or sequester) nutrients that are not needed. In some algal flagellates, toxin production increases under P stress (Granéli et al., 1998; John and Flynn, 2002).

Whether these toxins are used for defense or other purposes is irrelevant to their function in stoichiometric homeostasis. Among cyanobacteria, for example, various studies have related increasing N and increasing N:P ratios to increased toxicity of *Microcystis aeruginosa*. In Daechung Reservoir, Korea, *M. aeruginosa* toxicity was related not only to an increase in N in the water, but also to cellular N content (Oh et al., 2001). In P-limited chemostats, Oh et al. (2000) observed that while *M. aeruginosa* growth declined as the degree of P limitation increased, more microcystins were produced. Excess N has also been related to microcystin production

under controlled chemostat conditions (Van de Waal et al., 2009, 2010). In the dinoflagellate *Alexandrium tamarense*, saxitoxin production increased by 3- to 4-fold under P deficiency (Boyer et al., 1987; reviewed by Granéli, 2005; Granéli and Flynn, 2006; Fig.6). Similarly, toxin production by the dinoflagellates *Gymnodinium catenatum*, *Alexandrium excavatum* and the diatom *Pseudo-nitzschia multiseriata* also increased under P stress (Granéli and Flynn, 2006). In the case of the latter species, this effect was enhanced when the N source was NH_4^+ rather than NO_3^- (Granéli and Flynn, 2006).

In addition to allelochemical alteration of their environment, some species are able to effectively create their own microenvironment through other means. Having the ability to concentrate carbon by various mechanisms, for example, can dramatically alter the microhabitat. Carbon concentrating mechanisms allow cells to continue to take up carbon even when CO_2 is depleted, and involves altering the equilibrium of HCO_3^- and CO_2 (Merrett, 1991; Miller et al., 1991; Price and Badger, 1991; Raven and Johnson, 1991; Ratti et al., 2007). The advantage to nutrient acquisition is that by maintaining the ability to photosynthesize even under high-biomass, potentially otherwise carbon-limiting conditions, cell metabolism will elevate pH. In turn, the fundamental physical-chemical relationships related to P adsorption-desorption will be altered. Enhancement of sediment P release under elevated water-column pH has been observed in eutrophic lakes (Andersen, 1974; Drake and Heaney, 1987; Jensen and Andersen, 1992; Xie et al., 2003) and tidal fresh/oligohaline estuaries (Seitzinger, 1991). Indeed, PO_4^{3-} flux from Potomac Estuary sediments in experimental cores increased from $<5 \mu\text{mol}/\text{m}^2\cdot\text{h}$ to nearly $30 \mu\text{mol}/\text{m}^2\cdot\text{h}$ in $<24 \text{ h}$ when the pH increased from 7.8 to 9.5 (Bailey et al., 2006). Thus, maintaining a high rate of photosynthesis via carbon-concentrating mechanisms can allow cells to alter biogeochemical processes to mobilize the nutrient they require.

2.2 Low N:P environments

As with high N:P environments, there are also various mechanisms for coping successfully with an imbalance in N:P ratios when N is low relative to P availability: 1) Fix N_2 ; 2) Use a different form of N than used by competing organisms; 3) Use mixotrophy to obtain the requisite nutrient via phagotrophy rather than through absorption of dissolved substrates; 4) Acquire the required nutrient

and release what is not needed; and 5) Maintain defense strategies to maintain viability at low growth rates (described above).

The most well recognized mechanism for successful growth under N-limiting conditions is N_2 fixation. Only certain cyanobacteria have this capability, including some HA. Many ecosystems having low N:P ratios have been shown to be susceptible to blooms of N_2 -fixing cyanobacteria (Smith, 1983, 1990; Burkholder, 2002, 2009 and references therein). In fact, spectacular biomass accumulations can result from blooms of N_2 -fixing cyanobacteria, such as *Nodularia* blooms in the Baltic Sea (Zillén and Conley, 2010). Once N_2 -fixing cyanobacteria become established, other cyanobacteria may co-occur, allowing for mixed consortia of species (Paerl et al., 2001).

Under conditions when inorganic N is low and N:P stoichiometry is also “tilted” toward the low end, alternate forms of N are used by many phytoplankton. DON substrates are acquired by direct uptake, extracellular oxidation and hydrolysis, and pinocytosis (Glibert and Legrand, 2006; Lewitus, 2006). As reviewed by Burkholder et al. (2008), many HA, including dinoflagellates, cyanobacteria, pelagophytes (brown tide species), and others, use organic (dissolved or particulate) nutrient forms for some or all of their N, P, and/or C demands (Burkholder and Glasgow, 1997; Granéli et al., 1997, 1999; Berg et al., 1997, 2002; Lewitus et al., 1999; Stoecker, 1999; Kudela and Cochlan, 2000; Berman, 2001; Glibert et al., 2001, 2006a, b, 2007; Lomas et al., 2001, 2004; Mulholland et al., 2002, 2004; Gobler et al., 2005; Glibert and Legrand, 2006; Lewitus, 2006; Herndon and Cochlan, 2007; Kudela et al., 2008; Cochlan et al., 2008).

Urea is of special concern due to the global escalation in the anthropogenic use of this form of N and its association with many HABs, especially of cyanobacteria and dinoflagellates (Glibert et al., 2005a, 2006a). As reviewed by Solomon et al. (2010), urea supports a large fraction of the N demand of many HABs including the dinoflagellate *Lingulodinium polyedrum* off the coast of Mexico (Kudela and Cochlan, 2000), the dinoflagellate *Alexandrium catenella* in the Thau Lagoon of southern France (Collos et al., 2004), and the pelagophyte *Aureococcus anophagefferens* in Great South Bay and Peconic Bay, New York, USA (Lomas et al., 1996; Berg et al., 1997; Gobler et al., 2002). Published rates of urease activity in culture suggest that dinoflagellates have higher urease activities

on a per-cell basis, while cyanobacteria and *A. anophagefferens* have higher rates of urease activity on a per-cell-volume basis than other phytoplankton taxonomic groups (Solomon et al., 2010).

As reviewed by Burkholder et al. (2008), extracellular oxidation and hydrolysis of amino acids and proteins is also a common mechanism used by many HAB species to acquire N (Palenik and Morel, 1990; Mulholland et al., 1998, 2003; Stoecker and Gustafson, 2003; Dyhrman, 2005). For example, *Prymnesium parvum* uses cell-surface L-amino acid oxidases to oxidize amino acids and primary amines, and takes up the resulting NH_4^+ (Palenik and Morel, 1990). Stoecker and Gustafson (2003) demonstrated that leucine amino peptidase activity in a natural estuarine phytoplankton assemblage was associated with a dinoflagellate bloom, and in non-axenic cultures of bloom species *Akashiwo sanguinea*, *Gonyaulax grindleyi*, *Gyrodinium uncatenum*, *Karlodinium veneficum*, and *Prorocentrum minimum*, leucine amino peptidase activity was associated with dinoflagellates rather than bacteria. Mulholland et al. (2002) reported that peptide hydrolysis and amino acid oxidation were associated with the size fraction containing brown tide, *A. anophagefferens*, in natural samples from Long Island, New York.

Nitrogen fixation may also contribute to organic N availability for some HAB species. Blooms of *Karenina brevis* in the Gulf of Mexico have been hypothesized to initiate partly in response to organic N that becomes available following blooms of the cyanobacterium *Trichodesmium* (Walsh and Steidinger, 2001; Mulholland et al., 2006). *Trichodesmium* may release a significant fraction of its newly fixed N in the form of dissolved organic N (DON) (Glibert and Bronk, 1994), and estimates of this potential contribution suggest that DON from *Trichodesmium* may be sufficient to support moderately dense ($\leq 10^5$ cell/L) blooms of *K. brevis* (Mulholland et al., 2006). Dissolved organic N (DON) concentrations in West Florida estuaries and coastal waters are about 10-fold higher than inorganic N concentrations, and while inorganic N:P ratios are low, DON:DOP (dissolved organic P) ratios are consistently higher than the Redfield ratio (Heil et al., 2007; Fig.7).

Mixotrophy is another mechanism that some cells may use to acquire N, similar to that described above for acquisition of P. Mixotrophic dinoflagellates can ingest a wide variety of different prey items, including bacteria, other algae and protists, and fish tissue (Nygaard and Tobiesen, 1993; Stoecker,

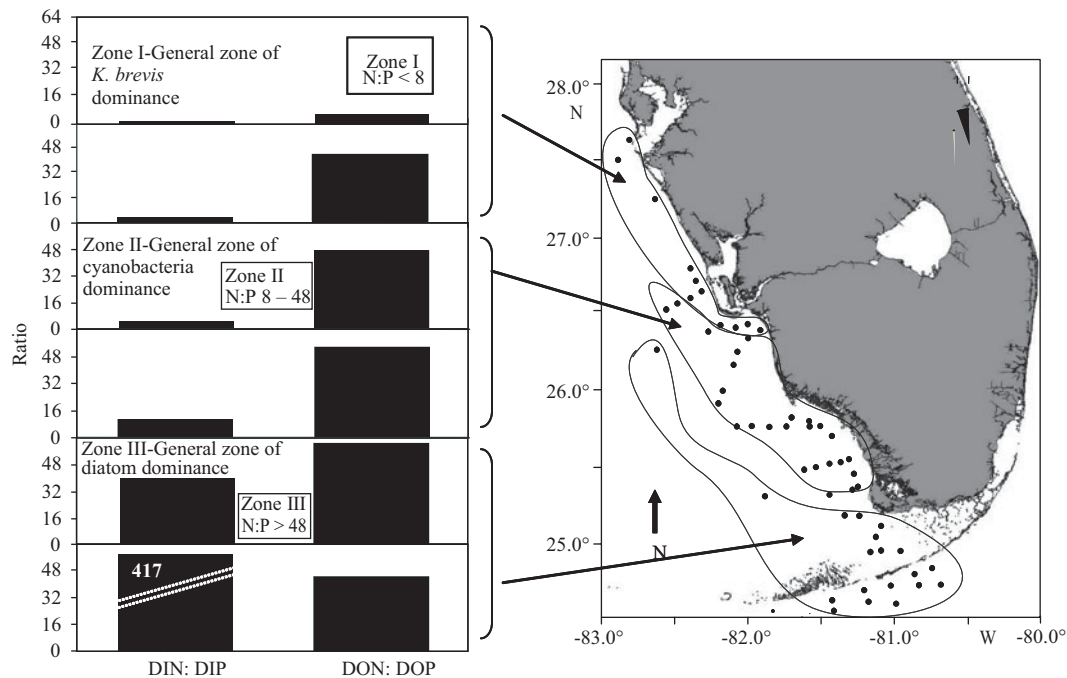


Fig.7 Mean dissolved inorganic and organic N:P ratios for each of the major riverine systems of western Florida shelf sampled during the dry season of May 2004

Each bar represents the means of the stations sampled along an individual riverine transect. The insert box indicates the mean particulate N:P ratio. The general zones are indicated on the companion map of southern Florida. Reproduced from Heisler et al. (2008), with permission of the publisher.

1999; Burkholder et al., 2001, 2008; Stoecker et al., 2006; Seong et al., 2006). The cyanobacterium *Synechococcus* has been suggested to be a source for N due to its ubiquitous nature in both offshore and coastal waters, its potential for high growth rates (Kana and Glibert, 1987), and its high N content relative to P (Finkel et al., 2011). *Synechococcus* co-occurs with many dinoflagellates (Tarran et al., 1999; Murrell and Lores, 2004). The Florida red tide dinoflagellate, *K. brevis* illustrates an important implication of mixotrophy. Not only does this species have the ability to graze *Synechococcus* at substantial rates (0.96–83.8 prey cell/*K. brevis*-h; Jeong et al., 2005a; Glibert et al., 2009), but also the growth rate of mixotrophic *K. brevis* was higher than growth under phototrophic conditions alone (Glibert et al., 2009). Numerous examples of other species, such as *Karlodinium veneficum* (Adolf et al., 2008) and *Cochlodinium polykrikoides* (Jeong et al., 2004) obtaining a growth rate benefit from mixotrophy have also been reported (Burkholder et al., 2008).

Lastly, as for P-limited cells, under N-limitation following enriched conditions, some harmful algae increase toxin production (Granéli and Hansen, 2006). Toxin production by the flagellates *P. parvum* and *C. polylepis* increases under N as well as

P stress, relative to toxin production in more stoichiometrically balanced growth conditions (Johansson and Granéli, 1999a, b; Granéli and Flynn, 2006) (Fig.6). However, the mechanism(s) of toxin production for stoichiometric balance under N limitation appears to be less common than under P limitation, perhaps in part because many toxins are N-rich (Granéli and Flynn, 2006).

3 MANAGEMENT IMPLICATION

Alterations in nutrient stoichiometry, either through disproportionate N and P loads, or through management actions whereby one nutrient is controlled without another, can have profound consequences on algal assemblages. In some cases, these consequences can cascade through the food web, broadly affecting the trophodynamics (Sterner and Elser, 2002; Glibert, 2010). Many HAB species, including toxic representatives, have the ability to acquire nutrients when in disproportionate supply, and also can produce nutrient-rich toxic compounds, making these species excellent competitors under non-Redfieldian conditions. The historic view of phytoplankton responses to eutrophication—increased nutrients promote increased chlorophyll and high-biomass blooms, leading to and losses in

habitat (Cloern, 2001; Wetzel, 2001)—is too simplistic for understanding ecosystem responses to the major changes in nutrient loads, forms, and stoichiometry that many systems are now sustaining. Nutrient forms and their proportions are important, and many HABs have physiological mechanisms that enable them to thrive in surface waters throughout the world that are being dramatically altered by human influence.

4 ACKNOWLEDGEMENT

This review is a contribution of the GEOHAB Core Research Project on HABs and Eutrophication. It is contribution number 4501 from the University of Maryland Center for Environmental Science.

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**: 119-128.
- Andersen J M. 1974. Nitrogen and phosphorus budgets and the role of sediments in six shallow Danish lakes. *Archiv. fur Hydrobiologia*, **74**: 528-550.
- Anderson D M. 1989. Toxic algal bloom and red tides: a global perspective. In: Okaichi T, Anderson D M, Nemoto T eds. *Red Tides: Biology, Environmental Science and Technology*. Elsevier. p.11-16.
- Anderson D A, Burkholder J M, Cochlan W P, Glibert P M, Gobler C, Heil C, Kudela R, Parsons M T, Trainer V, Vargo G. 2008. Harmful algal blooms in the United States: Linkages to eutrophication. *Harmful Algae*, **8**: 39-53.
- Anderson D M, Glibert P M, Burkholder J M. 2002. Harmful algal blooms and eutrophication: Nutrient sources, composition and consequences. *Estuaries*, **25**: 562-584.
- Armstrong-Howard M D, 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.
- Bailey E M, Owens M, Boynton W R, Cornwell J C, Kiss E, Smail P W, Soulen H, Buck E, Ceballos M. 2006. Sediment Phosphorus Flux: pH Interactions in the Tidal Freshwater Potomac River Estuary, Final Report. Technical Report Series. No.TS-505-06-CBL Ref. No. [UMCES] CBL06-005 of the University of Maryland Center for Environmental Science.
- Berg G M, Balode M, Purina I, Bekere S, Bechemin C, Maestrini S Y. 2003. Plankton community composition in relation to availability and uptake of oxidized and reduced nitrogen. *Aq. Microb. Ecol.*, **30**: 263-274.
- Berg G M, Glibert P M, Lomas M W, Burford M. 1997. Organic nitrogen uptake and growth by the chrysophyte *Aureococcus anophagefferens* during a brown tide event. *Mar. Biol.*, **129**: 377-387.
- Berg G M, Repeta D J, LaRoche J. 2002. Dissolved organic nitrogen hydrolysis rates in axenic cultures of *Aureococcus anophagefferens* (Pelagophyceae): Comparison with heterotrophic bacteria. *Appl. Environ. Microbiol.*, **68**: 401-404.
- Berman T. 2001. The role of DON and the effect of N:P ratios on occurrence of cyanobacterial blooms: Implications from the outgrowth of *Aphanizomenon* in Lake Kinneret. *Limnol. Oceanogr.*, **46**: 443-447.
- Bouwman A F, Beusen A H W, Billen G. 2009. Human alteration of the global nitrogen and phosphorus soil balances for the period 1970-2050. *Global Biogeochemical Cycles*, **23**: GB0A04, doi:10.1029/2009GB003576.
- Boyer J N, Fourqurean J W, Jones R D. 1999. Seasonal and long-term trends in the water quality of Florida Bay (1989-1997). *Estuaries*, **22**: 417-430.
- Boyer G L, Sullivan J J, Anderson R J, Harrison P J, Taylor F J R. 1987. Effects of nutrient limitation on toxin production and composition in the marine dinoflagellate *Protogonyaulax tamarensis*. *Mar. Biol.*, **96**: 123-128.
- Burkholder J M. 2002. Cyanobacteria. In: Bitton G ed. *Encyclopedia of Environmental Microbiology*. Wiley Publishers, New York. p.952-982.
- Burkholder J M, Dickey D A, Kinder C, Reed R E, Mallin M A, Melia G, McIver M R, Cahoon L B, Brownie C, Deamer N, Springer J, Glasgow H Jr, Toms D, Smith J. 2006. Comprehensive trend analysis of nutrients and related variables in a large eutrophic estuary: A decadal study of anthropogenic and climatic influences. *Limnol. Oceanogr.*, **51**: 463-487.
- Burkholder J M, Glasgow H B. 1997. *Pfiesteria piscicida* and other *Pfiesteria*-like dinoflagellates: behavior, impacts and environmental controls. *Limnol. Oceanogr.*, **42**: 1 052-1 075.
- Burkholder J M, Glasgow H B Jr, Deamer-Melia N J. 2001b. Overview and present status of the toxic *Pfiesteria* complex. *Phycologia*, **40**: 186-214.
- Burkholder J M, Glibert P M, Skelton H. 2008. Mixotrophy, a major mode of nutrition for harmful algal species in eutrophic waters. *Harmful Algae*, **8**(1): 77-93.
- Burkholder J M, Mallin M A, Glasgow H B, Larsen L M, McIver M R, Shank G C, Deamer-Melia N, Briley D S, Springer J, Touchette B W, Hannon E K. 1997. Impacts to a coastal river and estuary from rupture of a large swine waste holding lagoon. *J. Environ. Quality*, **26**: 1 451-1 466.
- Chróst R J. 1991. Environmental control of the synthesis and activity of aquatic microbial ectoenzymes. In: Chróst R J ed. *Microbial Enzymes in Aquatic Environments*. New York: Springer-Verlag. p.29-59.
- Cloern J E. 2001. Our evolving conceptual model of the coastal eutrophication problem. *Mar. Ecol. Prog. Ser.*, **210**: 223-253.
- 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.
- Cochlan W P, Herndon J, Ladizinsky N L, Kudela R M. 2005. Nitrogen uptake by the toxigenic diatom *Pseudo-nitzschia australis*. GEOHAB Open Science Meeting: HABs and Eutrophication, Baltimore, MD, USA.

- Collos Y, Gagne C, Laabir M, Vaquer A, Cecchi P, Souchu P. 2004. Nitrogenous nutrition of *Alexandrium catenella* (Dinophyceae) in cultures and in Thau lagoon, southern France. *J. Phycol.*, **40**: 96-103.
- Costanza J K, Marcinko S E, Goewer A E, Mitchell C E. 2008. Potential geographic distribution of atmospheric nitrogen deposition from intensive livestock production in North Carolina, USA. *Sci. Total Environ.*, **398**: 76-86.
- Cotner J B, Sada R H, Bootsma H, Johengen T, Cavaletto J F, Gardner W S. 2000. Nutrient limitation of heterotrophic bacteria in Florida Bay. *Estuaries*, **23**: 611-620.
- Dodds W K. 2003. Misuse of inorganic N and soluble reactive P concentrations to indicate nutrient status of surface waters. *J. N. Amer. Benthol. Soc.*, **22**: 171-181.
- Drake J C, Heaney S I. 1987. Occurrence of phosphorus and its potential remobilization in the littoral sediments of a productive English lake. *Freshwat. Biol.*, **17**: 513-23.
- Dugdale R C, Wilkerson F P, Hogue V E, Marchi A. 2007. The role of ammonium and nitrate in spring bloom development in San Francisco Bay. *Estuar. Coast. Shelf Sci.*, **73**: 17-29.
- Dumont E, Harrison J H, Kroeze C, Bakker E J, Seitzinger S P. 2005. Global distribution and sources of dissolved inorganic nitrogen export to the coastal zone: Results from a spatially explicit, global model. *Global Biogeochem. Cycles*, **19**: 1-14, doi:10.1029/2005GB002488.
- Dyhrman S T. 2005. Ectoenzymes in *Prorocentrum minimum*. *Harmful Algae*, **4**: 619-628.
- Dyhrman S T, Ruttenberg K C. 2006. Presence and regulation of alkaline phosphatase in eukaryotic phytoplankton from the coastal ocean: Implications for dissolved organic phosphorus remineralization. *Limnol. Oceanogr.*, **51**: 1381-1390.
- Finkel Z V, Beardall J, Flynn K J, Quiqq A, Rees T A, Raven J A. 2010. Phytoplankton in a changing world: Cells size and elemental stoichiometry. *J. Plankt. Res.*, **32**: 119-137.
- Food and Agriculture Organization of the United Nations (FAO). 2005. Current world fertilizer trends and outlook to 2009/2010. Rome. FAO Publishers.
- Fisher T R, Peele E R, Ammerman J W, Harding L W Jr. 1992. Nutrient limitation of phytoplankton in Chesapeake Bay. *Mar. Ecol. Prog. Ser.*, **82**: 51-63.
- Fistarol G O, Legrand C, Granéli E. 2003. Allelopathic effect of *Prynesium parvum* on a natural planktonic community. *Mar. Ecol. Prog. Ser.*, **255**: 115-125.
- Flynn K J. 2002. Modeling marine phytoplankton growth under eutrophic conditions. *J. Sea Res.*, **54**: 92-103.
- Flynn K J. 2010. Do external resource ratios matter? Implications for modeling eutrophication events and controlling harmful algal blooms. *J. Marine Systems*, **83**: 170-180.
- Flynn K, Franco J M, Fernández P, Reguera B, Zepata M, Wood G, Flynn K J. 1994. Changes in toxin content, biomass and pigments of the dinoflagellate *Alexandrium minutum* during nitrogen refeeding and growth into nitrogen and phosphorus stress. *Mar. Ecol. Prog. Ser.*, **111**: 99-109.
- Galloway J N, Cowling E B. 2002. Nitrogen and the world. *Ambio.*, **31**: 64-71.
- Galloway J N, Cowling E B, Seitzinger S P, Socolow R H. 2002. Reactive nitrogen: Too much of a good thing? *Ambio.*, **31**: 60-63.
- Glibert P M. 2010. Long-term changes in nutrient loading and stoichiometry and their relationships with changes in the food web and dominant pelagic fish species in the San Francisco Estuary, California. *Rev. Fish. Sci.*, **18**(2): 211-232.
- Glibert P M, Allen J I, Bouwman L, Brown C, Flynn K J, Lewitus A, Madden C. 2010a. Modeling of HABS and eutrophication: status, advances, challenges. *J. Mar. Systems*, **83**: 262-275.
- Glibert P M, Anderson D A, Gentien P, Granéli E, Sellner K G. 2005a. The global, complex phenomena of harmful algal blooms. *Oceanography*, **18**(2): 136-147.
- Glibert P M, Bronk D A. 1994. Release of dissolved organic nitrogen by the marine diazotrophic cyanobacteria, *Trichodesmium* spp. *Appl. Environ. Microbiol.*, **60**: 3996-4000.
- Glibert P M, Burkholder J M. 2006. The complex relationships between increasing fertilization of the Earth, coastal eutrophication, and HAB proliferation. In: Granéli E, Turner J eds. *The Ecology of Harmful Algae*. Springer-Verlag, New York, p.341-354.
- Glibert P M, Burkholder J M, Kana T M, Alexander J A, Schiller C, Skelton H. 2009. Grazing by *Karenia brevis* on *Synechococcus* enhances their growth rate and may help to sustain blooms. *Aquat. Microb. Ecol.*, **55**: 17-30.
- Glibert P M, Conley D J, Fisher T R, Harding L W Jr., Malone T C. 1995. Dynamics of the 1990 winter/spring bloom in Chesapeake Bay. *Mar. Ecol. Prog. Ser.*, **122**: 22-43.
- Glibert P M, Harrison J, Heil C, Seitzinger S. 2006a. Escalating worldwide use of urea—a global change contributing to coastal eutrophication. *Biogeochemistry*, **77**: 441-463.
- Glibert P M, Heil C A, Hollander D, Revilla M, Hoare A, Alexander J, Murasko S. 2004. Evidence for dissolved organic nitrogen and phosphorus uptake during a cyanobacterial bloom in Florida Bay. *Mar. Ecol. Prog. Ser.*, **280**: 73-83.
- Glibert P M, Heil C A, O'Neil J M, Dennison W C, O'Donohue M J H. 2006b. Nitrogen, phosphorus, silica and carbon in Moreton Bay, Queensland, Australia: Differential limitation of phytoplankton biomass and production. *Estuaries and Coasts*, **29**: 107-119.
- Glibert P M, Legrand C. 2006. The diverse nutrient strategies of HABS: Focus on osmotrophy. In: Granéli E, Turner J eds. *Ecology of Harmful Algae*. Springer. p.163-176.
- Glibert P M, Magnien R, Lomas M W, Alexander J, Fan C, Haramoto E, Trice T 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. 2005b. 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, Wazniak C E, Hall M, Sturgis B. 2007. Seasonal and interannual trends in nitrogen and brown tide in Maryland's Coastal Bays. *Ecol. Appl.*, **17**(S): S79-S87.

- Gobler C J, Lonsdale D J, Boyer G L. 2005. A synthesis and review of causes and impact of harmful brown tide blooms caused by the alga, *Aureococcus anophagefferens*. *Estuaries*, **28**: 726-749.
- Gobler C J, Renaghan M J, Buck N J. 2002. Impacts of nutrients and grazing mortality on the abundance of *Aureococcus anophagefferens* during a New York brown tide bloom. *Limnol. Oceanogr.*, **47**: 129-141.
- Granéli E. 2005. Harmful algal blooms. In: Wassmann P, Olli K eds. Drainage Basin Inputs and Eutrophication: An Integrated Approach. University of Tromsø, Norway, p.99-112. www.ut.ee/~olli/eutr/.
- Granéli E, Anderson D M, Carlsson P, Maestrini S Y. 1997. Light and dark carbon uptake by *Dinophysis* species in comparison to other photosynthetic and heterotrophic dinoflagellates. *Aquat. Microb. Ecol.*, **13**: 177-186.
- Granéli E, Carlsson P, Legrand C. 1999. The role of C, N and P in dissolved and particulate matter as a nutritional source for phytoplankton growth, including toxic species. *Aquat. Ecol.*, **33**: 17-27.
- Granéli E, Flynn K. 2006. Chemical and physical factors influencing toxin content. In: Granéli E, Turner J T eds. Ecology of harmful algae. Springer. The Netherlands. p.229-241.
- Granéli E, Johansson N, Panosso R. 1998. Cellular toxin contents in relation to nutrient conditions for different groups of phycotoxins. In: Reguera B, Blanco J, Fernandez M L, Wyatt T eds. Harmful Algae. Paris, France: Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO. p.321-324.
- Granéli E, Hansen P J. 2006. Allelopathy in harmful algae: A mechanism to compete for resources? In: Granéli E, Turner J T eds. Ecology of harmful algae. Springer. The Netherlands. p.189-201.
- Granéli E, Turner J T eds. 2006. Ecology of Harmful Algae. Springer. The Netherlands.
- Hansen P J. 1998. Phagotrophic mechanisms and prey selection in mixotrophic phytoflagellates. In: Anderson D M, Cembella A D, Hallegraeff G M eds. Physiological Ecology of Harmful Algal Blooms. NATO ASI Series G41, Springer-Verlag, Berlin Heidelberg. p.525-537.
- Harris G P. 1986. Phytoplankton Ecology: Structure, Function and Fluctuation. Chapman and Hall, London.
- Harrison J A, Caraco N, Seitzinger S P. 2005a. Global patterns and sources of dissolved organic matter export to the coastal zone: Results from a spatially explicit, global model. *Global Biogeochem. Cycles*, **19**: 1-16, doi:10.1029/2005GB002480.
- Harrison J A, Seitzinger S P, Bouwman A F, Caraco N F, Beusen A H W, Vörösmarty C J. 2005b. Dissolved inorganic phosphorus export to the coastal zone: Results from a spatially explicit, global model. *Global Biogeochem. Cycles*, **19**: 1-15, doi:10.1029/2004GB002357.
- Hecky R E, Kilham P. 1988. Nutrient limitation in freshwater and marine environments: A review of recent evidence on the effects of enrichment. *Limnol. Oceanogr.*, **33**: 796-822.
- Heil C A, Revilla M, Glibert P M, Murasko S. 2007. Nutrient quality drives phytoplankton community composition on the West Florida Shelf. *Limnol. Oceanogr.*, **52**: 1 067-1 078.
- Heil C A, Glibert P M, Murasko S, Alexander J S. 2009. Size-fractionated alkaline phosphatase activity along a gradient of nitrogen to phosphorus limitation in a carbonate dominated subtropical estuary. *Contrib. Mar. Sci.*, **38**: 37-48.
- Heisler J, Glibert P M, Burkholder J M, Anderson D A, Cochlan W P, Dennison W C, Dortch Q, Gobler C, Heil C A, Humphries E, Lewitus A, Magnien R, Marshall H, Sellner K, Stockwell D, Stoecker D, Suddleson M. 2008. Eutrophication and harmful algal blooms: A scientific consensus. *Harmful Algae*, **8**: 3-13.
- Herndon J, Cochlan W P. 2007. Nitrogen utilization by the raphidophyte *Heterosigma akashiwo*: growth and uptake kinetics in laboratory cultures. *Harmful Algae*, **6**: 260-270.
- Hodgkiss I J. 2001. The N:P ratio revisited. In: Ho K C, Wang Z D eds. Prevention and Management of Harmful Algal Blooms in the South China Sea. School of Science and Technology, the Open University of Hong Kong.
- Hodgkiss I J, Ho K C. 1997. Are changes in N:P ratios in coastal waters the key to increased red tide blooms? *Hydrobiologia*, **852**: 141-147.
- Howarth R W. 2008. Coastal nitrogen pollution: A review of sources and trends globally and regionally. *Harmful Algae*, **8**: 14-20.
- Howarth R W, Ramakrishna K, Choi E, Elmgren R, Martinelli L, Mendoza A, Moomaw W, Palm C, Boy R, Scholes M, Z Z L. 2005. Nutrient management, responses assessment. In: Ecosystems and Human Well-being. Vol. 3, Policy Responses, the Millennium Ecosystem Assessment. Washington, DC: Island Press. p.295-311.
- Howarth R W, Sharpley A, Walker D. 2002. Sources of nutrient pollution to coastal waters in the United States: Implications for achieving coastal water quality goals. *Estuaries*, **25**: 656-676.
- Huang B, Ou L, Wang X, Huo W, Li R, Hong H, Zhu M, Qi Y. 2007. Alkaline phosphatase activity of phytoplankton in East China Sea coastal waters with frequent harmful algal bloom occurrences. *Aquat. Microb. Ecol.*, **49**: 195-206.
- Irigoién X, Flynn K J, Harris R P. 2005. Phytoplankton blooms: a "loophole" in microzooplankton grazing impact? *J. Plank. Res.*, **27**: 313-321.
- Jensen H S, Andersen F O. 1992. Importance of temperature, nitrate, and pH for phosphate release from sediments of four shallow, eutrophic lakes. *Limnol. Oceanogr.*, **39**: 577-589.
- Jeong H J, Park J Y, Nho J H, Park M O, Ha J H, Seong K A, Jeng C, Seong C N, Lee K Y, Yih W H. 2005a. Feeding by red-tide dinoflagellates on the cyanobacterium *Synechococcus*. *Aquat. Microb. Ecol.*, **41**: 131-143.
- Jeong H J, Yoo Y D, Kim T H, Kim J H, Kang N S, Yih W H. 2004. Mixotrophy in the phototrophic harmful alga *Cochlodinium polykikoides* (Dinophyceae): prey species, the effects of prey concentration and grazing impact. *J. Eukaryot. Microbiol.*, **51**: 563-569.
- Jeong H J, Yoo Y D, Kim J S, Seong K A, Kang N S, Kim T H. 2010. Growth, feeding and ecological roles of the mixotrophic and heterotrophic dinoflagellates in marine planktonic food webs. *Ocean Sci. J.*, **45**(2): 65-91.

- Jeong H J, Yoo Y D, Park J Y, Song J Y, Kim S T, Lee S H, Kim K Y, Yih W H. 2005b. Feeding by phototrophic red-tide dinoflagellates: five species newly revealed and six species previously known to be mixotrophic. *Aquat. Microb. Ecol.*, **40**: 133-150.
- Jeong H J, Yoo Y D, Seong K A, Kim J H, Park J Y, Kim S, Lee S Y, Ha J H, Yih W H. 2005c. Feeding by the mixotrophic red-tide dinoflagellate *Gonyaulax polygramma*: mechanisms, prey species, effects of prey concentration, and grazing impact. *Aquat. Microb. Ecol.*, **38**: 249-257.
- Ji X Q, Han X T, Zheng L, Yu Z M, Yang B J, Zou J Z. 2011. Allelopathic interactions between *Prorocentrum micans* and *Skeletonema costatum* or *Karenia mikimotoi* in laboratory cultures. *Chinese J. Oceanology Limnol.*, This issue.
- Johansson N, Granéli E. 1999a. Cell density, chemical composition and toxicity of *Chrysochromulina polylepis* (Haptophyta) in relation to different N:P supply ratios. *Mar. Biol.*, **135**: 209-217.
- Johansson N, Granéli E. 1999b. Influence of different nutrient conditions on cell density, chemical composition and toxicity of *Prymnesium parvum* (Haptophyta) in semi-continuous cultures. *J. Exp. Mar. Biol. Ecol.*, **239**: 243-258.
- 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.
- Kana T M, Glibert P M. 1987. Effect of irradiances up to 2000 $\mu\text{E m}^{-2} \text{sec}^{-1}$ on marine *Synechococcus* WH7803: I. Growth, pigmentation, and cell composition. *Deep-Sea Res.*, **34**: 479-495.
- Karl D M, Björkman K M. 2001. Phosphorus cycle in seawater: Dissolved and particulate pool inventories and selected phosphorus fluxes. *Methods in Microbiol.*, **30**: 239-270.
- Klausmeier C A, Litchman E, Daufresne T, Levin S A. 2004. Optimal N:P stoichiometry of phytoplankton. *Nature*, **429**: 171-174.
- Kudela R M, Cochlan W P. 2000. Nitrogen and carbon uptake kinetics and the influence of irradiance for a red tide bloom off southern California. *Aquat. Microb. Ecol.*, **21**: 31-47.
- 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**(1): 103-110.
- Legrand C, Rengefors K, Granéli E, Fistarol G O. 2003. Allelopathy in phytoplankton—biochemical, ecological and evolutionary aspects. *Phycologia*, **42**: 406-419.
- Lewitus A J. 2006. Osmotrophy in marine microalgae. In: Subba-Rao DV ed. *Algal cultures, Analogues of blooms and applications*. Science Publishers, Enfield, NH, USA. p.343-383.
- Lewitus A J, Burkholder J M, Glasgow H B Jr, Glibert P M, Willis B M, Hayes K C. 1999. Mixotrophy and nitrogen uptake by *Pfiesteria piscicida* (Dinophyceae). *J. Phycol.*, **35**: 1 430-1 437.
- Li H, Veldhuis M J W, Post A F. 1998. Alkaline phosphatase activities among planktonic communities in the northern Red Sea. *Mar. Ecol. Prog. Ser.*, **173**: 107-115.
- Li J, Glibert P M, Zhou M, Lu S, Lu D. 2009. Relationships between nitrogen and phosphorus forms and ratios and the development of dinoflagellate blooms in the East China Sea. *Mar. Ecol. Prog. Ser.*, **383**: 11-26.
- Lomas M W, Glibert P M, Berg G M, Burford M. 1996. Characterization of nitrogen uptake by natural populations of *Aureococcus anophagefferens* (Chrysophyceae) as a function of incubation duration, substrate concentration, light, and temperature. *J. Phycol.*, **32**: 907-916.
- Lomas M W, Glibert P M, Clougherty D A, Huber D R, Jones J, Alexander J, Haramoto E. 2001. Elevated organic nutrient ratios associated with brown tide blooms of *Aureococcus anophagefferens* (Pelagophyceae). *J. Plank. Res.*, **23**: 1 339-1 344.
- Lomas M W, Kana T M, MacIntyre H L, Cornwell J C, Nuzzi R, Waters R. 2004. Interannual variability of *Aureococcus anophagefferens* in Quantuck Bay, Long Island: natural test of the DON hypothesis. *Harmful Algae*, **3**: 389-402.
- Mallin M A. 2000. Impacts of industrial-scale swine and poultry production on rivers and estuaries. *Amer. Scientist*, **88**: 26-37.
- Merrett M J. 1991. Inorganic carbon transport in some marine microalgal species. *Can. J. Bot.*, **69**: 1 032-1 039.
- Miller A G, Espie G S, Canvin D T. 1991. Active CO_2 transport in cyanobacteria. *Can. J. Bot.*, **69**: 925-935.
- Mitra A, Flynn K J. 2006. Promotion of harmful algal blooms by zooplankton predatory activity. *Biol. Lett.*, **2**: 194-197.
- Mulholland M R, Bernhardt P W, Heil C A, Bronk D A, O'Neil J M. 2006. Nitrogen fixation and release of fixed nitrogen by *Trichodesmium* spp. in the Gulf of Mexico. *Limnol. Oceanogr.*, **51**: 1 762-1 776.
- Mulholland M R, Glibert P M, Berg G M, Van Heukelem L, Pantoja S, Lee C. 1998. Extracellular amino acid oxidation by phytoplankton and cyanobacteria: a cross-ecosystem comparison. *Aq. Microb. Ecol.*, **15**: 141-152.
- Mulholland M R, Gobler C J, Lee C. 2002. Peptide hydrolysis, amino acid oxidation, and nitrogen uptake in communities seasonally dominated by *Aureococcus anophagefferens*. *Limnol. Oceanogr.*, **47**: 1 094-1 108.
- Mulholland M R, Heil C A, Bronk D A, O'Neil J M, Bernhardt P. 2004. Does nitrogen regeneration from the N_2 fixing cyanobacteria *Trichodesmium* spp. fuel *Karenia brevis* blooms in the Gulf of Mexico? In: Steidinger K A, Landsberg J H, Tomas C R, Vargo G A eds. *Harmful Algae 2002*. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography and Intergovernmental Oceanographic Commission of UNESCO. p.47-49.
- Mulholland M R, Lee C, Glibert P M. 2003. Extracellular enzyme activity and uptake of carbon and nitrogen along an estuarine nutrient and salinity gradient. *Mar. Ecol. Prog. Ser.*, **258**: 3-17.
- Murrell M C, Lores E M. 2004. Phytoplankton and zooplankton seasonal dynamics in a subtropical estuary: importance of cyanobacteria. *J. Plankt. Res.*, **26**: 371-382.
- National Research Council (NRC). 2000. *Clean Coastal Waters—Understanding and Reducing the Effects of*

- Nutrient Pollution. National Academy Press, Washington, DC.
- Nygaard K, Tobiesen A. 1993. Bacterivory in algae: a survival strategy during nutrient limitation. *Limnol. Oceanogr.*, **38**: 273-279.
- Oh H M, Lee S J, Jang M H, Yoon B D. 2000. Microcystin production by *Microcystis aeruginosa* in a phosphorus-limited chemostat. *Appl. Envir. Microbiol.*, **66**: 176-179.
- Oh H M, Lee S J, Kim J H, Kim H S, Yoon B D. 2001. Seasonal variation and indirect monitoring of microcystin concentrations in Daechung Reservoir, Korea. *Appl. Envir. Microbiol.*, **67**: 1 484-1 489.
- Paerl H W, Fulton R S, Moisaner P H, Dyble J. 2001. Harmful freshwater algal blooms, with an emphasis on cyanobacteria. *The Scientific World*, **1**: 76-113.
- Palenik B, Morel F M M. 1990. Comparison of cell-surface L-amino acid oxidases from several marine phytoplankton. *Mar. Ecol. Prog. Ser.*, **59**: 195-201.
- Palenik B, Brahmsha B, Larimer F W, Land M, Hauser L, Chain P, Lamerdin J, Regala W, Allen E E, McCarren J, Paulsen I, Dufresne A, Partensky F, Webb E A, Waterbury J. 2003. The genome of a motile marine *Synechococcus*. *Nature*, **424**: 1 037-1 042.
- Parrow M W, Burkholder J M. 2003. Estuarine heterotrophic cryptoperidiniopoids (Dinophyceae): Life cycle and culture studies. *J. Phycol.*, **39**: 678-696.
- Price G D, Badger M R. 1991. Evidence for the role of carboxysomes in the cyanobacterial CO₂-concentrating mechanism. *Can. J. Bot.*, **69**: 963-973.
- Ratti S, Giordano M, Morse D. 2007. CO₂-concentrating mechanisms of the potentially toxic dinoflagellate *Protoceratium reticulatum* (Dinophyceae, Gonyaulacales). *J. Phycol.*, **43**: 693-701.
- Raven J A, Johnston A M. 1991. Mechanisms of inorganic carbon acquisition in marine phytoplankton and their implications for the use of other resources. *Limnol. Oceanogr.*, **36**: 1 701-1 714.
- Redfield A C. 1934. On the proportions of organic derivatives in sea water and their relation to the composition of plankton. In: James Johnstone Memorial Volume. Liverpool: University of Liverpool Press. p.176-192
- Redfield A C. 1958. The biological control of chemical factors in the environment. *Amer. Scientist*, **46**: 205-221.
- 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.
- Romdhane M S, Eilertsen H C, Yahia O K D, Yahia M N D. 1998. Toxic dinoflagellate blooms in Tunisian lagoons: Causes and consequences for aquaculture. In: Reguera B, Blance J, Fernandez M L, Wyatt T eds. Harmful Algae. Xunta de Galicia and the Intergovernmental Oceanographic Commission of United Nations Educational, Scientific and Cultural Organization, Paris, France. p.80-83.
- Rothenberger M A, Burkholder J M, Wentworth T. 2009. Multivariate analysis of phytoplankton and environmental factors in a eutrophic estuary. *Limnol. Oceanogr.*, **54**: 2 107-2 127.
- Schindler D W. 1977. Evolution of phosphorus limitation in lakes. *Science*, **196**: 260-262.
- Seitzinger S P. 1991. The effect of pH on the release of phosphorus from Potomac Estuary sediments: Implications for blue-green algal blooms. *Est. Coast. Shelf Sci.*, **33**: 409-418.
- Seitzinger S P, Harrison J A, Dumont E, Beusen A H W, Bouwman A F. 2005. Sources and delivery of carbon, nitrogen and phosphorus to the coastal zone: An overview of global nutrient export from watersheds (NEWS) models and their application. *Global Biogeochem. Cycles*, **19**: 9, doi:10.1029/2005GB002606, 1-11.
- Seitzinger S P, Kroeze C. 1998. Global distribution of nitrous oxide production and N inputs in freshwater and coastal marine ecosystems. *Global Biogeochem. Cycles*, **12**: 93-113.
- Seitzinger S P, Kroeze C, Bouwman A F, Caraco N, Dentener F, Styles R V. 2002. Global patterns of dissolved inorganic and particulate nitrogen inputs to coastal systems: Recent conditions and future projections. *Estuaries*, **25**: 640-655.
- Seitzinger S P, Mayorga E, Kroeze C, Bouwman A F, Beusen A H W, Billen G, Van Drecht G, Dumont E, Fekete B M, Garnier J, Harrison J, Wisser D, Wollheim W M. 2009. Global nutrient river export trajectories 1970-2050: A Millennium Ecosystem Assessment scenario analysis. *Global Biogeochem. Cycles*, 2009GB003587.
- Seong K A, Jeong H J, Kim S, Kim G H, Kang J H. 2006. Bacterivory by co-occurring red-tide algae, heterotrophic nanoflagellates, and ciliates. *Mar. Ecol. Prog. Ser.*, **322**: 85-97.
- Skelton H M, Parrow M W, Burkholder J M. 2006. Phosphatase activity in the heterotrophic dinoflagellate, *Pfiesteria shumwayae* (Dinophyceae). *Harmful Algae*, **5**: 395-406.
- Smayda T J. 1990. Novel and nuisance phytoplankton blooms in the sea: Evidence for a global epidemic. In: Granéli E, Sundstrom B, Edler L, Anderson DM eds, Toxic Marine Phytoplankton. Elsevier, New York. p.29-40.
- Smayda T J. 1997. Harmful phytoplankton blooms: their ecophysiology and general relevance. *Limnol. Oceanogr.*, **42**: 1 137-1 153.
- Smil V. 2001. Enriching the Earth: Fritz Haber, Carl Bosch, and the Transformation of World Food. The MIT Press, Cambridge, United Kingdom.
- Smith V H. 1983. Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. *Science*, **221**: 669-671.
- Smith V H. 1990. Nitrogen, phosphorus and nitrogen fixation in lacustrine and estuarine ecosystems. *Limnol. Oceanogr.*, **35**: 1 852-1 859.
- Solomon C M, Collier J L, Berg G M, Glibert P M. 2010. Role of urea in microbes in aquatic systems: a biochemical and molecular review. *Aquat. Microb. Ecol.*, **59**: 67-88.
- Stephens D W, Krebs J R. 1986. Foraging Theory. Princeton University Press, Princeton, NJ.
- Stibor H, Sommer U. 2003. Mixotrophy of a photosynthetic flagellate viewed from an optimal foraging perspective. *Protist.*, **154**: 91-98.
- Stoecker D. 1999. Mixotrophy among dinoflagellates. *J. Eukaryot. Microbiol.*, **46**: 397-401.

- Stoecker D K, Gustafson D E Jr. 2003. Cell-surface proteolytic activity of photosynthetic dinoflagellates. *Aquat. Microb. Ecol.*, **30**: 175-183.
- Stoecker D K, Tillmann U, Granéli E. 2006. Phagotrophy in harmful algae. In: Granéli E, Turner J T eds. *Ecology of Harmful Algae*. Springer-Verlag, Berlin. p.177-187.
- Sterner R W, Elser J J. 2002. *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere*. Princeton, NJ: Princeton University Press.
- Tarran G A, Burkhill P H, Edwards E S, Woodward E M S. 1999. Phytoplankton community structure in the Arabian Sea during and after the SW monsoon 1994. *Deep-Sea Res. II*, **46**: 655-676.
- Tilman D. 1977. Resource competition between planktonic algae: an experimental and theoretical approach. *Ecology*, **58**: 338-348.
- Trainer V L, Le Eberhart B T, Wekell J C, Adams N G, Hanson L, Cox F, Dowell J. 2003. Paralytic shellfish toxins in Puget Sound, Washington. *J. Shellfish Res.*, **22**: 213-223.
- Uchida T. 1992. Alkaline phosphatase and nitrate reductase activities in *Prorocentrum micans* Ehrenberg. *Bull. Plank. Soc. Japan*, **38**: 85-92.
- Uchida T. 2001. The role of cell contact in the life cycle of some dinoflagellate species. *J. Plankt. Res.*, **23**: 889-891.
- Van de Waal D B, Tonk L, Van Donk E, Matthijs H C P, Visser P S, Huisman J. 2010. Climate change and the impact of C:N stoichiometry on toxin production by harmful cyanobacteria. 14th International Conference on Harmful Algae, Hersonissos-Crete, Greece, November 2010. Abstract.
- Van de Waal D B, Verspagen J M, Lurling M, Van Donk E, Visser P M, Huisman J. 2009. The ecological stoichiometry of toxins produced by harmful cyanobacteria: An experimental test of the carbon-nutrient balance hypothesis. *Ecol. Lett.*, **12**: 1 326-1 335.
- Van Drecht G, Bouwman A F, Harrison J, Knoop J M. 2009. Global nitrogen and phosphate in urban waste water for the period 1970-2050. *Global Biogeochemical Cycles*, **23**: GB0A03, doi:10.1029/2009GB003458.
- Van Mooy B A S, Fredricks H F, Pedler B E, Dyhrman S T, Karl D M, Koblizek M, Lomas M W, Mincer T J, Moore L R, Moutin T, Rappe M S, Webb E A. 2009. Phytoplankton in the ocean use non-phosphorus lipids in response to phosphorus scarcity. *Nature*, **458**: 69-72.
- Van Nieuwenhuysse E. 2007. Response of summer chlorophyll concentration to reduced total phosphorus concentration in the Rhine River (Netherlands) and the Sacramento-San Joaquin Delta (California, USA). *Can. J. Fish. Aqu. Sciences*, **64**: 1 529-1 542.
- Walsh J, Steidinger K. 2001. Saharan dust and Florida red tides: the cyanophyte connection. *J. Geophys. Res.*, **106**: 11 597-11 612
- Wassmann P. 2005. Cultural eutrophication: perspectives and prospects. In: Wassmann P, Olli K eds. *Drainage Basin Inputs and Eutrophication: An Integrated Approach*. University of Tromsø, Norway. p.224-234. www.ut.ee/~olli/eutr/.
- Wetzel R G. 2001. *Limnology*, 3rd Edition. Academic Press, New York.
- Xie L Q, Xie P, Tang H J. 2003. Enhancement of dissolved phosphorus release from sediment to lake water by *Microcystis* blooms—an enclosure experiment in a hypereutrophic, subtropical Chinese lake. *Envir. Poll.*, **122**: 391-399.
- Zhou M J, Yan T, Zou J Z. 2003. Preliminary analyses of the characteristics of red tide areas in Changjiang River estuary and its adjacent sea. *Chinese J. Appl. Ecol.*, **14**: 1 031-1 038. (in Chinese with English abstract)
- Zillén L, Conley D J. 2010. Hypoxia and cyanobacterial blooms are not natural features of the Baltic Sea. *Biogeosciences Discuss.* **7**: 1 783-1 812.