13 The Diverse Nutrient Strategies of Harmful Algae: Focus on Osmotrophy

P. M. Glibert and C. Legrand

13.1 Introduction and Terminology

Ever since the discovery of microscopic flagellates, the manner in which they sustain themselves has been a topic of interest. Indeed, in 1677 Anthoni van Leeuwenhoek proposed to call the flagellates that he observed 'animalcules' after observing their motion, "When these animalcules bestirred 'emselves, they sometimes stuck out two little horns, which were continually moved, after the fashion of a horse's ears" (re-quoted by Milius 1999). Today, there is much interest in identifying the mode of nutrition of plankton, especially harmful algae, for a variety of reasons. As many harmful algal blooms (HABs) have been associated with eutrophication and/or alterations in the amount and form of nutrients supplied (reviewed by Glibert et al. 2005), linking HABs nutrition with specific nutrient forms is a key management issue. Additionally, as models are developed for the prediction of HABs, accurately relating growth to the nutrient supply is essential. Lastly, there is much to be learned about adaptive physiology from cells that have a diversity of nutritional mechanisms.

Many HABs rely strictly upon photosynthesis for their carbon, and use inorganic nutrients for their nutrition: these are the *autotrophs*. Some phototrophic species, however, have alternative pathways for acquiring carbon or nutrients: these are the *mixotrophs*. They use dissolved or particulate organic substances to renew their cellular reserves of carbon, macronutrients, amino acids, trace elements or phospholipids (Raven 1997; Stoecker 1999; Granéli et al. 1999). In applying this terminology to nutrition, mixotrophy encompasses several processes, including *osmotrophy*, nutrition by direct absorption and uptake of organic molecules, and *phagotrophy*, ingestion of prey or other food particles. When carbon is incorporated, the process is *heterotrophy*.

Many mixotrophic species display a continuum of dependence on these alternative pathways. Some species only display osmotrophy or phagotrophy

Ecological Studies, Vol. 189
Edna Granéli and Jefferson T. Turner (Eds.)
Ecology of Harmful Algae

when particular cellular requirements can apparently not be met by autotrophy. Other species have lost the ability for autotrophy and rely exclusively on phagotrophy and osmotrophy (Jones 1994). Heterotrophic dinoflagellates are among the species that have lost the ability for autotrophy. In fact, roughly half of all dinoflagellates are obligate heterotrophs (Smayda 1997). *Noctiluca* is a well-recognized example of a phagotrophic, heterotrophic dinoflagellate (Kimor 1979). Some heterotrophic dinoflagellates (e.g., *Pfiesteria*) can 'borrow' or 'steal' functional chloroplasts from its prey; this strategy is termed *kleptochloroplastidy* (Skovgaard 1998). The continuum of processes also includes such strategies as *dasmotrophy*, as found for the genus *Chrysochromulina*, in which extracellular toxins perforate the cell membrane of prey, inducing osmosis and leakage of organic compounds available for uptake or incorporation (Estep and MacIntyre 1989).

This chapter focuses on our current understanding of osmotrophy in HABs. This chapter is not intended to be a review of the uptake of organic compounds by algae (see recent review by Berman and Bronk 2003), but rather an introduction to the importance and diversity of this nutritional strategy for HABs.

13.2 Osmotrophy Pathways and Methods to Explore Them

One of the factors complicating the ability to trace the pathways of uptake of organic nutrients is the fact that in nature, the range of organic compounds is large, including dissolved amino acids, nucleic acids, polypeptides, polyphenolic substances and polysaccharides. Many are unknown or not well characterized, many are refractory, and others are highly variable in time and space (Antia et al. 1991; Bronk 2002). Ultra-filtration techniques have been used to characterize DOM by broad molecular weight fraction (e.g., Benner et al. 1997), but such approaches do not allow characterization or identification of individual compounds. Using ^{15}N-NMR spectroscopy, it has recently been found that there are different pools of high molecular weight DON in the oceans and they may have different chemical and biological reactivity (Aluwihare et al. 2005). This approach has yet to be applied to eutrophic coastal regions and may be useful in characterizing which DOM compounds are bioavailable during HAB outbreaks.

For the determination of the rates of uptake of specific organic compounds, stable isotopic or radiotracer techniques can be used. These are analogous to the techniques used for inorganic nutrient uptake. These techniques are limited, however, because only selected compounds can be enriched with a stable or radioactive tracer. Studies in which radioisotopes have been used to trace the uptake of specific organic compounds have a long history in the literature (e.g., Wheeler et al. 1974). Some natural dissolved organic nitrogen

(DON) compounds have been prepared with ^{15}N labels, but this involves growing natural assemblages or cultures with a ^{15}N substrate, isolating and concentrating the released DON and using this concentrate as a tracer in subsequent experiments (e.g., Bronk and Glibert 1993; Veuger et al. 2004). These experiments have not been widely conducted because of the difficulty and variability in the DON isolates. Other investigators have used organic compounds that are labeled with two tracers, such as ^{15}N and ^{13}C to assess the extent to which an organic compound may be used for nutrient or carbon (e.g., Mulholland et al. 2004).

Osmotrophy pathways are numerous and include direct uptake as well as extracellular oxidation and hydrolysis, and pinocytosis (Fig. 13.1). Enzymatic measurements have been used to determine some of the pathways involved in the incorporation and degradation of organic compounds (Chrost 1991). Several enzymes illustrate these pathways. Urease is an intracellular enzyme that has been characterized in relation to the uptake of urea. Urease activity appears to be constitutive for many algal species but may be higher in many

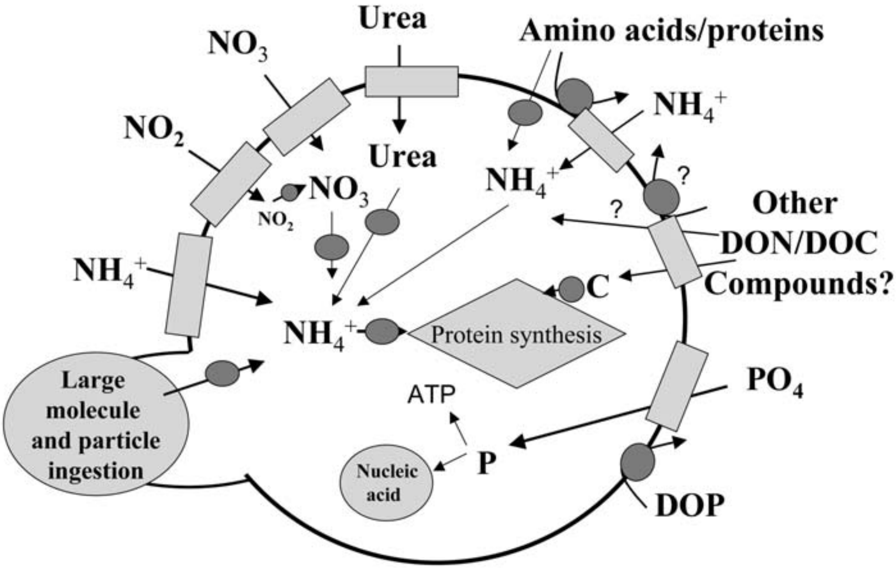

Fig. 13.1. Schematic of the various pathways by which HABs may acquire their nutrients. All phytoplankton can transport nitrate, nitrite, ammonium, urea and phosphate across cell membranes via passive diffusion or active transport. Some species can transport large organic molecules across their cell membrane, and some have cell surface enzymes for breaking down larger organic molecules before transporting the nutrients. Some species have the ability to phagocytize other cells, particles and/or large molecules. Although many pathways are shown, this is not intended to imply that all HABs have all capabilities. Pathways that involve enzymatic reactions are indicated with a *circle*. Pathways for which there is much uncertainty are indicated with a *question mark*

HAB species compared to non-HABs. For example, urease activity has been found to be sufficiently high in *Aureococcus anophagefferens* and *Prorocentrum minimum* to meet the cellular nitrogen demand for growth but insufficient to meet this demand in the diatom *Thalassiosira weissflogii* (Fan et al. 2003). In *Alexandrium fundyense* urease activity was shown to be seasonally variable and related to the toxin content of the cells (Dyhrman and Anderson 2003).

Another ecologically important enzyme is alkaline phosphatase (AP). This enzyme is responsible for the hydrolysis of organic phosphorus sources, such as sugar phosphates, phospholipids, and nucleotide phosphates, among other compounds. This enzyme is located on the cell surface, membrane-bound. In general, activity of AP increases upon phosphate stress and thus is an excellent ecological indicator of phosphorus limitation (Dyhrman 2005). Polyclonal antibodies have been developed to AP in selected species and whole-cell immunolabeling approaches have been applied in the field to determine the AP activity of *A. fundyense* in mixed assemblages (Dyhrman and Palenik 1999). Such approaches allow for species–specific activities to be determined, and advance our understanding of the nutrient status of individual species within mixed assemblages.

Some species have the capability for the breakdown of other organic compounds at the cell surface. These species have enzymes that can oxidize or hydrolyze amino acids or proteins. Extracellular amino acid oxidation has been shown to be expressed to a greater degree by dinoflagellates compared to some diatoms, and furthermore, to be expressed to a greater degree when inorganic nutrients are at or near depletion (Palenik and Morel 1990; Mulholland et al. 1998). Also occurring at the cell surface of many flagellates is peptide hydrolysis. Both peptide hydrolysis and amino acid oxidation may be important in some HABs, e.g., *A. anophagefferens* in natural communities (Mulholland et al. 2002). Leucine amino peptidase is another protease that hydrolyses peptide bonds and liberates amino acids (Dyhrman 2005). It is measured by assessing the rate of hydrolysis of an artificial substrate, and has been shown to be of potential significance in dinoflagellates and other HAB classes. Stoecker and Gustafson (2003), for example, demonstrated in Chesapeake Bay that Leu-amino-peptidase activity was associated with a dinoflagellate bloom, and that in non-axenic cultures of *Akashiwo sanguinea, Gonyaulax grindleyi, Gyrodinium uncatenum, Karlodinium micrum* and *P. minimum* the activity was associated with the dinoflagellates and not the bacteria.

The direct uptake of larger organic molecules by osmotrophic dinoflagellates is largely unknown, but there is evidence that the mechanism involves pinocytosis (endocytotic uptake), at least in some species. For example, *Prorocentrum micans* can accumulate fluorescently labeled proteins in the pusule, an organelle that functions in osmoregulation, waste disposal and/or nutrition (Klut et al. 1987). Fluorescently labeled high molecular weight dextrans were also found to be taken up by *Alexandrium catenella* and to accumulate in small vesicles in the cells (Legrand and Carlsson 1998). In the same species, Doblin et al. (2001), using autoradiography, found that radioactively labeled humic substances were also taken up by the cells. The process of pinocytosis begins to blur the distinction between osmotrophy and phagotrophy and many species, such as *Alexandrium* spp., can use the same pathway to capture large organic molecules or prey.

Several new methods are being applied to the study of the large suite of individual compounds in natural mixes of dissolved organic matter (DOM) and their uptake by HABs (Glibert et al. 2005). Electrospray mass spectrometry is a tool that can be used to identify and quantify the presence of a broad suite of compounds in a natural sample and to track their rate of change under experimental conditions (Seitzinger et al. 2005). Other advances in flow cytometry, epifluorescence microscopy and the detection of gene expression, using quantitative reverse transcription PCR, show promise in detecting expression of enzymes and can help to isolate the source of the signal in a natural sample (Dyhrman 2005). New in situ nutrient monitoring systems provide data on both inorganic and organic nutrient fluxes on the time and space resolution necessary to define the ephemeral changes that may be significant to phytoplankton cells. There are thus many new tools to address the composition and fluxes of DOM and the physiological capability of cells to use distinct fractions of available DOM. Although many approaches are now available, there is a need to integrate both qualitative and quantitative approaches to estimate the importance of osmotrophy to HABs in nature.

13.3 Cellular Costs and Benefits of Osmotrophy

The photosynthetic apparatus constitutes up to 50 % of the cell biomass and uses a high proportion of the cellular budget of energy and nutrients. All autotrophs have the ability to assimilate organic molecules since they possess enzymes in the Krebs cycle. Thus, the costs and maintenance of transplasma membranes, lysosomes and specific enzymes used in the uptake and digestion of organic molecules is similar in osmotrophs and strict autotrophs. In contrast, strict heterotrophs have higher growth efficiencies than strict autotrophs and mixotrophs because they do not have the cellular maintenance costs of the photosynthetic apparatus (Raven 1997; Stoecker 1999). There is some evidence that the energetic costs for mixotrophy may be higher in brackish waters than in marine environments, due to the physiological constraints of osmotic stress in a brackish system (Moorthi 2004). Although there may be higher cellular costs to maintaining mixotrophy, there are obvious advantages. Facultative mixotrophs can switch from autotrophic to heterotrophic nutrition under unfavorable environmental conditions (light, nutrients), resulting in survival and potentially increased growth compared to those species unable to accommodate to unfavorable conditions. It has thus been hypothesized that osmotrophy in HABs is an advantage that may help them thrive during periods when inorganic nutrients may not be sufficient.

13.4 Ecological Significance of Osmotrophy

For osmotrophy to be of significance ecologically, the organisms present must have the capability for uptake of complex organic nutrients and/or carbon, and such compounds must be available in the environment. To illustrate the ecological importance of osmotrophy, several examples are described, which show both the diversity of the sources supporting HAB osmotrophy and the significance of these sources in their proliferation.

Blooms of the dinoflagellate *Karenia brevis* generally originate offshore, in oligotrophic waters, and may be stimulated by sources of nutrients that are not of anthropogenic origin. Indeed, it is thought that these blooms are stimulated by organic matter produced by blooms of yet another HAB species of the cyanobacterium genus *Trichodesmium* (Walsh and Steidinger 2001). *Trichodesmium* spp. are diazotrophs that can form large blooms in tropical and subtropical regions of the world's oceans (LaRoche and Breitbarth 2005). When these cells are actively fixing N_2 they release ammonium and DON, mostly in the form of low molecular weight compounds (Capone et al. 1994; Glibert and Bronk 1994). The release of DON during blooms can enrich the ambient DON within blooms by 50–500 % (Karl et al. 1992; Glibert and O'Neil 1999). Using isotopic techniques in which the *Trichodesmium* were first enriched with $^{15}N_2$, and the released DON was subsequently collected and provided to natural *K. brevis*, the direct uptake of this source of nitrogen was documented (Bronk et al. 2004).

DON stimulates another dinoflagellate, *P. minimum*, during its early stages of development, but in this case, DON is typically of terrestrial or anthropogenic origin. The occurrence of large *P. minimum* blooms has often been linked to eutrophication in coastal environments (Heil et al. 2005). A large proportion of river nutrient loading is in the form of humic substances, high molecular weight organic compounds which, while generally considered refractory, may stimulate *P. minimum*. In mesocosm experiments, Granéli et al. (1985) showed that dinoflagellate populations, including *P. minimum*, were stimulated by inorganic nitrogen only when added in combination with humic acids. Carlsson et al. (1999) found that humic additions stimulated the growth of this dinoflagellate, and that *P. minimum* used 35 % of the DON from the added humics. Heil (2005) also found that organic additions stimulated growth rates and cell yields of *P. minimum*, but the extent of stimulation varied with the organic fraction examined and its molecular weight: stimulation was greatest with humic and fulvic acids and lowest with hydrophilic acids.

Aureococcus anophagefferens provides yet another model of the role of DON in stimulating brown-tides. Development of these blooms has previously been correlated with years of low rainfall (LaRoche et al. 1997), in turn leading to higher ratios of organic: inorganic nitrogen. Studies in natural blooms have substantiated high uptake rates of organic nitrogen (e.g., Berg et al. 1997). Lomas et al. (2004) identified the benthos as an important source of both organic nitrogen and phosphorus in the Long Island, New York embayments where these blooms are most common. Recent reports underscore the importance of DON, specifically low molecular weight DON (urea and amino acids), in promoting growth of these cells and therefore the development of blooms, particularly under low light (Pustizzi et al. 2004; Mulholland et al. 2004). Peptides, acetamide and urea contribute significantly to the growth of *A. anophagefferens* under axenic conditions (Berg et al. 2002).

Urea has been related to several HAB outbreaks. Urea is a simple organic molecule with significant ecological implications because of the growing use of urea as an agricultural fertilizer (Glibert et al. 2006). Blooms of cyanobacteria have been found to be directly related to urea availability or rates of urea uptake (e.g., Berman 1997; Glibert et al. 2004). In Florida Bay, the fraction of the algal community composed of cyanobacteria was positively correlated with the fraction of urea-N uptake and negatively correlated with the fraction of nitrate-N uptake (Glibert et al. 2004). In the Baltic Sea, urea was among the nitrogen compounds shown to contribute significantly to the growth of the filamentous cyanobacteria *Aphanizomenon* sp. and *Nodularia spumigena* (Panosso and Granéli 2000; Berg et al. 2003). Urea has also been found to promote faster growth than other nitrogen sources in *A. ovalisporum* and *T. theibautii* (Berman and Chava 1999; Mulholland et al. 1999). Urea has also been found to be significant for several HAB dinoflagellate outbreaks, such as in blooms of *Lingulodinium polyedrum* off the coast of California (Kudela and Cochlan 2000) and *Alexandrium catenella* from the Thau lagoon, Mediterranean Sea (Collos et al. 2004).

Several HAB events are related to an elevation in the ratio of dissolved organic carbon (DOC):DON. Conditions where dissolved organic nutrients and DOC concentrations are high will likely favor osmotrophs over strictly photosynthetic protists. Three separate blooms of *Pfiesteria piscicida, P. minimum* and *A. anophagefferens* in Chesapeake Bay were all correlated with elevated DOC:DON ratios relative to the long-term mean (Glibert et al. 2001). The elevation in this ratio for these particular blooms was a reflection of both elevated levels of DOC as well as a depletion of DON. Lomas et al. (2001) showed this relationship to be robust for numerous brown tide blooms in Long Island, New York. Furthermore, during a bloom of *Gymnodinium* spp. in Kuwait Bay, the ratios of DOC:DON for stations collected within the bloom were approximately twice those determined for non-bloom stations with a mixed phytoplankton assemblage (Heil et al. 2001).

The extent to which carbon from DOC is used by osmotrophs is highly variable. Amino acid carbon may be used more readily than urea-carbon in *A. anophagefferens* (Mulholland et al. 2004). It is thought that DOC is important in the growth and maintenance of blooms of many *Dinophysis* species, including those responsible for diarrhetic shellfish poisoning (DSP). These blooms are often found in light limited environments, such as off the French coast or in the Baltic Sea, where they proliferate in thin layers in the region of the thermocline where light is less than 1 % (Gisselson et al. 2002; Gentien et al. 2005). Utilization of DOC in these species has been documented by the uptake of ^{14}C in organic compounds in the dark (Granéli et al. 1997) and enrichment experiments with natural dinoflagellate communities (Purina et al. 2004). These results suggest that *Dinophysis* can compensate for reduced irradiance by feeding heterotrophically.

13.5 A Comment on Evolutionary Aspects of Osmotrophy

The endosymbiotic theory of eukaryote evolution was introduced by Margulis (1975) and more formally described by Margulis (1981). The concept that symbiotic relationships gave rise to many organelles, including chloroplasts, has now been vastly extended. Whereas the primary plastids all likely trace back to a cyanobacterial endosymbiotic event, the phylogenetic evidence now suggests that early branching of the eukaryotic phototrophs gave rise to two major clades, the “green algae”, which contain chlorophyll-*b* (chl-*b*) and the “red algae” which contain chlorophyll-*c* (chl-*c*) (Delwiche 1999; Grzebyk et al. 2003). With respect to the dinoflagellates, there is evidence for secondary plastid endosymbiotic events, leading to dinoflagellates with either chl-*b* or *c* (Delwiche 1999). Future genomic analyses will be of value in resolving the number and nature of endosymbiotic plastid events (Palmer 2003).

Even with the uncertainties in understanding plastid endosymbiotic evolution, the evolution of mixotrophy is more complex than autotrophy. Raven (1997) suggested that phagotrophy is a primitive character and its absence in photosynthetic protists a derived trait. Osmotrophs take up organic substances via importers localized in the plasma membrane, just like phagotrophs, but they do not have proper food vacuoles and digestion enzymes are likely excreted into the environment. This suggests that in an evolutionary sense, osmotrophy may have preceded phagotrophy because without membrane importers food vacuoles would have no function (Martin et al. 2003). However, this idea is not without controversy, given the traditional view that phagocytosis was necessary for eukaryotic evolution. Mixotrophy is found in a large number of planktonic groups, suggesting independent evolution (Jones 1994). How eukaryotes became osmotrophs in the first place remains unclear, and genomic analyses are needed to see if eukaryotic genes involved in osmotrophy (e.g., cell membrane transport proteins involved in the uptake of specific organic compounds) share similarities with those found among bacteria.

13.6 Conclusions

Organic forms of nutrients originate from various sources, natural and anthropogenic. Organic nutrients are, in turn, used by many HAB species that have multiple acquisition mechanisms. The strategies for nutrient and carbon acquisition by HABs are thus far more complex than were thought a decade or two ago. With the application of the host of methods now available to characterize and quantify organic matter and to measure nutrient and carbon fluxes, the role of osmotrophy in HAB dynamics will increasingly be recognized. However complex, these processes must be better understood, quantified and incorporated into models in order to advance our ability to understand population and food web dynamics and to predict the occurrences of HABs.

Acknowledgements. The authors thank E. Granéli for the invitation to prepare this chapter. This is contribution number 3895 from the University of Maryland Center for Environmental Science. P. Glibert acknowledges financial support from Maryland Sea Grant and NOAA MERHAB. C. Legrand acknowledges financial support from the Swedish Research Council and The European Commission through the FP 6-THRESHOLDS project (GOCE 003933).

References

Antia NJ, Harrison PJ, Oliveira L (1991) The role of dissolved organic nitrogen in phytoplankton nutrition, cell biology and ecology. Phycologia 30:1–89

Aluwihare LI, Repeta DJ, Pantoja S, Johnson CG (2005) Two chemically distinct pools of organic nitrogen accumulate in the ocean. Science 308:1007–1010

Benner RB, Biddanda B, Black B, McCarthy M (1997) Abundance, size distribution, and stable carbon and nitrogen isotopic compositions of marine organic matter isolated by tangential-flow ultrafiltration. Mar Chem 57:243–263

Berg GM, Repeta DJ, 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

Berg GM, Balode M, Purina I, Bekere S, Béchemin C, Maestrini SY (2003) Plankton community composition in relation to availability and uptake of oxidized and reduced nitrogen. Aquat Microb Ecol 30:263–274

Berg GM, Glibert PM, Lomas MW, Burford MA (1997) Organic nitrogen uptake and growth by the chrysophyte *Aureococcus anophagefferens* during a brown tide event. Mar Biol 129:377–387

Berman T (1997) Dissolved organic nitrogen utilization by an *Aphanizomenon* bloom in Lake Kinneret. J Plankton Res 19:577–586

Berman T, Bronk DA (2003) Dissolved organic nitrogen: a dynamic participant in aquatic ecosystem. Aquat Microb Ecol 31:279–305

Berman T, Chava S (1999) Algal growth on organic compounds as nitrogen sources. J Plankton Res 21:1423–1437

Bronk DA (2002) Dynamics of DON. In: Hansell DA, Carlson CA (eds) Biogeochemistry of marine dissolved organic matter. Academic Press, San Diego, pp 153–247

Bronk DA, Glibert PM (1993) Application of a new ^{15}N tracer method to the study of dissolved organic nitrogen uptake during spring and summer in Chesapeake Bay. Mar Biol 115:501–508

Bronk DA, Sanderson MP, Mulholland MR, Heil CA, O’Neil JM (2004) Organic and inorganic nitrogen uptake kinetics in field populations dominated by *Karenia brevis*. In: Steidinger KA, Landsberg JH, Tomas CR, Vargo GA (eds) Harmful algae 2002. Florida Fish and Wildlife Conservation Comm, Fl Inst Oceanogr, IOC-UNESCO, St. Petersburg, USA, pp 80–82

Capone DG, Ferrier MD, Carpenter EJ (1994) Amino acid cycling in colonies of the planktonic marine cyanobacterium *Trichodesmium thiebautii*. Appl Environ Microbiol 60:3989–3995

Carlsson P, Granéli E, Segatto AZ (1999) Cycling of biological available nitrogen in riverine humic substances between marine bacteria, a heterotrophic nanoflagellate and a photosynthetic dinoflagellate. Aquat Microb Ecol 18:23–36

Chrost RJ (1991) Microbial enzymes in aquatic environments. Springer, Berlin Heidelberg New York, 317 pp

Collos Y, Gagné 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

Delwiche CF (1999) Tracing the web of plastid diversity through the tapestry of life. Am Nat 154:S164–S177

Doblin M, Legrand C, Carlsson P, Hummert C, Granéli E, Hallegraeff G (2001) Uptake of radioactively labeled humic substances by the toxic dinoflagellate *Alexandrium catenella*. In: Hallegraeff GM, Blackburn SI, Bolch CJ, Lewis RJ (eds) Harmful algal blooms 2000. IOC-UNESCO, Paris, pp 336–339

Dyhrman ST (2005) Ectoenzymes in *Prorocentrum minimum*. Harmful Algae 4:619–627

Dyhrman ST, Anderson DM (2003) Urease activity in cultures and field populations of the toxic dinoflagellate *Alexandrium*. Limnol Oceanogr 48:647–655

Dyhrman ST, Palenik BP (1999) Phosphate stress in cultures and field populations of the dinoflagellate *Prorocentrum minimum* detected using a single cell alkaline phosphatase activity assay. Appl Environ Microbiol 65:3205–3212

Estep K, MacIntyre F (1989) Taxonomy, life cycle, distribution and dasmotrophy of *Chrysochromulina*: a theory accounting for scales, haptonema, muciferous bodies and toxicity. Mar Ecol Prog Ser 57:11–21

Fan C, Glibert PM, Alexander J, Lomas MW (2003) Characterization of urease activity in three marine phytoplankton species. Mar Biol 142:949–958

Gentien P, Donoghay P, Yamazaki H, Raine R, Reguera B, Osborn T (2005) Harmful algal blooms in stratified environments. Oceanography 18:172–183

Gisselson L-Å, Carlsson P, Granéli E, Pallon J (2002) *Dinophysis* blooms in the deep euphotic zone of the Baltic Sea: do they grow in the dark? Harmful Algae 1:401–418

Glibert PM, Bronk DA (1994) Release of dissolved organic nitrogen by the marine diazotrophic cyanobacterium *Trichodesmium* spp. Appl Environ Microbiol 60:3996–4000

Glibert PM, O'Neil JM (1999) Dissolved organic nitrogen release and amino acid oxidase activity by *Trichodesmium* spp. In: Charpy L, Larkum T (eds), Marine cyanobacteria. Bull Inst Océanogr, special no. 19:265–271

Glibert PM, Harrison J, Heil C, Seitzinger S (2006) Escalating worldwide use of urea – a global change contributing to coastal eutrophication. Biogeochemistry (in press)

Glibert PM, Heil CA, 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 PM, Magnien R, Lomas MW, Alexander J, Fan C, Haramoto E, Trice TM, Kana TM (2001) Harmful algal blooms in the Chesapeake and Coastal Bays of Maryland, USA: Comparison of 1997, 1998, and 1999 events. Estuaries 24:875–883

Glibert PM, Seitzinger S, Heil CA, Burkholder JM, Parrow MW, Codispoti LA, Kelly V (2005) The role of eutrophication in the global proliferation of harmful algal blooms. Oceanography 18:198–209

Granéli E, Anderson DM, Carlsson P, Maestrini SY (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, Edler L, Gedziorowska D, Nyman U (1985) Influence of humic and fulvic acids on *Prorocentrum minimum* (Pav) J Schiller. In: Anderson DM, White AW, Baden DG (eds) Toxic dinoflagellates. Elsevier, New York, pp 201–206

Grzebyk D, Schofield O, Vetriani C, Falkowski PG (2003) The Mesozoic radiation of eukaryotic algae: The portable plastid hypothesis. J Phycol 39:259–267

Heil CA (2005) Influence of humic, fulvic and hydrophilic acids on the growth, photosynthesis and respiration of the dinoflagellate *Prorocentrum minimum* (Pavillard) Schiller. Harmful Algae 4:603–618

Heil CA, Glibert PM, Al-Sarawi MA, Faraj M, Behbehani M, Husain M (2001) First record of a fish-killing *Gymnodinium* sp. bloom in Kuwait Bay, Arabian Sea: Chronology and potential causes. Mar Ecol Prog Ser 214:15–23

Heil CA, Glibert PM, Fan C (2005) *Prorocentrum minimum* (Pavillard) Schiller –A review of a harmful algal bloom species of growing worldwide importance. Harmful Algae 4:449–470

Jones RI (1994) Mixotrophy in planktonic protists as a spectrum of nutritional strategies. Mar Microb Food Webs 8:87–96

Karl DM, Letelier R, Hebel RV, Bird DF, Winn CD (1992) *Trichodesmium* blooms and new nitrogen in the North Pacific Gyre. In: Carpenter EJ, Capone DG, Reuter JG (eds) Marine pelagic cyanobacteria: *Trichodesmium* and other diazotrophs. NATO ASI Series 362. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 219–238

Kimor B (1979) Predation by *Noctiluca miliaris* Souriray on *Acartia tonsa* Dana eggs in the inshore waters of southern California. Limnol Oceanogr 24:568–572

Klut ME, Bisalputra T, Antia NJ (1987) Some observations on the structure and function of the dinoflagellate pusule. Can J Bot 65:736–744

Kudela RM, Cochlan WP (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

Legrand C, Carlsson P (1998) Uptake of high molecular weight dextran by the dinoflagellate *Alexandrium catenella*. Aquat Microb Ecol 16:81–86

LaRoche J, Breitbarth E (2005) Importance of the diazotrophs as a source of new nitrogen in the ocean. J Sea Res 53:67–91

LaRoche J, Nuzzi R, Waters R, Wyman K, Falkowski PG, Wallace DWR (1997) Brown tide blooms in Long Island's coastal waters linked to variability in groundwater flow. Global Change Biol 3:397–410

Lomas MW, Glibert PM, Clougherty DA, Huber DR, Jones J, Alexander J, Haramoto E (2001) Elevated organic nutrient ratios associated with brown tide algal blooms of *Aureococcus anophagefferens* (Pelagophyceae). J Plankton Res 23:1339–1344

Lomas MW, Kana TM, MacIntyre HL, Cornwell JC, 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

Margulis L (1975) Symbiotic theory of the origin of eukaryotic organelles; criteria for proof. Symp Soc Exp Biol. 29:21–38

Margulis L (1981) Symbiosis in cell evolution. W. H. Freeman and Company, San Francisco, pp 206–227

Martin W, Rotte C, Hoffmeister M, Theissen U, Gelius-Dietrich G, Ahr S, Henze K (2003) Early cell evolution, eukaryotes, anoxia, sulfide, oxygen, fungi first (?), and a tree of genomes revisited. IUBMB Life 55:193–204

Milius S (1999) Myriad monsters confirmed in water droplets. Science News Online, vol 156, www.sciencenews.org/pages/sn_arc99/12_18_99b/fob1.htm

Moorthi S (2004) Mixotrophic flagellates in coastal marine sediments: quantitative role and ecological significance. PhD Thesis, Leibniz Institute of Marine Sciences, Kiel University, Germany, 128 pp

Mulholland MR, Boneillo G, Minor EC (2004) A comparison of N and C uptake during brown tide (*Aureococcus anophagefferens*) blooms from two coastal bays on the east coast of the USA. Harmful Algae 3:361–376

Mulholland MR, Gobler CJ, Lee C (2002) Peptide hydrolysis, amino acid oxidation and N uptake in communities seasonally dominated by *Aureococcus anophagefferens*. Limnol Oceanogr 47:1094–1108

Mulholland MR, Glibert PM, Berg GM, Van Heukelem L, Pantoja S, Lee C (1998) Extracellular amino acid oxidation by microplankton: a cross-ecosystem comparison. Aquat Microb Ecol 15:141–152

Mulholland MR, Ohki K, Capone DG (1999) Nitrogen utilization and metabolism relative to patterns of N_2 fixation in cultures of *Trichodesmium* NIBB1067. J Phycol 35:977–988

Palenik B, Morel FMM (1990) Comparison of cell-surface L-amino acid oxidases from several marine phytoplankton. Mar Ecol Prog Ser 59:195–201

Palmer JD (2003) The symbiotic birth and spread of plastids: how many times and whodunit? J Phycol 39:4–11

Panosso R, Granéli E (2000) Effect of dissolved organic matter on the growth of *Nodularia spumigena* (Cyanophyceae) cultivated under N and P deficiency. Mar Biol 136:331–336

Purina I, Balode M, Béchemin C, Pöder T, Maestrini S (2004) Influence of dissolved organic matter from terrestrial origin on the changes of dinoflagellates species composition in the Gulf of Riga, Baltic Sea. Hydrobiologia 514:127–137

Pustizzi F, MacIntyre H, Warner ME, Hutchins DA (2004) Interactions of nitrogen source and light intensity on the growth and photosynthesis of the brown tide alga *Aureococcus anophagefferens*. Harmful Algae 3:343–360

Raven JA (1997) Phagotrophy in phototrophs. Limnol Oceanogr 42:198–205

Seitzinger SP, Hartnett HE, Lauck R, Mazurek M, Minegishi T, Spyres G, Styles RM (2005) Molecular level chemical characterization and bioavailability of dissolved organic matter in stream water using ESI mass spectrometry. Limnol Oceanogr 50:1–12

Skovgaard A (1998) Role of chloroplast retention in a marine dinoflagellate. Aquat Microb Ecol 15:293–301

Smayda TJ (1997) Harmful algal blooms: their ecophysiology and general relevance to phytoplankton blooms in the sea. Limnol Oceanogr 42:1137–1153

Stoecker DK (1999) Mixotrophy among dinoflagellates. J Eukaryot Microbiol 46:397–401

Stoecker DK, Gustafson D (2003) Cell-surface proteolytic activity of photosynthetic dinoflagellates. Aquat Microb Ecol 30:175–183

Veuger B, Middelburg JJ, Boschker HTS, Nieuwenhuize J, van Rijswijk P, Rochelle-Newall EJ, Navarro N (2004) Microbial uptake of dissolved organic and inorganic nitrogen in Randers Fjord. Est Coast Shelf Sci 61:507–515

Walsh JJ, Steidinger KA (2001) Saharan dust and Florida red tides: the cyanophyte connection. J Geophys Res 106:11597–11612

Wheeler PA, North BB, Stephens GC (1974) Amino acid uptake by marine phytoplankters. Limnol Oceanogr 19:249–259