

The Role of Eutrophication in the Global Proliferation of Harmful Algal Blooms

NEW PERSPECTIVES AND NEW APPROACHES

BY PATRICIA M. GLIBERT, SYBIL SEITZINGER,
CYNTHIA A. HEIL, JOANN M. BURKHOLDER, MATTHEW W. PARROW,
LOUIS A. CODISPOTI, AND VINCE KELLY

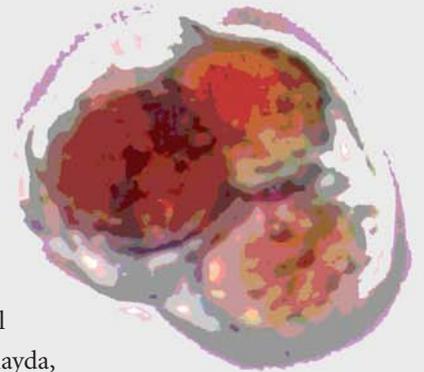
Eutrophication is now recognized to be one of the important factors contributing to habitat change and to the geographical and temporal expansion of some harmful algal bloom species...

Cultural eutrophication, the pollution of coastal waters by nutrients, is a result of population growth, food production (agriculture, animal operations and aquaculture), and energy production and consumption, and is considered one of the largest pollution problems globally (Howarth et al., 2002). Population growth and food production result in major changes to the landscape, in turn, increasing sewage discharges and runoff from agriculture and populated lands. In addition to population growth, eutrophication arises from the large increases in the use of chemical fertilizers that began in the 1950s and which are projected to continue to escalate in the coming decades (Smil, 2001). Both nitrogen and phosphorus are of concern in eutrophication, but nitrogen has received far more attention because it often limits primary production in estuaries and coastal waters and because the global application of nitrogen from synthetic fertilizers is far greater than that of phosphorus (more information available at www.ut.ee/~olli/eutr/).

Although cultural eutrophication is occurring globally, nutrient export is not evenly distributed (Seitzinger et al., 2002a). For example, inorganic nitrogen export to coastal waters is estimated to be highest from European and Asian lands, although significant discharge also occurs from the United States and other parts of the world. Furthermore, the rate of nutrient export to coastal waters has increased dramatically in recent years in some parts of the world. China, which consumed less than 5 million tonnes of nitrogen fertilizer in the 1970s, now consumes more than 20 million tonnes per year, representing 25 percent of global nitrogen fertilizer consumption (Figure 1) (more information available at www.fertilizer.org), leading to significant increased nitrogen pollution of its coastal waters.

Global increases in population growth and food production, and consequently eutrophication, have important impacts on the environment, affecting the microbiota of adjacent waters,

for example. Eutrophication is now recognized to be one of the important factors contributing to habitat change and to the geographical and temporal expansion of some harmful algal bloom (HAB) species (Smayda, 1990; Anderson et al., 2002).



Some of the clearest examples of the relationship between HAB frequency and increases in total nutrient loadings to coastal waters come from China. Since the 1970s, when escalation in use of chemical fertilizer began in China, the number of HAB outbreaks has increased over 20-fold, with blooms that now are of greater geographic extent, more toxic, and more prolonged (Anderson et al., 2002). Other examples of a positive relationship between nutrient loading and HAB outbreaks can be found throughout the world. In northern European waters, blooms of the mucus-forming HAB species *Phaeocystis globosa* have been shown to be directly related to the nitrate content of riverine and coastal waters (Lancelot, 1995). In the United States, a relationship between increased nutrient loading from the Mississippi River to the Louisiana shelf and increased abundance of the toxic diatom *Pseudo-nitzschia pseudodelicatissima* has been documented from examination of the diatom frustules preserved in sediment cores (Parsons et al., 2002).

Indeed, strong positive relationships have been reported between increasing human population and the intensity of some HAB outbreaks. In Puget Sound, in the U.S. Pacific Northwest, where consistent shellfish monitoring has been carried out for decades, a strong positive relationship between the frequency of one algal toxin, paralytic shellfish toxin (PST), and the growth

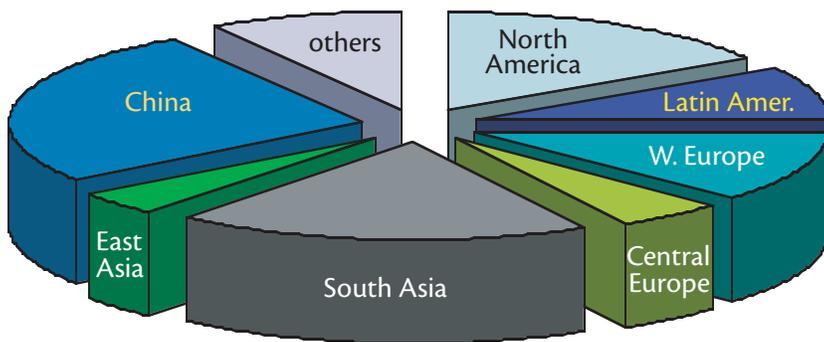
in human population has been reported (Trainer et al., 2003) (Figure 2). In this case, eutrophication was considered to be one of the important factors contributing to the increased incidence of PST as intensive monitoring efforts have been in place for many decades.

One mechanism by which eutrophication can shift coastal community structure toward the promotion of HAB species is by changing the ratio of individual nutrients relative to other nutrients. Many studies have examined the relationships between HABs and inorganic

nutrient ratios, such as nitrogen:phosphorus (N:P) or nitrogen:silicon (N:Si) (e.g., Smayda, 1990; 1997), and some successes have been achieved in relating shifts in these ratios to HAB outbreaks (Anderson et al., 2002). In Tolo Harbor, Hong Kong, during the 1980s, the molar ratio of N:P decreased nearly 50 percent as both human population and sewage loading increased several-fold and, concomitantly, the numbers of dinoflagellate HAB outbreaks increased almost ten-fold (Lam and Ho, 1989). Altered nutrient ratios also have been correlated with shifts from diatom-dominated to flagellate-dominated assemblages in some U.S., European, and Asian coastal waters. Even on short time scales, changing nutrient ratios can be related to HAB outbreaks, as in Tunisian aquaculture lagoons where toxic dinoflagellates develop seasonally when N:P ratios change (Romdhane et al., 1998).

While these examples demonstrate the relationship of several HAB species with both total nutrient loading or changes in specific nutrient ratios, many other dinoflagellate species involved in HAB events have not appeared to follow these patterns, leading to much confusion about the role of eutrophication in their development. One of the difficulties in linking nutrient loading to HABs is the multiplicity of factors contributing to HAB species responses to nutrient loading. The inability to universally apply a single criterion, such as the N:P (or other nutrient) ratio, to determine whether eutrophication is stimulating HABs does not negate the utility of this approach. Rather, it underscores the interdependence of environmental factors, physiological factors, and trophic interactions in the out-

Contribution by global region to the consumption of the world's annual current use of 85 million tonnes of nitrogen



Estimate of the increase in nitrogen fertilizer consumption through the Year 2030

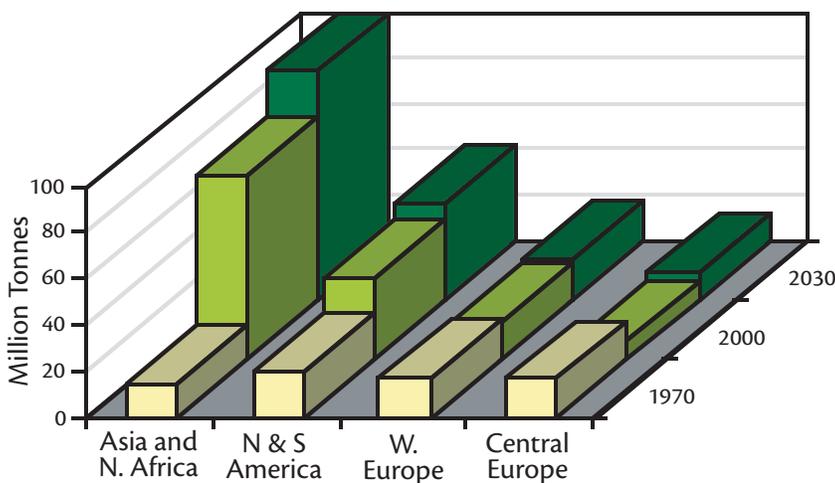


Figure 1. The use of nitrogen fertilizer varies considerably around the globe. Of the 85 million tonnes of nitrogen consumed annually, Asia uses about half. The greatest projected increases in global nitrogen fertilizer are also expected to be in Asia over the next 25 years. Data from the International Fertilizer Industry.

come of any species succession, as well as the importance of appropriately scaled temporal and spatial data (Glibert et al., this issue). The old paradigm (that HABs are stimulated by nutrient over-enrichment only if there is a direct and visibly evident increase in their biomass) is giving way to an appreciation for the fact that nutrient enrichment can also indirectly stimulate HABs in more subtle but significant ways over the long term—for example, by increasing hypoxia that, in turn, eliminates filter-feeding shellfish that would have consumed HABs but that can no longer survive in the habitat (Burkholder, 2001).

INORGANIC NUTRIENTS ARE NOT THE ONLY NUTRIENT SOURCE FOR MANY HABs

Inputs of the major nutrients include both inorganic (ammonium, nitrate, nitrite, phosphate, silicate) and organic forms (dissolved organic and particulate matter). The magnitude and changing composition of nutrients, both inorganic and organic, discharged to the coastal zone are beginning to be better understood and quantified. For example, about half of the dissolved nitrogen transported by rivers to the coastal ocean is in the form of dissolved organic nitrogen (DON) (Meybeck, 1982). Dissolved organic matter (DOM), the more inclusive term for organic material containing nutrients as well as carbon, is exported from all natural and anthropogenic land uses in watersheds, but the magnitude and composition of this export is changing as land use changes. While fertilizer use throughout the world is increasing (Vitousek et al., 1997), the fraction of total fertilizer that is composed of or-

ganic material and manures is increasing at a greater rate (Smil, 2001). Worldwide use of urea as a nitrogen fertilizer and feed additive has increased more than 50 percent in the past decade (Glibert et al., in press). Based on fertilizer pro-

duction and application statistics, it is now estimated that urea represents more than 50 percent of world nitrogen fertilizer use, a dramatic global change in the composition of nitrogen in one decade (Smil, 2001; Glibert et al., in press). The

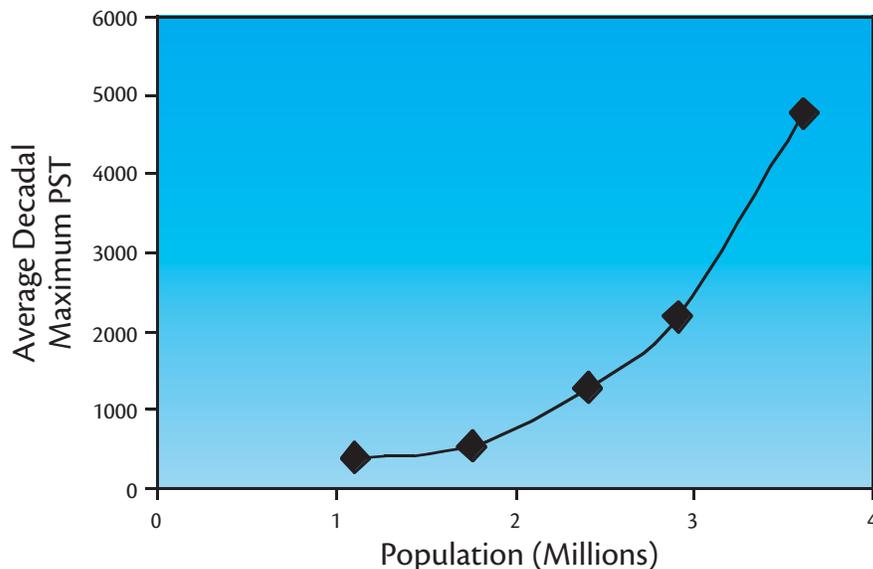


Figure 2. An example of the relationship between population growth and HABs. These data are from Puget Sound, Washington, USA, where continuous monitoring of paralytic shellfish poisoning has been ongoing since the mid 1950s. Plotted here is the relationship between human population in the region for the past 40 years (data were derived from the U.S. census) and the average decadal maximum recorded amount of paralytic shellfish toxins (PST). Replotted from Trainer et al. (2003).

Patricia M. Glibert (glibert@hpl.umces.edu) is Professor, University of Maryland Center for Environmental Science, Horn Point Laboratory, Cambridge, MD, USA. **Sybil Seitzinger** is Visiting Professor, Rutgers University, Institute of Marine and Coastal Sciences, Rutgers/NOAA CMER Program, New Brunswick, NJ, USA. **Cynthia A. Heil** is Senior Research Scientist, Fish and Wildlife Research Institute, Florida Fish and Wildlife Conservation Commission, St. Petersburg, FL, USA. **JoAnn M. Burkholder** is Professor and Director, Center for Applied Aquatic Ecology, North Carolina State University, Raleigh, NC, USA. **Matthew W. Parrow** is Postdoctoral Research Associate, Center for Applied Aquatic Ecology, North Carolina State University, Raleigh, NC, USA. **Louis A. Codispoti** is Research Professor, University of Maryland Center for Environmental Science, Horn Point Laboratory, Cambridge, MD, USA. **Vince Kelly** is Senior Research Assistant, University of Maryland Center for Environmental Science, Horn Point Laboratory, Cambridge, MD, USA.

development of concentrated animal feed operations in many countries, many located near coastal areas, has also led to massive amounts of organic waste produced in relatively small areas of land that cannot absorb it over the long term (Mallin, 2000).

The mode of delivery of both inorganic and organic nutrients (especially nitrogen) is not restricted to aquatic sources; atmospheric input is also important. In many estuarine and coastal waters the atmosphere may contribute up to 40 percent of the total inorganic plus organic nitrogen inputs (Howarth et al., 2002), and local emissions near large-animal operations and feedlots may be even greater. It has been further demonstrated that DON from atmospheric deposition can stimulate coastal phytoplankton production (Seitzinger and Sanders, 1999), but much remains to be understood in terms of the role of this nutrient source in HAB proliferation.

The chemical composition of DOM exported from agricultural watersheds is not known, but up to 50 percent can be taken up directly or indirectly by estuarine plankton communities (Seitzinger et al., 2002b). The chemical composition, bioavailability, and effects of DOM on coastal plankton communities vary depending on the source of the DOM, the plankton community composition, and the season. ***We are beginning to recognize the magnitude of export of DOM, but quantifying its variable composition and the extent to which it is contributing to eutrophication effects within coastal environments is one of the large challenges facing oceanographers today.***

The significance of organic nutrients

to HAB nutrition is also now beginning to be recognized. Many HAB species rely strictly upon photosynthesis for their energy and use inorganic nutrients (e.g., nitrogen, phosphorus, carbon). Other species, however, use not only photosynthesis but also dissolved or particulate organic nutrients for some or all of their nutrient demands (e.g., Granéli et al., 1999; Stoecker, 1999). Organic nutrients have been shown to be important in the development of blooms of various HAB species, in particular cyanobacteria and dinoflagellates (e.g., Paerl, 1988; Glibert et al., 2001), and the importance of this phenomenon is beginning to be documented around the world. A recent study found that in Florida Bay the cyanobacterial fraction of the algal community was positively correlated with the fraction of nitrogen taken up as urea, and negatively correlated with the fraction of nitrogen taken up from the inorganic source, nitrate (Glibert et al., 2004). Other studies have shown that some cyanobacteria grow faster on urea than on other nitrogen sources (e.g., Berman and Chava, 1999).

In addition to direct uptake, many phytoplankton have other mechanisms to acquire nutrients. These phytoplankton are referred to as mixotrophs (i.e., species that “mix” their strategies for taking up nutrients). They use complex organic molecules for both nutrients and energy, and their pathways for obtaining these nutrient forms include extracellular oxidation and hydrolysis (Figure 3). Some phytoplankton, including an increasing number of HAB species, are now also recognized as able to acquire their nutrients or energy via particle ingestion: they eat! These spe-

cies can consume (phagocytize) other living organisms or substances produced by them, as illustrated in Figure 3. Many of the physiological and behavioral mechanisms involved in these modes of nutrition are difficult to quantify, and even more difficult to relate to species shifts or dominance. Nevertheless, the list of HAB species with reported mixotrophic capability is long, and includes species such as *Chrysochromulina polylophoides*, *Dinophysis norvegica* and *D. acuminata*, *Pfiesteria spp.*, and *Prorocentrum minimum* and *Karlodinium micrum*. Yet, the extent to which they eat depends on other factors that regulate their nutrition. *Prorocentrum minimum*, for example, appears to become proportionately more mixotrophic when it becomes inorganic nutrient-limited (Stoecker et al., 1997); however, it can take up some organic nutrients, including humic substances, when supplied, regardless of nutrient status (Granéli et al., 1985). As would be expected for most organisms that feed, feeding activity by *Pfiesteria spp.* decreases as the cells become engorged with prey.

The complex mixture of organic nutrients in the environment can also have other stimulatory effects on HABs. Organic nutrients may stimulate bacteria or other heterotrophs that, in turn, can stimulate the development of heterotrophic or mixotrophic HAB species by favorably altering the micro-environment. Thus, organic forms of nutrients may have many sources, from synthetic fertilizer, to natural terrestrial sources, to release by other organisms. Organic nutrients are, in turn, used by many HAB species that have multiple acquisition mechanisms.

STRAINS ARE NOT CREATED EQUAL

Many generalizations about how HAB species respond to nutrient enrichment have been based upon culture studies of only one or a few populations, or strains, of a given species, cultured from a single cell isolated from the natural environment. It is often assumed that the characteristics of one strain maintained for years under highly artificial laboratory conditions are representative of all strains of that species in the natural environment. Not so. Different strains within the same species have been shown to differ

markedly in fundamental traits such as growth characteristics, toxicity, bloom-forming behavior, and responses to nutrients and other environmental conditions.

Consideration of strain variations within a given HAB species is fundamentally important because many strains can occur in the natural environment and because laboratory studies using cultured strains are heavily relied upon to interpret, and to predict, how natural populations respond to eutrophication. Among many examples of significant differences among strains in response to nutrients, Doblin et al. (2000) described

differences between strains of the toxic dinoflagellate *Gymnodinium catenatum* in their requirement for the micronutrient, selenium. Strom and Bright (2003) found that strains of the dimethylsulphide-producing haptophyte *Emiliana huxleyi* differed in their growth response when given inorganic (nitrate, ammonium) and organic (amino acids) nitrogen sources. All four strains tested grew on nitrate and ammonium, but one strain grew only on the inorganic nitrogen sources and, apparently, could not utilize the organic nitrogen sources. The other three strains showed growth when

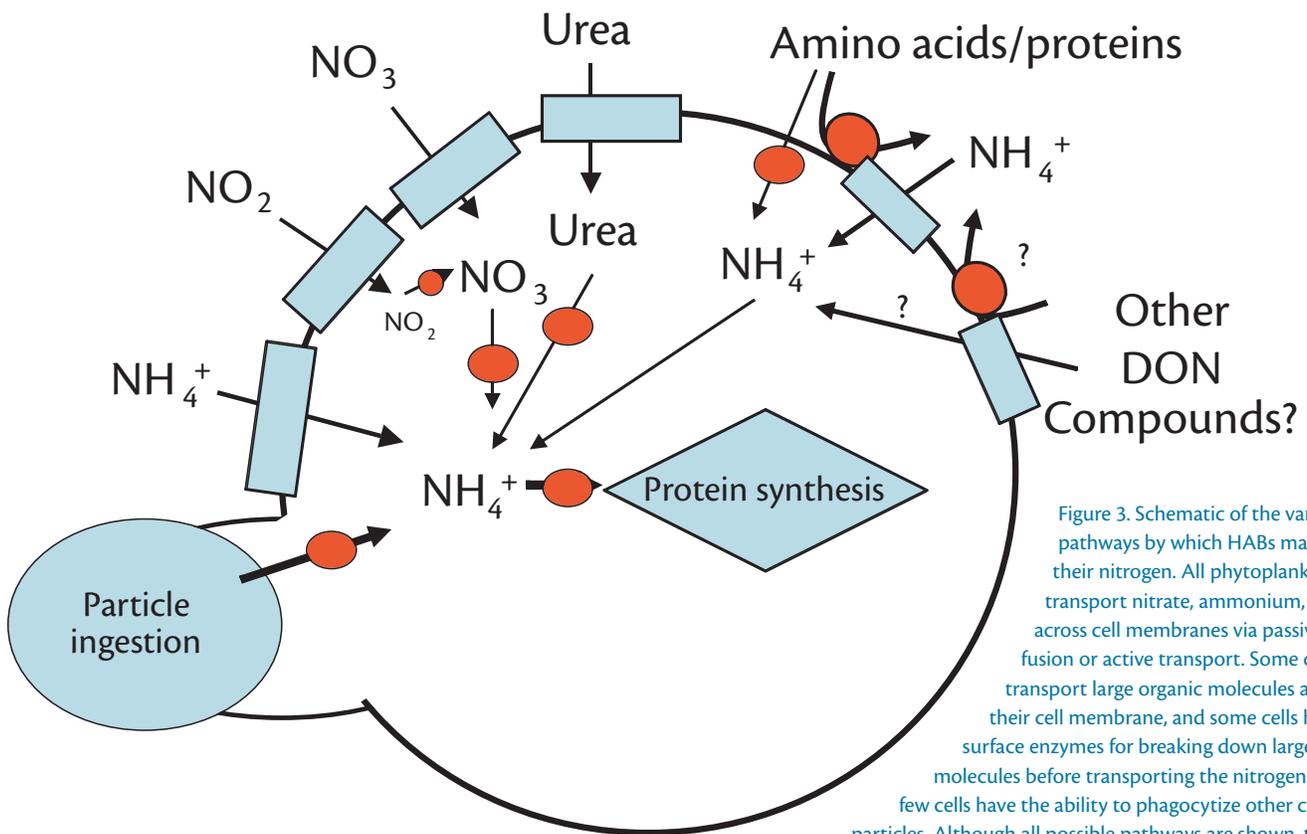


Figure 3. Schematic of the various pathways by which HABs may acquire their nitrogen. All phytoplankton can transport nitrate, ammonium, or urea across cell membranes via passive diffusion or active transport. Some cells can transport large organic molecules across their cell membrane, and some cells have cell surface enzymes for breaking down larger organic molecules before transporting the nitrogen. Only a few cells have the ability to phagocytize other cells and particles. Although all possible pathways are shown, this is not intended to imply that all cells have all capabilities. Pathways that involve enzymatic reactions are indicated with a red circle. Pathways for which there is much uncertainty are indicated with a question mark.

given amino acids, but differed in the amino acids that supported growth—two of the strains grew on glutamine but not alanine, while the third strain grew on alanine but not glutamine. Such data show the importance of including multiple strains in research to understand the nutritional ecology and physiology of HAB species.

PHYSIOLOGY IS COMPLICATED BY ENVIRONMENTAL CONDITIONS, TIMING, AND TROPHIC INTERACTIONS

The ultimate success of a given species depends upon its ability to exploit the quantity and quality of available nutrients, the timing and intensity of the nutrient supplies, and the interactions with other environmental factors and competitor or consumer species. Further complicating the interpretation of these effects is the response of individual species that may also be separated in time and space from the source of nutrient delivery, as nutrients are consumed, recycled, and transformed. Attempts to relate concentrations of nutrients to HAB abundance may fail even in cases where certain nutrients are stimulatory during bloom development if these nutrients are fully consumed by the time maximum biomass is established.

Many aspects of cellular physiology also impact the affinity of a cell for a particular form of nutrient and the immediate fate of that nutrient once taken up. Thus, a nutrient pulse may be assimilated by cells of the same species at different rates depending on the strains present, their nutritional history, and their growth rates. The timing and intensity of nutrient loading may also be impor-

tant in maintaining dominance of one species versus stimulating competitor or consumer species. For example, whether nutrients are delivered in the spring with freshwater runoff or in the summer (as is the case in dry years) will impact downstream total algae accumulation, as grazers are more likely to be poised for rapid algal consumption during the warmer months. In addition, it is important to consider whether nutrients are delivered in a pulsed manner, such as that which would follow heavy rainfall, or a more continuous manner, such as may occur from continuous sewage discharge. For example, in the Chesapeake Bay (Glibert et al., 2001) and the Neuse Estuary (both located on the U.S. East Coast) (Springer et al., 2005), blooms of *Prorocentrum minimum* have occurred following nitrogen delivery in heavy spring rains, but this species does not achieve dense blooms during more dry years.

NEW TOOLS ARE YIELDING NEW INSIGHTS

The potential for advances in understanding the impacts of nutrient pollution on and relating eutrophication to HABs and their proliferation is large. New and better tools are providing methodologies for addressing questions related to the complexity of the nutrient pool, the complexities of the time and space scales on which nutrients are delivered, and the complexity of algal nutrition. Three of many examples are briefly highlighted here.

Electrospray ionization mass spectrometry (ESI-MS) is one such new tool. In contrast to past methodologies for studying the DOM pool—which were mostly limited to bulk analysis of total

carbon or nitrogen, analyses of a few individual compounds (e.g., urea, amino acids, specific proteins), or compound classes that account for a small percent of total DOM (e.g., total carbohydrates, total proteins)—ESI-MS permits examination of a large suite of individual compounds in natural mixes of DOM. Ultrafiltration techniques have been used to characterize DOM by broad molecular weight fraction, but such approaches do not allow characterization or identification of individual compounds. ESI-MS makes it possible to determine which compounds are used by organisms, and which are not, and it also makes it possible to follow rates of utilization of specific compounds. This technique also provides molecular weight information of ionizable compounds (in the form of a mass-to-charge ratio [m/z], which equals molecular weight for singly charged compounds) and a measure of concentration as ion abundance.

One such data set, in which changes in DOM were followed over a 14-day period, demonstrates very different patterns of use for different compounds. Those with an m/z of 137 and 203 were consumed by the natural plankton community, while the compound with an m/z of 253 was not used (Figure 4). As more researchers use this instrument, compound libraries will be developed, allowing characterization of individual compounds and comparison among patterns in use of specific compounds in comparable ecosystems and by comparable species in different environments.

A classic dilemma in assessing whether and to what extent nutrients are stimulatory to a given HAB is that the reporting and sampling of bloom events typically

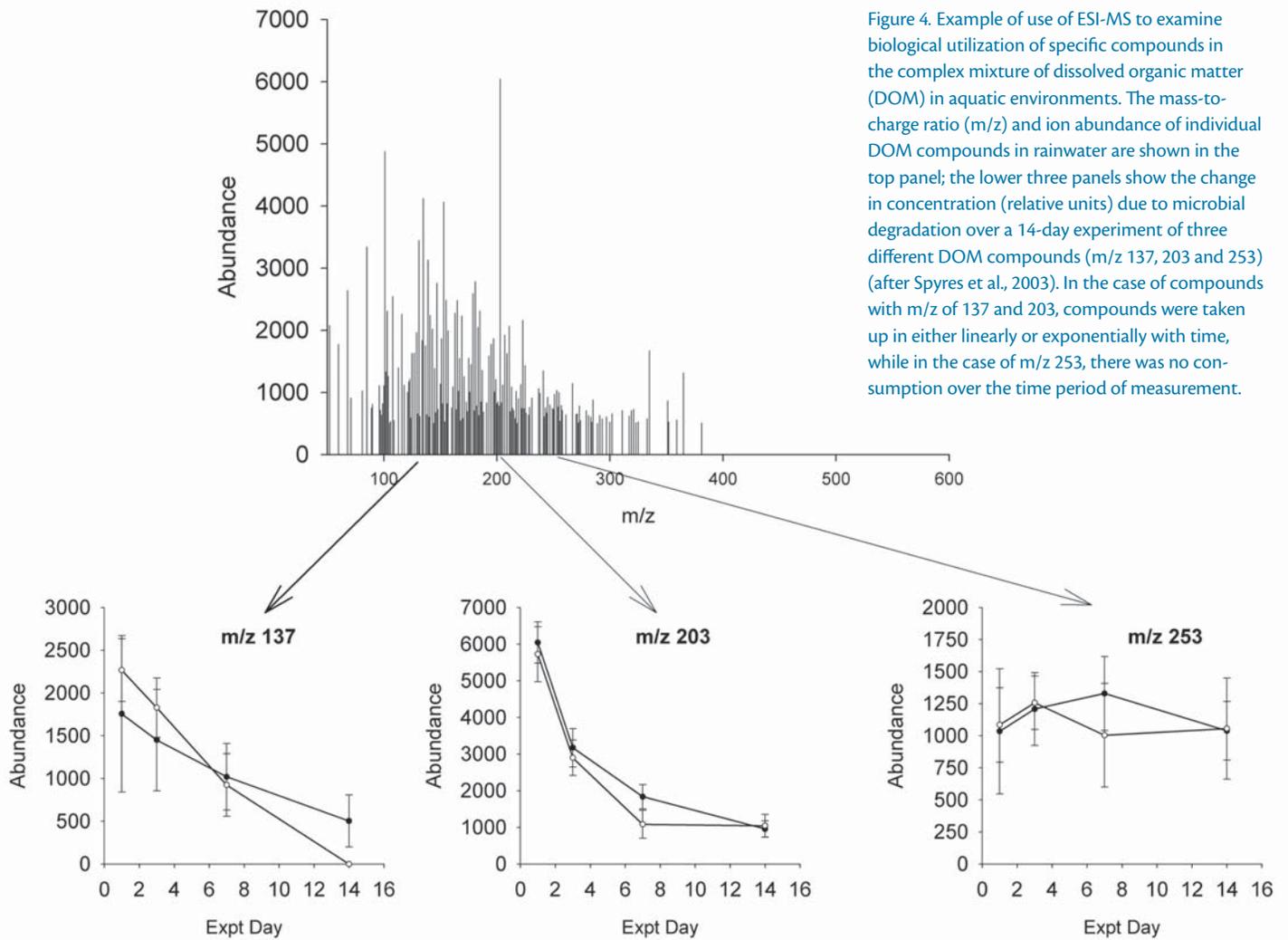


Figure 4. Example of use of ESI-MS to examine biological utilization of specific compounds in the complex mixture of dissolved organic matter (DOM) in aquatic environments. The mass-to-charge ratio (m/z) and ion abundance of individual DOM compounds in rainwater are shown in the top panel; the lower three panels show the change in concentration (relative units) due to microbial degradation over a 14-day experiment of three different DOM compounds (m/z 137, 203 and 253) (after Spyres et al., 2003). In the case of compounds with m/z of 137 and 203, compounds were taken up in either linearly or exponentially with time, while in the case of m/z 253, there was no consumption over the time period of measurement.

occur *after* the HAB has developed. By that time, many nutrients are consumed, leading to the potentially erroneous conclusion that nutrients are unavailable and therefore unimportant. Traditional approaches for collecting information on nutrient distributions, such as weekly or bimonthly sampling, are also inadequate to detect the short-lived nutrient pulses that often follow meteorological events. Development of *in situ* nutrient monitors has provided a tool to begin resolv-

ing the all-important antecedent conditions and to provide sufficient resolution of the temporal scale. Deployments of these monitors in the tributaries draining agricultural lands in the Chesapeake Bay watershed have shown trends in nutrient concentrations that vary with different nutrient sources and that are heavily driven by rainfall events (Figure 5). In one such deployment, coincident with the rainfall events, phosphate “spikes” were recorded, lasting only hours, after

which concentrations decreased but remained elevated for several days in comparison to conditions prior to the precipitation events. In the same study, maximum nitrate + nitrite concentrations lagged behind rainfall events by several days, suggesting either a groundwater discharge following the rain or microbial transformation of reduced nitrogen (e.g., ammonium and urea) following its delivery. Fine-scale temporal resolution in nutrient concentrations also makes it

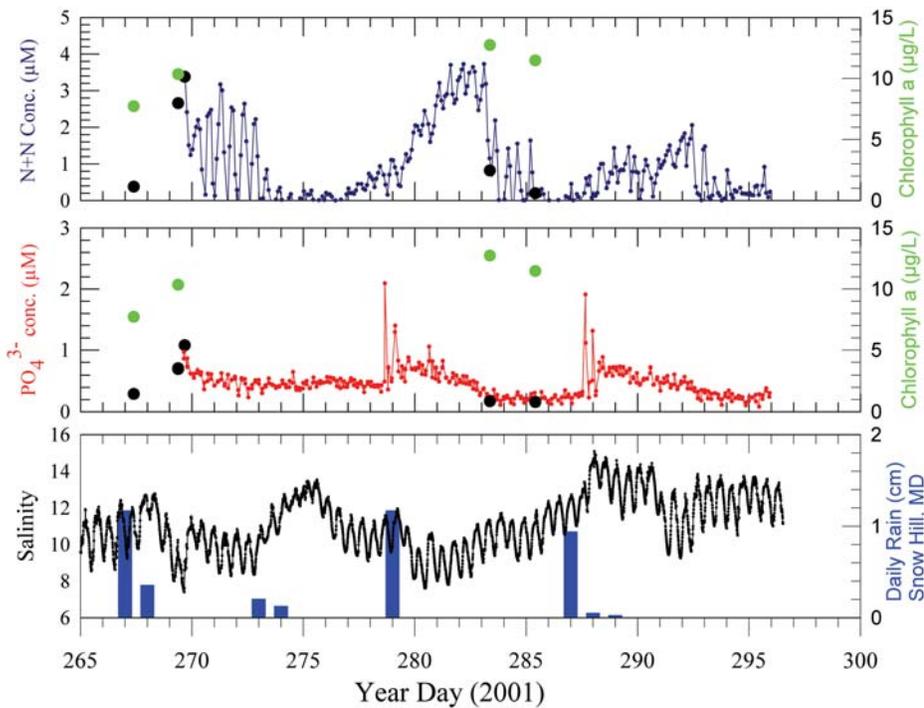


Figure 5. Time series of nitrate + nitrite (upper panel), phosphate (middle panel), and rainfall and salinity (lower panel) in the Pocomoke River, one tributary of the Chesapeake Bay, in which *in situ* nutrient sensors were used for the dates indicated in 2001. The solid black points are reference samples that were collected simultaneously by a small boat. In the upper two panels, the solid green points are the values for chlorophyll *a* determined from the same samples collected from the boat. The lower panel displays rainfall events (as measured at Snow Hill, Maryland, USA) and the fluctuations in salinity. Data from L. Codispoti and colleagues, University of Maryland Center for Environmental Science, Horn Point Laboratory, Cambridge, Maryland, unpublished.

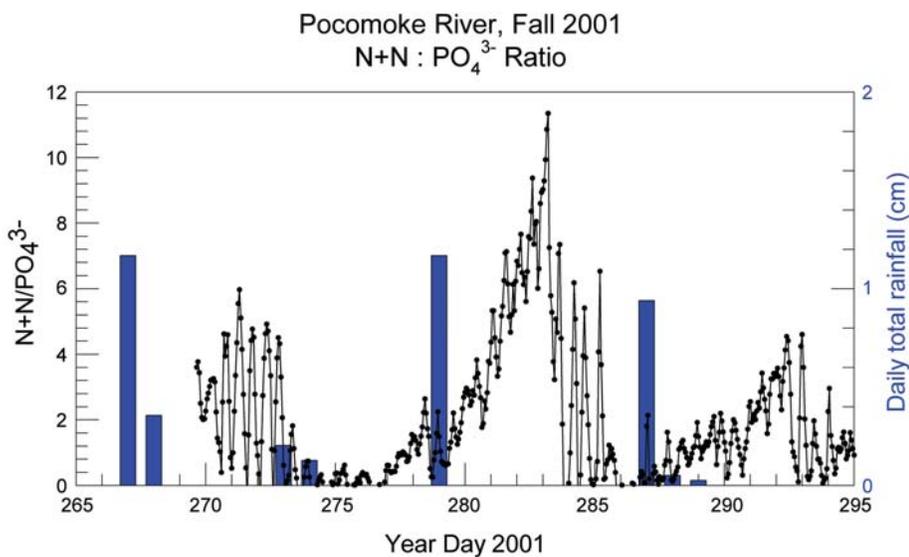


Figure 6. Ratio of (nitrate + nitrite): phosphate³⁻ and daily rainfall during the same *in situ* nutrient instrument deployment shown in Figure 5.

possible to calculate the N:P (in this case, nitrate + nitrite:phosphate) ratio of the dissolved inorganic nutrients (Figure 6). In this example, N:P ratios ranged from less than 1 to greater than 10 over a several-day time span. Most interesting is the fact that chlorophyll *a* concentrations increased several-fold after the short-term increase in nitrogen availability. While increases in nitrate + nitrite following the rainfall drove the ratio higher, it was the draw-down of nitrogen that resulted in sharp declines in this ratio—and increases in chlorophyll *a* based on weekly sampling—following each short-term N spike. Use of these types of *in situ* instruments in conjunction with other remote methods for resolving additional environmental characteristics (Babin et al., this issue) will yield the resolution necessary to define the ephemeral changes in nutrients that can be significant to phytoplankton cells.

Flow cytometry, a methodology for rapidly measuring intrinsic or applied (stained) optical characteristics of individual cells, has been used to study the occurrence, abundance, physiology, and diversity of aquatic microorganisms for the past two decades. This technology has been increasingly applied to the study of HAB species, for example, to generally enumerate and specifically identify harmful algal cells in cultures and natural samples. More recently, flow cytometry also has been used as a tool for tracking changes in the complex structure of aquatic microbial communities (including viruses, pathogenic bacteria, and harmful algae) as related to seasonal cycles, nutrients, or bloom age. Such studies, including recent *in situ* flow cytometric investigations of phy-

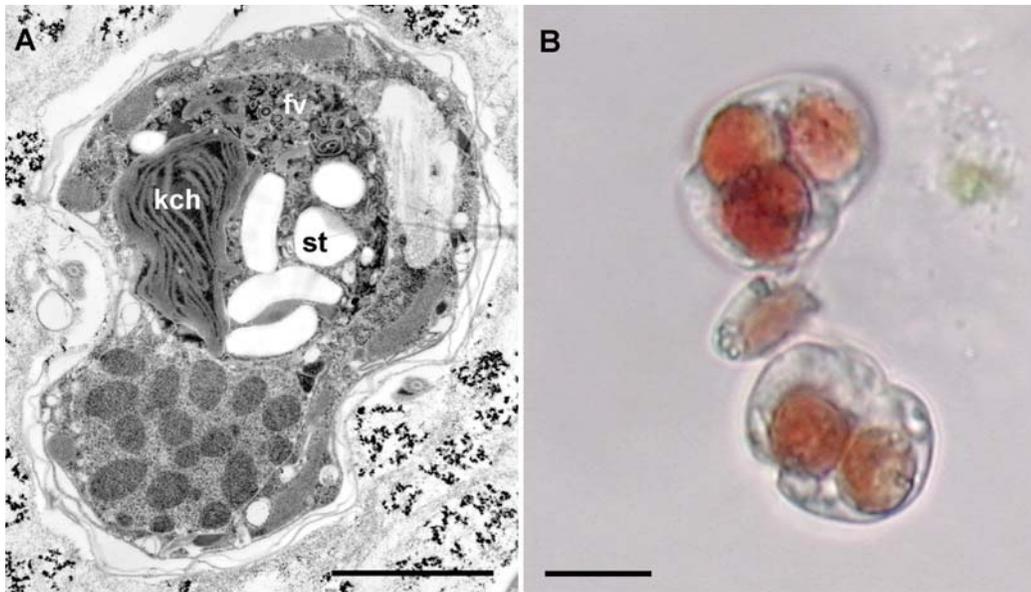


Figure 7. (A) Thin-section electron micrograph of a *Pfiesteria piscicida* flagellated cell showing the large food vacuole in the upper portion of the cell (fv), a kleptochloroplast in the food vacuole (kch), and starch grains (st) apparently produced by the kleptochloroplast. The kleptochloroplast had been ingested from a cryptomonad algal prey cell, and had been retained inside the food vacuole for at least 7 days. Scale bar = 5 μm (modified from Lewitus et al., 1999). (B) Two cryptoperidiniopsis dinoflagellates with food vacuoles containing ingested chloroplast material (red) from cryptomonad algal prey. Scale bar = 10 μm (modified from Parrow and Burkholder, 2004).

toplankton assemblages, are providing valuable insights about biological factors (e.g., infection, competition, grazing) that influence the occurrence and distribution of HABs.

Recently, the cell sorting capacity of some flow cytometers has been applied, as well, to assess modes of nutrition by HAB species. For example, one group of HABs that exhibits complex nutritional assimilation is *Pfiesteria* and the related *pfiesteria*-like species—small heterotrophic dinoflagellates that can kill finfish and shellfish by a combination of toxin production and feeding activity (Burkholder et al., 2001). These dinoflagellates do not have their own chloroplasts, but can consume them from algal prey (Lewitus et al., 1999) (Figure 7A). The consumed “kleptochloroplasts”—or stolen chloroplasts—may benefit these cells for short periods (hours to days) by allow-

ing photosynthesis to occur without the cellular expenditure of manufacturing the organelle. Other closely related species, such as some strains of cryptoperidiniopsoids, consume algal prey but may not retain the chloroplasts. Cryptoperidiniopsoids that have eaten algal prey are pigmented because of the algal pigments consumed, whereas those that have not fed are colorless. Flow cytometry has been used to sort live cryptoperidiniopsoids after feeding trials with cryptomonad algae (Figure 7B), and to separate the pigmented cryptoperidiniopsoid cells from the non-pigmented cells (Figure 8). The pigmented dinoflagellates lost or digested the chloroplast material in their food vacuoles within 48 hours. Overall, these experiments show that heterotrophic HAB species may sometimes benefit from photosynthesis derived from ingested chloroplasts (Le-

witus et al., 1999), while in other cases they may not (Parrow and Burkholder, 2003). Differentiation between kleptoplastidy and simple digestion is problematic, but application of flow cytometry is leading to a better understanding of these phenomena in different species and strains, and the possible metabolic benefit derived from such behavior.

CONCLUSIONS

The complexities of nutrient sources, timing of delivery, nutritional mechanisms, complexities of the interactions among members of the microbial community, and intrinsic differences in physiology and behavior among strains within HAB species often make it difficult to differentiate the role of eutrophication in development of HABs from other mechanisms that structure populations. Consideration of the full suite of

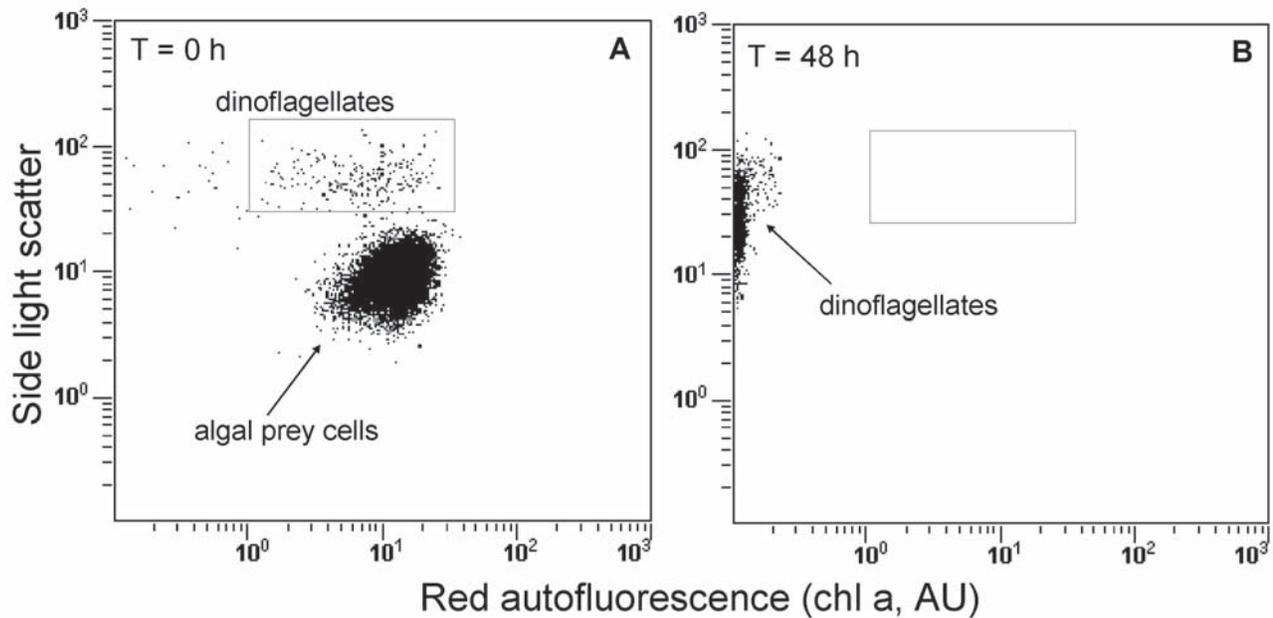


Figure 8. Flow cytometric histograms of side angle light scatter versus red autofluorescence (indicating chlorophyll a, chl a). Each data point denotes 1 cell. (A) Time = 0, a mixed culture containing algal prey cells and feeding dinoflagellates. The boxed region demonstrates the sorting parameters used to select dinoflagellates with red autofluorescence of similar intensity to that of the algal prey. The dinoflagellates meeting these criteria were sorted and collected. (B) The sorted populations, reanalyzed under the same parameters 48 h later exhibited no detectable chl a autofluorescence and therefore clustered at the y-axis. AU = arbitrary units (from Parrow and Burkholder, 2003).

organic and inorganic nutrient forms, the timing of inputs, and the range of nutritional mechanisms, including mixotrophy and heterotrophy, will advance understanding of when and where certain species may dominate under specific nutrient regimes. Impacts on the coastal zone from the increasing use of synthetic and manure fertilizers are projected to worsen worldwide, especially in regions that are already sustaining increased proliferations of HABs.

ACKNOWLEDGEMENTS

The ideas and research presented in this paper were developed under several funding sources, including the NOAA

ECOHAB and MERHAB programs, Maryland and New Jersey Sea Grant, and NOAA South Florida Ecosystem Research and Monitoring Program. We wish to thank J. Alexander and C. Solomon for contributing to the work presented here, and V. Trainer for sharing graphics. This is Contribution No. 128 from the ECOHAB Program, NJSJG-05-586 from NOAA Sea Grant, and 3837 from the University of Maryland Center for Environmental Science. ☒

REFERENCES

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

Berman, T. and S. Chava. 1999. Algal growth on organic compounds as nitrogen sources. *Journal of Plankton Research* 21:1423-1437.

Burkholder, J.M. 2001. Beyond algal blooms, oxygen deficits and fish kills: Chronic, long-term impacts of nutrient pollution on aquatic ecosystems. Pp. in *Waters in Peril*, L. Bendell-Young and P. Gallagher, eds. Kluwer Academic Publisher, Norwell, Massachusetts, USA.

Burkholder, J.M., H.B. Glasgow, and N.J. Deamer-Melia. 2001. Overview and present status of the toxic *Pfiesteria* complex. *Phycologia* 40:186-214.

Doblin, M.A., S.I. Blackburn, and G.M. Hallegraeff. 2000. Intraspecific variation in the selenium requirement of different geographic strains of the toxic dinoflagellate *Gymnodinium catenatum*. *Journal of Plankton Research* 22:421-432.

Glibert, P.M., R. Magnien, M.W. Lomas, J. Alex-

- ander, C. Fan, E. Haramoto, T.M. Trice, and T.M. Kana. 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, P.M., C.A. Heil, D. Hollander, M. Revilla, A. Hoare, J. Alexander, and S. Murasko. 2004. Evidence for dissolved organic nitrogen and phosphorus uptake during a cyanobacterial bloom in Florida Bay. *Marine Ecology Progress Series* 280:73-83.
- Glibert, P.M., J. Harrison, C. Heil, and S. Seitzinger. In press. Escalating worldwide use of urea—a global change contributing to coastal eutrophication. *Biogeochemistry*.
- Granéli, E., P. Carlsson, and C. Legrand. 1999. The role of C, N and P in dissolved and particulate organic matter as a nutrient source for phytoplankton growth, including toxic species. *Aquatic Ecology* 33:17-27.
- Granéli, E., L. Edler, D. Gedziorowska, and U. Nyman. 1985. Influence of humic and fulvic acids on *Prorocentrum minimum* (Pav.) J. Schiller. Pp. 201-206 in *Toxic Dinoflagellates*, D.M. Anderson, A.W. White, and D.G. Baden, eds. Elsevier Science Publishing Co., North-Holland, New York, USA.
- Howarth, R.W., A. Sharpley, and D. Walker. 2002. Sources of nutrient pollution to coastal waters in the United States: Implications for achieving coastal water quality goals. *Estuaries* 25:656-676.
- Lancelot, C. 1995. The mucilage phenomenon in the continental coastal waters of the North Sea. *Science of the Total Environment* 165:83-102.
- Lam, C.W.Y., and K.C. Ho. 1989. Red tides in Tolo Harbour, Hong Kong. Pp. 49-52 in *Red Tides: Biology, Environmental Science, and Toxicology*, T. Okaichi, D. M. Anderson, and T. Nemoto, eds. Elsevier, New York, New York, USA.
- Lewitus, A.J., H.G. Glasgow, and J.M. Burkholder. 1999. Kleptoplastidy in the toxic dinoflagellate, *Pfiesteria piscicida* (Dinophyceae). *Journal of Phycology* 35:303-312.
- Mallin, M.A. 2000. Impacts of industrial animal production on rivers and estuaries. *American Scientist* 88:2-13.
- Meybeck, M. 1982. Carbon, nitrogen, and phosphorus transport by world rivers. *American Journal of Science* 282:401-450.
- Paerl, H.W. 1988. Nuisance phytoplankton blooms in coastal, estuarine, and inland waters. *Limnology and Oceanography* 33:823-847.
- Parrow, M.W. and J.M. Burkholder. 2003. Estuarine heterotrophic cryptoperidopsoids (Dinophyceae) life cycle and culture studies. *Journal of Phycology* 39:678-696.
- Parrow, M.W. and J. M. Burkholder. 2004. The sexual life cycles of *Pfiesteria piscicida* and cryptoperidiniopsoids (Dinophyceae). *Journal of Phycology* 40:664-673.
- Parsons, M.L., Q. Dortch, and R.E. Turner. 2002. Sedimentological evidence of an increase in *Pseudo-nitzschia* (Bacillariophyceae) abundance in response to coastal eutrophication. *Limnology and Oceanography* 47:551-558.
- Romdhane, M.S., H.C. Eilertsen, O.K.D. Yahia, and M.N.D. Yahia. 1998. Toxic dinoflagellate blooms in Tunisian lagoons: causes and consequences for aquaculture. Pp. 80-83 in *Harmful Algae*, B. Reguera, J. Blance, M. L. Fernandez, and T. Wyatt, eds. Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO, Paris, France.
- Seitzinger, S.P. and R.W. Sanders. 1999. Atmospheric inputs of dissolved organic nitrogen stimulate estuarine bacteria and phytoplankton. *Limnology and Oceanography* 44:721-730.
- Seitzinger, S.P., C. Kroeze, A.F. Bouwman, N. Caraco, F. Dentener and R.V. Styles. 2002a. Global patterns of dissolved inorganic and particulate nitrogen inputs to coastal systems: Recent conditions and future projections. *Estuaries* 25:640-655.
- Seitzinger, S.P., R.W. Sanders, and R.V. Styles. 2002b. Bioavailability of DON from natural and anthropogenic sources to estuarine plankton. *Limnology and Oceanography* 47:353-366.
- Smayda, T.J. 1990. Novel and nuisance phytoplankton blooms in the sea: Evidence for a global epidemic. Pp. 29-40 in *Toxic Marine Phytoplankton*, E. Granéli, B. Sundström, L. Edler, and D.M. Anderson, eds. Elsevier, New York, New York, USA.
- Smayda, T.J. 1997. Harmful algal blooms: Their ecophysiology and general relevance to phytoplankton blooms in the sea. *Limnology and Oceanography* 42:1137-1153.
- Smil, V. 2001. *Enriching the Earth: Fritz Haber, Carl Bosch, and the Transformation of World Food*. The MIT Press, Cambridge, United Kingdom.
- Springer, J.J., J.M. Burkholder, P.M. Glibert, and R.E. Reed. 2005. Use of a real-time monitoring network (RTRM) and shipboard sampling to characterize a dinoflagellate bloom in the Neuse Estuary, North Carolina, U.S.A. *Harmful Algae* 4:553-574.
- Spyres, G., S.P. Seitzinger, H.E. Hartnett, and R.J. Lauck. 2003. Bioavailability of dissolved organic matter in urban and rural rainwater. Abstract in *The Earth's Eyes: Aquatic Sciences Through Space and Time*, Annual ASLO Meeting held in Salt Lake City, Utah. American Society of Limnology and Oceanography, Waco, Texas, USA.
- Stoecker, D.K. 1999. Mixotrophy among dinoflagellates. *Journal of Eukaryotic Microbiology* 46:397-401.
- Stoecker, D.K., A. Li, D.W. Coats, D.E. Gustafson, and M.K. Nannen. 1997. Mixotrophy in the dinoflagellate *Prorocentrum minimum*. *Marine Ecology Progress Series* 152:1-12.
- Strom S.L. and K. Bright. 2003. Effects of planktonic food webs of organic nitrogen use by bloom-forming phytoplankton. Abstract in *Proceedings of the Biennial Meeting of the Estuarine Research Federation*, Seattle, Washington, USA. Estuarine Research Federation, Port Republic, Maryland, USA.
- Trainer, V.L., B-T. Le Eberhart, J.C. Wekell, N.G. Adams, L. Hanson, F. Cox, and J. Dowell. 2003. Paralytic shellfish toxins in Puget Sound, Washington. *Journal of Shellfish Research* 22:213-223.
- Vitousek, P.M., J. Aber, R.W. Howarth, G.E. Likens, P.A. Matson, D.W. Schindler, W.H. Schlesinger, and G.D. Tilman. 1997. Human alteration of the global nitrogen cycle: Causes and consequences. *Ecological Applications* 7:737-750.