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

NEW PERSPECTIVES AND NEW APPROACHES

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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-

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 organic 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 production 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

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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., Pærl, 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 species 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 polyellips*, *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 toxicogenic dinoflagellate *Gymnodinium catenatum* in their requirement for the micronutrient, selenium. Strom and Bright (2003) found that strains of the dimethylsulphide-producing haptophyte *Emiliania 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...
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 important 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
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 resolving 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 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.
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-
top plankton 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 *pf esteria*-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 “kletochloroplasts”—or stolen chloroplasts—may benefit these cells for short periods (hours to days) by allowing 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 (Lewitus et al., 1999), while in other cases they may not (Parrrow 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
organic and inorganic nutrient forms, the timing of inputs, and the range of nutritional mechanisms, including mixtothery 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.

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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).