Phytoplankton Photopigments as Indicators of Estuarine and Coastal Eutrophication

HANS W. PAERL, LEXIA M. VALDES, JAMES L. PINCKNEY, MICHAEL F. PIEHLER, JULIANNE DYBLE, AND PIA H. MOISANDER

Human development of coastal watersheds has greatly increased nutrient loading and accelerated estuarine and coastal eutrophication. These waters are also affected by climatic perturbations (e.g., droughts, hurricanes, floods), which may be increasing. The ecological effects of these stressors are often most evident at the microbial level, where the bulk of primary production and biogeochemical cycling occurs. Phytoplankton dominate coastal primary production and thus may be indicative of eutrophication and other major perturbations underlying coastal ecosystem change. Using photopigments that are diagnostic for phytoplankton functional groups (chlorophytes, cryptophytes, cyanobacteria, diatoms, and dinoflagellates), we examined the relative responses of these taxonomic groups to nutrient and hydrologic alterations and evaluated their use as indicators of ecological change in the Neuse River Estuary, North Carolina, and Galveston Bay, Texas. Photopigment indicators can be routinely incorporated in water-quality monitoring programs to assess environmental controls on ecosystem structure and function over varying spatial and temporal scales.

Keywords: photopigments, bioindicators, nutrients, phytoplankton, coastal eutrophication

More than three-quarters of the world's human population lives in coastal watersheds (Vitousek et al. 1997). Not surprisingly, the amounts, types, and ecological impacts of pollutants discharged to estuarine and coastal waters are closely linked to population growth in these watersheds (Peierls et al. 1991, Nixon 1995). Deterioration of coastal ecosystems as a result of anthropogenic nutrient overenrichment is a serious concern (NRC 2000), yet there is a paucity of information on how changes at the base of the food web mediating productivity and nutrient cycling are driven by environmental perturbations. Substantial changes have been attributed to nutrient-enhanced production of organic matter, or eutrophication (Nixon 1995); habitat degradation (Diaz and Solow 1999); and changes in the structure and function of food webs (Jackson et al. 2001). Anthropogenic nutrient enrichment and food web alterations, including a reduction in the consumers of phytoplankton, have dramatically increased coastal phytoplankton stocks (Boesch et al. 2001, Jackson et al. 2001). This has caused significant changes in coastal nutrient (carbon, nitrogen, phosphorus, and silicon) cycling, water quality, biodiversity, and overall ecosystem health (Conley 2000, NRC 2000, Cloern 2001).

Coastal ecological change has been documented using paleoecological indicators (Brush 1986, Bianchi et al. 2000), loss of water clarity (Harding and Perry 1997), increased frequencies of hypoxic (low-oxygen) bottom waters (Diaz and Solow 1999, Rabalais and Turner 2001), altered stoichiometry and shifts in nutrient limitation (Conley 2000, Rabalais and Turner 2001), algal bloom expansion (Paerl 1988, Richardson 1997), and increases in opportunistic and introduced grazers (Carlton 1999). Coastal ecosystems are also subject to natural perturbations, including hurricanes and flooding, the intensity and frequency of which are predicted to increase in the foreseeable future (Goldenberg et al. 2001). Distinguishing climatic from human pollution impacts on ecosystem structure and function is critical to understanding environmental drivers of changes in water quality, habitat, and biotic resources.

Human and natural perturbations are often most apparent and most easily detected at the microbial level, where the bulk of ecosystem energy and nutrient flow is mediated. Within the microbial community, planktonic eukaryotic algae and blue-green algae (cyanobacteria), jointly termed *phytoplankton,* comprise a large portion of the primary producers in most coastal ecosystems. They are particularly sensitive "canaries," capable of responding to changes in nutrient and toxin input, hydrology (i.e., water residence time), sedimentation, irradiance, and temperature regimes over a wide range of time scales (hours to many years).

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Phytoplankton can be partitioned into functional taxonomic groups (chlorophytes, cryptophytes, cyanobacteria, diatoms, and dinoflagellates) that play major roles in coastal production, nutrient cycling, and food web dynamics (Pinckney et al. 2001). General characteristics of these groups can be explored to make them potentially useful indicators of ecosystem function and change. For example, as a group, diatoms are indicative of acceptable water quality (i.e., low levels of nutrient loading, good water clarity). Most diatoms (a notable exception being the genus *Pseudonitzschia,* which produces the neurotoxin domoic acid) are good food sources for filter-feeding zooplankton, benthic invertebrates, and larval fish (Richardson 1997). In contrast, cyanobacteria include numerous toxic or inedible species that generally thrive under nutrient-enriched, eutrophic conditions. Some cyanobacteria fix inert atmospheric nitrogen (N_2) into ammonium, which can significantly contribute to nitrogen budgets and can contribute to nitrogen overenrichment problems. Dinoflagellates are generally considered important food sources for higher trophic levels, but there are exceptions, such as the organism that produces toxic red tide. Even with these exceptions, and even though species within these phytoplankton functional groups (PFGs) may vary in their response to environmental perturbations, there are broad functional differences and environmental preferences among the PFGs that may prove useful in determining water quality, fertility, resourcefulness, and overall ecological condition.

Determining phytoplankton functional groups using diagnostic photopigments

The ecological importance and dynamic nature of PFGs call for rapid, unequivocal, quantifiable, and broadly applicable means of identifying and characterizing their responses to environmental stressors. High inherent spatiotemporal variability at the ecosystem scale requires that the analytical approach be able to process large numbers of samples quickly and economically. Unfortunately, the only reliable technique for identifying and enumerating phytoplankton species or groups is microscopy, a time-consuming and costly procedure that requires a high level of expertise.

Alternatively, phytoplankton biomass may be estimated from photopigment content. Chlorophyll *a* (Chl *a)* measurements have been used for this purpose for many years. Chlorophyll *a* is contained in all photosynthetic algae, cyanobacteria, and higher plants, and it is easily measured at high sensitivities. However, Chl *a*–based techniques cannot distinguish between phytoplankton groups. To circumvent this problem, high-performance liquid chromatography (HPLC) coupled with in-line photodiode array spectrophotometry can be used to characterize phytoplankton community structure at the PFG level, on the basis of separation and quantification of a variety of photopigments (chlorophylls and carotenoids) that have distinct spectral absorbance characteristics. These photopigments (e.g., fucoxanthin, peridinin, chlorophyll *b,* zeaxanthin, and alloxanthin) are diagnostic of specific phytoplankton functional groups (diatoms, dinoflagellates, chlorophytes, cyanobacteria, and cryptophytes, respectively) and in a few cases can determine phytoplankton composition at the species level (e.g., the common "Florida red tide" dinoflagellate *Karenia brevis);* thus, they can be used as sensitive indicators of phytoplankton community structure (table 1; Wright et al. 1991, Millie

Table 1. Chemotaxonomic photopigments (chlorophylls and carotenoids) diagnostic for phytoplankton functional groups in natural communities, determined from high-performance liquid chromatography.

Algal groups	Photopigments																	
	Chlorophyll a	diV-Chl a	Chlorophyll b	diV-Chl b	Chlorophyll c_1c_2	Chlorophyll c_3	Alloxanthin	Anhteraxanthin	ß-Carotene	Canthaxanthin	Diadinoxanthin	Diatoxanthin	Echinenone	but-Fucoxanthin	Fucoxanthin	hex-Fucoxanthin	Gyroxanthin	Lutein
Chlorophytes	■		■					▨	■									■
Chrysophytes (Chrysophyta)	■				■	■			▨		■	▨		■	■			
Cryptophytes	■				■		■		■									
Cyanobacteria (pelagic)	■							■	■									
Cyanobacteria (benthic)	■							■	■	■			■					
Diatoms	■				■				▨		■	▨			■			
Dinoflagellates	■				■				▨		■	▨						
Euglenophytes	■		■					▨	■		■	▨						
Eustigmatophytes	■								■									
Prymnesiophytes (Haptophyta)	■				■				▨		■	▨		■	■	■		
Pelagophytes	■				■				■		■	▨		■	■			
Prasinophytes	■		■						■									■
Prochlorophytes		■		■					■									
Raphidophytes (Chloromonads) (Chrysophyta)	■				■				■		■	▨			■			
Karenia brevis	■				■	■					■	▨		■	■	■	■	

Note: Cross-hatched squares indicate that pigments occur in low concentrations for the algal group; filled squares indicate a major pigment for the group.

et al. 1993, Jeffrey et al. 1997). Statistical procedures (CHEMTAX; Mackey et al. 1996) can then be applied using the HPLC-derived pigment concentrations to partition the total pool of Chl *a* (total phytoplankton community biomass) into the Chl *a* contributed by each functional group (Pinckney et al. 2001). HPLC-based pigment analysis is easily executed and applicable to a wide variety of resource types (streams, lakes, rivers, estuaries, oceans) over large spatial scales. In addition, HPLC measurements can provide calibration and verification for remotely sensed imagery of phytoplankton distributions in relation to large-scale physical and chemical perturbations.

Here we evaluate PFGs as indicators of coastal environmental change using diagnostic photopigments. We consider how anthropogenic stressors interact with natural forcing features to determine the distribution, activities, and interactions of PFGs in two geographically and hydrologically distinct US coastal ecosystems: Neuse River–Pamlico Sound, North Carolina, and Galveston Bay, Texas. To varying degrees, these systems are affected by human discharge of nutrients and other pollutants, by hydrologic alterations (water supply diversions), and by climate change (droughts, hurricanes, floods). These systems are also being targeted for nutrient reduction strategies, including the formulation of total maximum daily loads (TMDLs) of nitrogen (USEPA 1997). We will examine and evaluate the use and application of diagnostic PFG photopigments for assessing coastal water quality, trophic state, and overall condition, against which ecological change may be gauged. Finally, we will discuss the advantages and disadvantages of using PFGs versus finer-scale, species-level phytoplankton indicators in coastal waters.

Eutrophication dynamics of the Neuse River–Pamlico Sound ecosystem

The Neuse River Estuary is a key tributary of North Carolina's Albemarle Pamlico Sound system, the second largest estuarine complex in the United States (figure 1). This estuary drains some of North Carolina's most productive and rapidly expanding agricultural, urban, and industrial regions. As noted for many other coastal ecosystems (Nixon 1995), excessive nitrogen loading in the Neuse River Estuary has been linked to eutrophication (Paerl 1987, Paerl et al. 1995). Symptoms include nuisance algal blooms, with surface-dwelling cyanobacteria dominating upstream, dinoflagellate blooms in midestuarine mesohaline regions, and cryptomonad blooms in the downstream segments (figure 2; Paerl et al. 1995). Once they senesce, all three types of algal blooms provide the fuel for bottom water hypoxia, a causative agent of fish kills and large-scale biogeochemical changes (figure 2; Paerl et al. 1998).

In addition, this system is prone to major climatic perturbations. During fall 1999, three sequential hurricanes (Dennis, Floyd, and Irene) inundated coastal North Carolina with up to 1 meter (m) of rainfall in a 6-week period, causing a 100- to 500-year flood in the Pamlico Sound watershed and turning the Neuse River Estuary completely fresh. Floodwater discharge displaced more than 80% of the volume of Pamlico Sound, depressed salinity by 70%, and accounted for half the annual nitrogen load to this nitrogen-sensitive system (figure 3). Biogeochemical and ecological effects included hypoxic bottom waters, accompanied by major

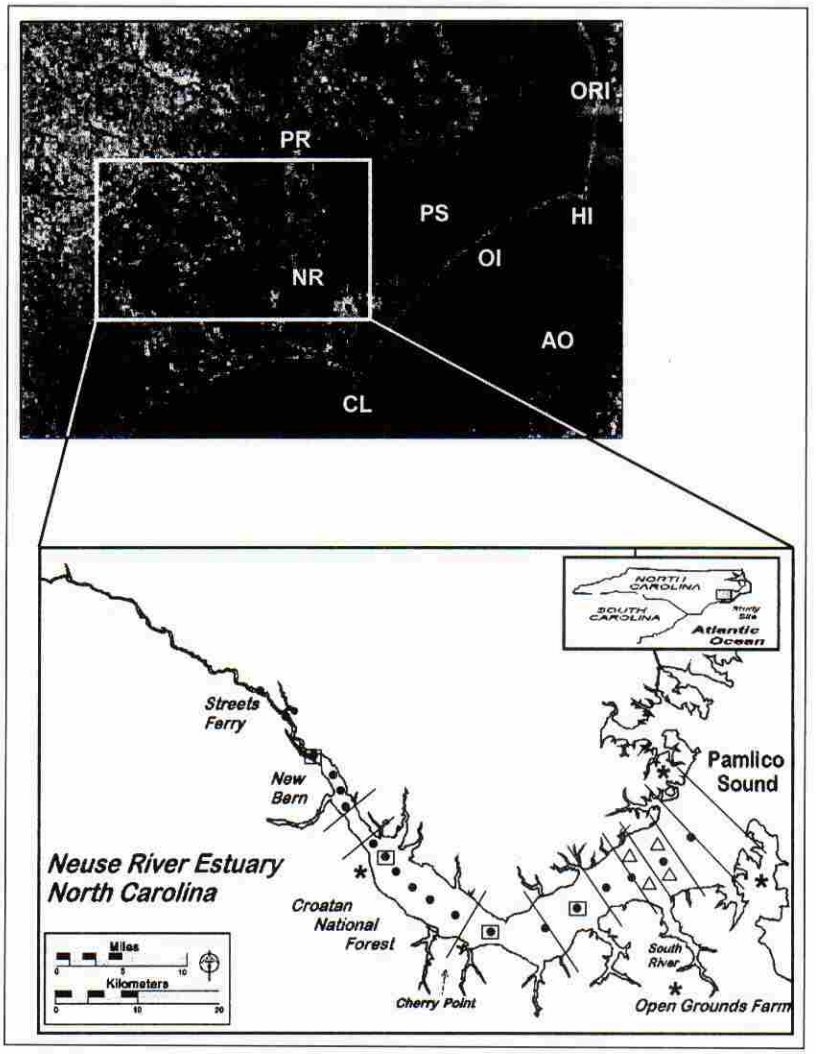

Figure 1. Location of the Neuse River Estuary and Pamlico Sound, North Carolina. Shown are the Atlantic Ocean (AO); the Oregon, Hatteras, and Ocracoke Inlets (ORI, HI, and OI, respectively); Cape Lookout (CL); Pamlico Sound (PS); and the Pamlico and Neuse Rivers (PR and NR). The Neuse River Estuary sampling sites for midriver water quality (19 filled circles) and continuous in-stream monitoring (four open boxes) are shown. Triangles indicate sites for diel studies and other periodic studies during which additional samples are collected.

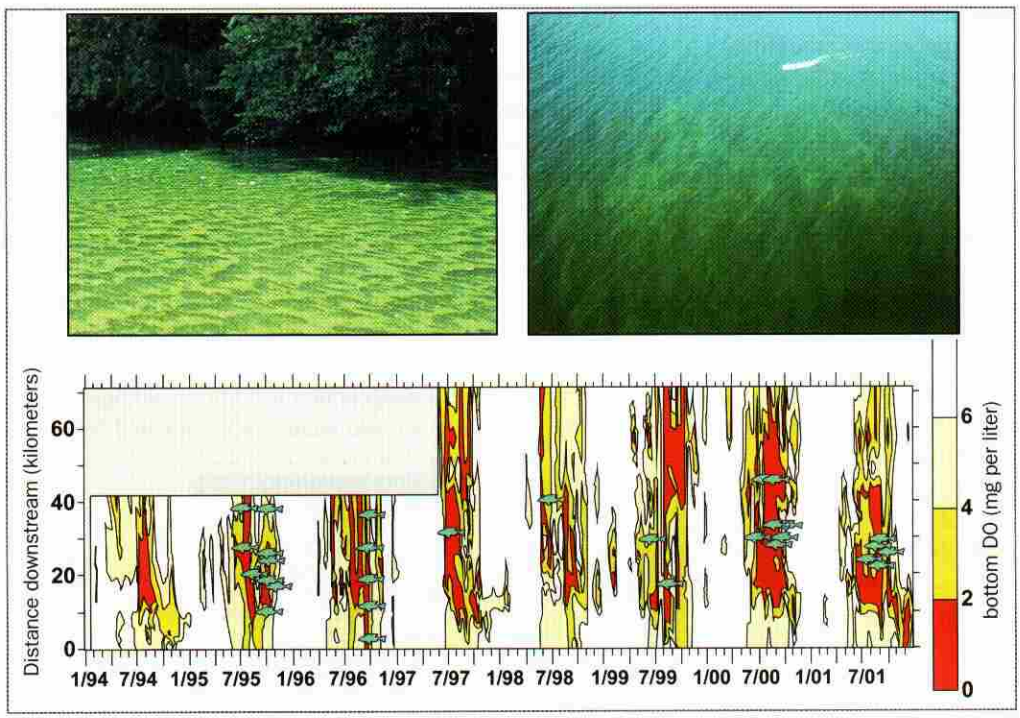

Figure 2. Upper left: Cyanobacterial bloom in the upstream, freshwater segment of the Neuse River Estuary. Upper right: Dinoflagellate bloom at a mesohaline, midestuarine location. Lower frame: Spatiotemporal relationship between bottom water hypoxia and fish kills, plotted for 1994–2001 in a mesohaline segment of the Neuse River Estuary between New Bern and Cherry Point, North Carolina (see figure 1). Dissolved oxygen (DO) data (in milligrams per liter) were obtained by the University of North Carolina at Chapel Hill Neuse River Modeling and Monitoring Program (ModMon;* **www.marine.unc.edu/neuse/modmon**). *The fish kill events (more than 500 fish counted) were recorded by the North Carolina Department of Environment and Natural Resources, Division of Water Quality (www.esb.enr.state.nc.us/Fishkill/fishkillmain.htm***). White area indicates that data were not collected. Photographs: Hans W. Paerl.***

changes in nutrient (nitrogen, phosphorus, and carbon) cycling, a threefold increase in algal biomass, and shifts in microbial, invertebrate, and fish community structure and function (Paerl et al. 2001).

To stem eutrophication, the North Carolina legislature (based on a scientific consensus) mandated a 30% reduction in external nitrogen loading to the Neuse River Estuary; this reduction should be in effect by the end of 2004. The US Environmental Protection Agency has designated an allowable TMDL that also calls for a 30% reduction in nitrogen loading. These management goals have provided an ecosystem-scale "experiment" in which the impacts of nitrogen reductions on phytoplankton community structure and function can be documented. Although many of the ramifications of mandated nitrogen reductions remain unknown, nitrogen loading may have significant impacts beyond a simple reduction in phytoplankton productivity and biomass, including shifts in composition and selection for species adapted to low nitrogen-to-phosphorus (N:P) ratios. For example, the phytoplankton community could become more dominated by N_2-fixing cyanobacteria, circumventing nitrogen limitation (Piehler et al. 2002).

Phytoplankton exhibit species- or group-specific growth responses to different nutrient concentrations and formulations (Collos 1989, Stolte et al. 1994). Changes in N:P and other nutrient (e.g., silicon) input ratios can also affect phytoplankton community structure (Smith 1990, Stolte et al. 1994, Sterner and Elser 2002). Nutrient manipulation experiments, in which the biologically available dissolved inorganic nitrogen (ammonium and nitrate) and dissolved organic nitrogen (urea) pools were varied, illustrate the potential impacts these manipulations may have on phytoplankton community structure and biomass (figure 4). Given human population growth and changing activities in the Neuse River Estuary and other coastal watersheds, it is likely that total amounts and types of nitrogen and other nutrients (e.g., phosphorus, silicon) entering the ecosystem will change over time.

The potential ecological ramifications include alterations of the natural phytoplankton community composition, which may in turn change ecosystem food web and nutrient cycling dynamics. For example, if the growth of more readily grazed PFGs (e.g., diatoms) is favored, trophic transfer and nutrient cycling will take place largely in the water column, with enhanced export of the assimilated algae (as fish) to the coastal ocean. In contrast, if the nutrient formulation favors PFGs that may not be readily grazed (e.g., cyanobacteria and dinoflagellates), trophic transfer will be poor, and relatively large amounts of unconsumed algal biomass will settle to the bottom. This unconsumed biomass will stimulate microbial decomposition and oxygen consumption, exacerbating the potential for hypoxia (figure 5).

Since 1994, diagnostic photopigments have been used for long-term assessments of PFGs in response to environmental change. During this time, the Neuse River Estuary has experienced the combined stresses of anthropogenic nutrient enrichment, droughts, and, starting in 1996, elevated tropical storm and hurricane activity. These distinct perturbations have provided opportunities for examining the

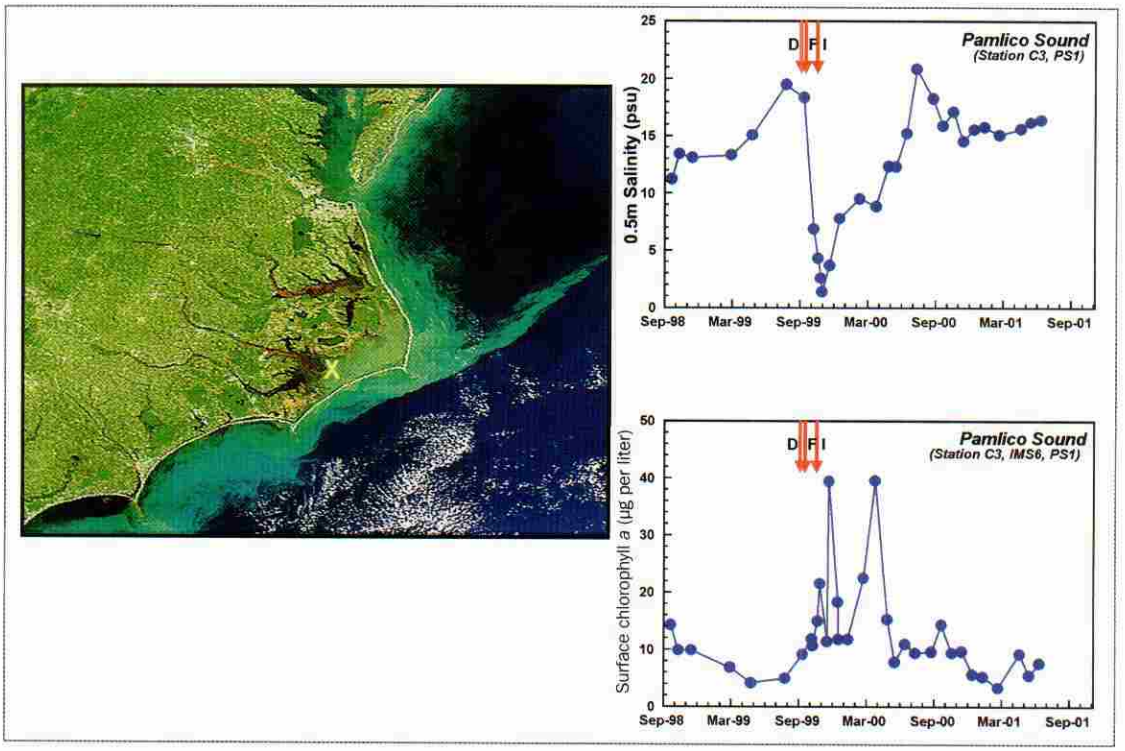

Figure 3. Left: Satellite image of Hurricane Floyd's floodwaters inundating Pamlico Sound on 23 September 1999, one week after the hurricane's landfall. Upper right: Floodwater impacts on salinity at a reference station located in the western Pamlico Sound. Lower right: Impacts on phytoplankton biomass (as chlorophyll* a*, micrograms per liter) at the same reference station. Photograph: National Aeronautics and Space Administration, Sea-viewing Wide Field-of-View Sensor Project.

impacts of both anthropogenic stressors (nutrient influx) and natural stressors (freshwater discharge) on PFGs. The effects of hydrologic condition on PFGs are illustrated for a midriver long-term monitoring station in the Neuse River Estuary (figure 6). Seasonal and hurricane-induced variations in river discharge, and the resulting changes in flushing rates and hence in estuarine residence times, appear to have differentially affected PFGs as a function of their contrasting growth characteristics. For instance, during periods of elevated river flow associated with major storms, the total Chl *a* pool in the Neuse River Estuary was composed primarily of chlorophytes and cryptophytes. We hypothesize that these groups dominated during high freshwater discharge conditions because of their efficient growth rates, enhanced nutrient uptake rates, and tolerance of low salinity conditions. Cyanobacteria also responded in an individualistic manner. They normally prefer low salinity conditions, but because their growth rates are generally slow compared with those of chlorophytes and cryptomonads, cyanobacteria lagged behind these groups in terms of responding to enhanced freshwater discharge. For example, despite the decreased salinity and increased nutrient loading after the 1999 flooding event, cyanobacteria did not increase in abundance until spring 2000, when typical flow conditions were restored. Once longer residence times were again established, cyanobacteria took advantage of the decreased salinity and increased nutrient loading, reaching levels of abundance that have rarely been observed in the Neuse River Estuary since 1994. Like cyanobacteria, dinoflagellates demonstrated greater relative biomass when flushing was minimal and residence times were longer.

Changes in hydrologic condition are hypothesized to have significantly altered phytoplankton community structure in the Neuse River Estuary. Historical trends in the abundance of PFGs show that, since 1996, increases in the frequency and magnitude of tropical storms and hurricanes have coincided both with decreases in the occurrence of winter–spring dinoflagellate blooms and with increases in the abundance of chlorophytes and cryptophytes (figures 6, 7). The relatively slow growth rates of dinoflagellates may have led to their reduced abundance during these high river-discharge events. Furthermore, the Chl *a* concentrations contributed by each PFG, and thus total community Chl *a*, were significantly greater in 2000 than in previous years, which could be a consequence of the increased nutrient loading associated with the fall 1999 hurricanes. These elevated nutrient concentrations may have exceeded the filtering capacity of the estuary and remained in the system through the following year, thereby promoting the observed increase in phytoplankton biomass in 2000. These results indicate that phytoplankton composition has been altered since 1994, most likely in response to a sudden increase in hurricane activity accompanied by

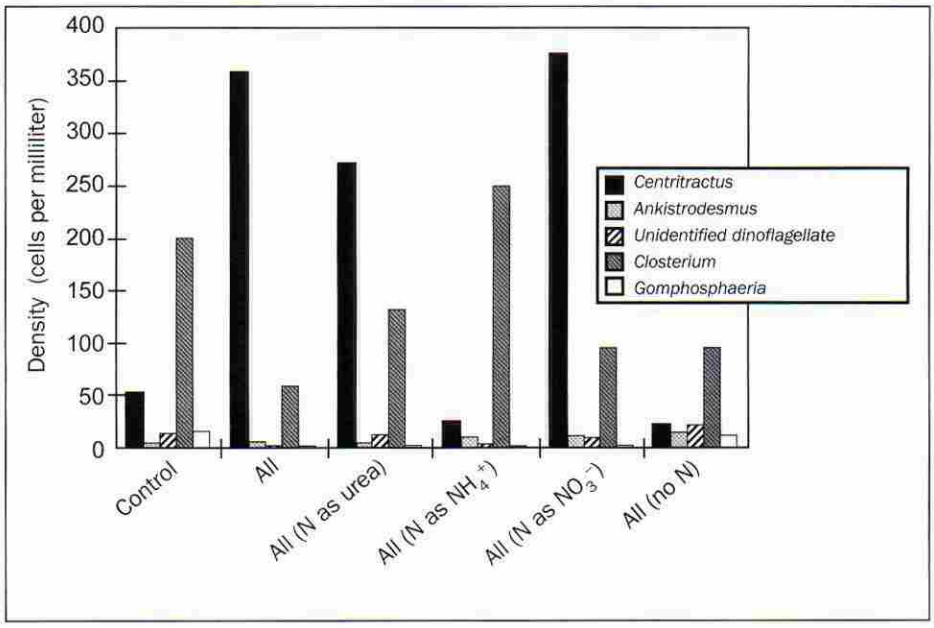

Figure 4. Microscopic counts of phytoplankton species composition in the Neuse River Estuary following 36-hour in situ bioassays to manipulate available forms of nitrogen (N). Treatments included a control (unamended estuarine water sample), all nutrients (N, phosphorus, vitamins, trace metals, and silica), all with urea as the N form, all with ammonium (NH_4^+) as the N form, all with nitrate (NO_3^-) as the N form, and all with no N. Bars represent the mean density of cells present (three replicate counts for each treatment).

hydrologic changes (figures 6, 7). These changes in phytoplankton community structure could potentially alter trophodynamics and nutrient cycling in the Neuse River Estuary.

Nutrient-enhanced phytoplankton blooms and pink oysters in Galveston Bay

Galveston Bay, the second largest estuary in the Gulf of Mexico, encompasses 1554 square kilometers of water surrounded by 526 square kilometers of marshland. The bay is shallow (about 2 m deep) and receives freshwater inputs from the Trinity (83%) and San Jacinto (8%) rivers. These rivers transport organic and inorganic materials with moderate to high dissolved organic matter (5 to 8 milligrams [mg] carbon per liter [L]) and suspended particulate matter (4 to over 180 mg per L) to the bay (Guo and Santschi 1997). The tidal range in the bay averages 40 centimeters, is primarily diurnal, and fosters the long hydraulic residence time of the estuary (40 to 88 days) (Santschi 1995). Winds are more important than tides for circulation and resuspension of sediments in Galveston Bay. Freshwater and nutrient inputs from the Trinity River extend well into Trinity Bay and Galveston Bay, especially during periods of elevated spring discharge (Santschi 1995). Nitrate concentrations are inversely correlated with salinity, and benthic regeneration of phosphorus leads to a phosphate maximum in late summer (Santschi 1995).

In May 1999, we established a biweekly sampling program to collect water-quality data (including nutrient concentrations) and phytoplankton spatiotemporal dynamics at seven locations in Galveston Bay and Trinity Bay (figure 8). The most common PFGs in Galveston Bay were diatoms, cyanobacteria, chrysophytes, and cryptophytes (Örnólfsdóttir 2002). Dinoflagellates, chlorophytes, and euglenoids were occasionally abundant. Periodic diatom blooms occurred during the summer. Nutrient addition bioassays indicated nitrogen-limited conditions throughout the year (Örnólfsdóttir 2002, Pinckney et al. 2002).

Galveston Bay supports a large commercial fishery for the eastern oyster *(Crassostrea virginica)*, with annual harvests near 400 metric tons. Phytoplankton is the primary food source for oysters, and the algal species that comprise the phytoplankton community vary in nutritional quality. Variations in food availability and composition are primary factors determining oyster condition and the prevalence of disease. *Perkinsus marinus* (dermo) is an endoparasitic protozoan that infects a large portion of the oyster population in Galveston Bay. Model simulations of Galveston Bay oysters suggest a strong association between phytoplankton levels, environmental conditions (mainly temperature and salinity), dermo infection intensity, and oyster condition index (Hofmann et al. 1995). Therefore, ecological factors that regulate phytoplankton community composition are indirectly linked to oyster condition.

Texas oystermen have recently expressed concern over a peculiar pinkish coloration of oysters from some commercial reefs in Galveston Bay. Although the conspicuous color of these "pink oysters" has no obvious effect on their condition and is not known to pose a human health hazard, it adversely affects consumer acceptance and hence oyster marketability. An "off taste" further detracts from the pink oysters' marketability. The magnitude and frequency of pink oyster events appear to be increasing in Galveston Bay and may be a symptom of nutrient-driven eutrophication of this and other estuaries in the Gulf of Mexico.

Pink oysters have been reported intermittently from October through January over the past 14 years. The intensity of events varies from year to year. The most intense events seem to follow heavy rainfall and riverine runoff in September and October. The area of the bay that is most heavily affected is along the eastern side of the Houston Ship Channel in the midbay region. The number of pink oysters harvested by commercial oystermen throughout the bay was monitored in the 1999–2002 seasons. No pink oysters were reported in winter 1999–2000. In the 2000–2001 season,

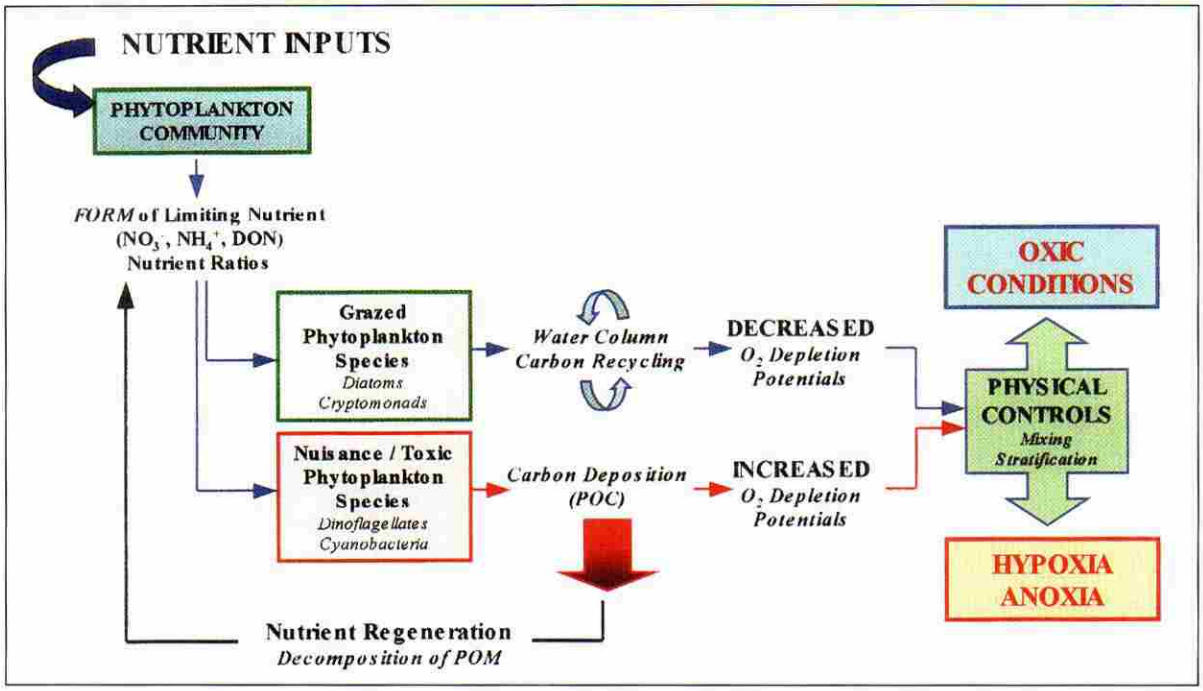

Figure 5. Conceptual diagram showing the linkages of nutrient input formulations and amounts, phytoplankton functional group (PFG) responses, trophodynamics, and biogeochemical cycling in an estuary. Two contrasting scenarios are shown: (1) Growth of readily grazed phytoplankton groups, including diatoms and cryptomonads. Their biomass is mainly utilized and cycled in the water column. These phytoplankton are effectively transferred up the food chain and exported to the coastal ocean as invertebrates and fish. (2) Proliferation of nuisance (i.e., not effectively grazed because of toxicity or poor food value) PFGs, including some cyanobacterial and dinoflagellate species. Because they are not effectively grazed, relatively large proportions of these taxa are deposited and decomposed in the sediments, adding to sediment oxygen demand, hypoxia, and anoxia. In systems with long water residence times, like the Neuse Estuary–Pamlico Sound, repeated nuisance bloom events within a single season can greatly exacerbate hypoxia and sediment nutrient release, perpetuating further bloom events. Abbreviations: DON, dissolved organic nitrogen; NH_4^+, ammonium; NO_3^-, nitrate; O_2, molecular oxygen; POC, particulate organic carbon; POM, particulate organic matter. Source: Pinckney et al. 2001.

however, nearly 75% of the oysters exhibited bright pink coloration in the gut and mantle. Pink oysters were also reported in winter 2001–2002, but their numbers were lower than in the previous year. Preliminary field and laboratory evidence suggested that the pink coloration was caused by the phytoplankton upon which the oysters were feeding. Accessory photosynthetic pigments (carotenoids, phycobilins) associated with the algae accumulate in the oyster gut and liver, causing the red or pink coloring.

During the pink oyster event in December 2000, gut contents of both normal and pink oyster types were analyzed with HPLC to determine phytoplankton group composition. Although the analysis of gut pigments was qualitative, a comparison of the two oyster types revealed a higher concentration of peridinin in the guts of pink oysters than in those of normal oysters. Peridinin is a bright red carotenoid accessory pigment that indicates the presence of dinoflagellates (Jeffrey et al. 1997). Microscopic examinations of water samples during this period revealed that the most abundant dinoflagellate was *Prorocentrum minimum*. This evidence suggested that *Pr. minimum* could be causing the pink coloration. However, gut pigment analysis can be misleading, because pigment degradation rates differ depending on the type of pigment and chemical conditions in the gut. Cryptophytes, which also have red accessory pigments (water-soluble phycoerythrin), were likewise present in high abundance during December 2000, and small amounts of alloxanthin (the indicator carotenoid pigment for cryptophytes) were detected in gut contents. The HPLC method used for these analyses cannot detect phycoerythrin. However, the presence of alloxanthin in the oyster guts suggests that the oysters were also feeding on the phycoerythrin-containing cryptophytes.

An examination of the water-quality conditions and phytoplankton community composition during the entire period (1999–2002) offered insights into potential causal mechanisms for the occurrence and magnitude of pink oyster events. The salinity was relatively high in Galveston Bay during fall and winter 1999 (figure 9). A tropical storm in May 2000, high rainfall, and subsequent freshwater input from the Trinity River in late September 2000 resulted in lower salinities within the bay. Similarly, high rainfall in September and October 2001 lowered salinities. These riverine freshwater

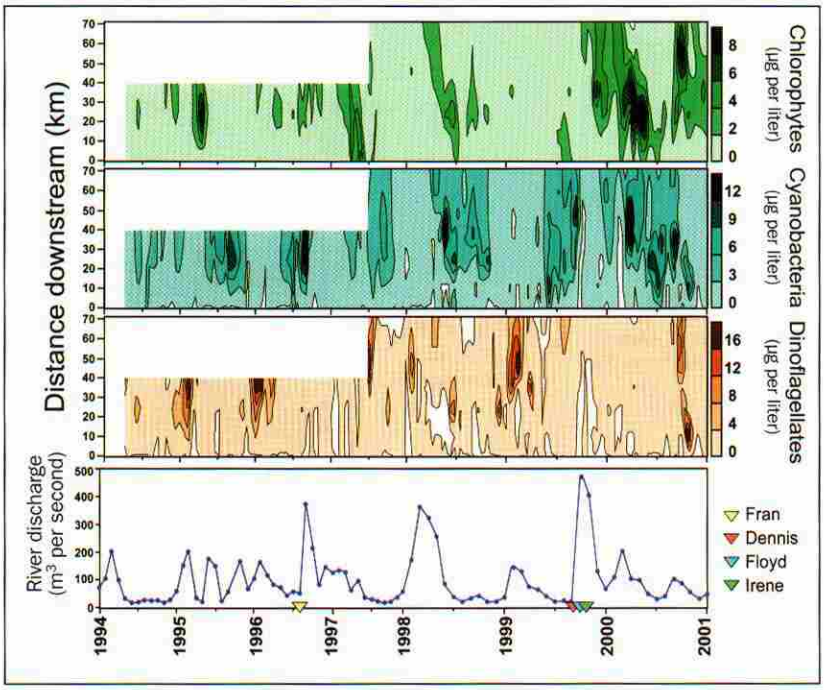

Figure 6. Spatiotemporal surface concentrations of chlorophyll a *(micrograms per liter) contributed by chlorophytes, cyanobacteria, and dinoflagellates at a mesohaline location (Station 120) of the Neuse River Estuary, 1994–2000. Distance downstream refers to distance in kilometers from Station 0. Values were derived from ChemTax analysis of photopigment concentration data. Data were collected biweekly and were temporally and spatially extrapolated. White areas indicate instances where data were not collected. ChemTax data were plotted along with freshwater discharge (cubic meters per second) at the head of the estuary. The dates of landfall of the four major hurricanes that have significantly affected flow since mid-1996 are shown. Data are from the US Geological Survey, Raleigh, North Carolina.*

inputs resulted in elevated concentrations of dissolved inorganic nitrogen in excess of 25 micromoles, which fostered phytoplankton blooms in Galveston Bay (figure 9). The location of these blooms overlapped with the commercial oyster reefs in the central region of the bay.

Phytoplankton community composition was determined using the combined HPLC ChemTax pigment analyses (figure 10). Cryptophytes and peridinin-containing dinoflagellates were the most abundant phytoplankton groups present when pink oysters were harvested. The occurrence of pink oysters seems to be linked more closely with cryptophyte blooms than with dinoflagellate blooms. The dinoflagellate blooms may, however, be linked to the cryptophyte blooms, because cryptophytes are a potential food source for the mixotrophic *Pr. minimum*, the major dinoflagellate bloom species. Assuming a conservative oyster filtration rate of 1 milliliter of water per second and cryptophyte biomass of 4 micrograms (µg) per L (the lower threshold concentrations in figure 9), an oyster would consume approximately 350 µg of cryptophyte biomass (in Chl *a* units) per day. This amount of material is more than sufficient to produce pink coloration.

Although the observations and data described above supply only correlative and circumstantial evidence for the causal mechanisms behind pink oyster production in Galveston Bay, these data will be invaluable for guiding future research programs. Controlled laboratory experiments, using oysters and manipulated combinations of phytoplankton species, will be necessary to definitively prove the causal mechanisms for pink oyster events. Understanding the linkages between system-level driving features for phytoplankton blooms is highly relevant for oyster fishery management and commercial harvests in Galveston Bay. Significant changes in the phytoplankton community composition resulting from increased nutrient loading and phytoplankton densities would have major impacts on higher trophic levels in Galveston Bay. This estuary, like many others in the Gulf of Mexico, supports large recreational and commercial fisheries. Therefore, a thorough understanding of the anthropogenic factors (e.g., nutrients, xenobiotics) that regulate phytoplankton species and biomass dynamics is a prerequisite for developing effective mitigation and management strategies for Galveston Bay and similar shallow estuaries in the Gulf of Mexico.

Future applications to coastal eutrophication studies

Coastal waters are experiencing unprecedented ecological change, attributable to both climatic perturbations and human alteration of their watersheds. To accurately and broadly assess change at the base of planktonic food webs where phytoplankton dominate, researchers need indicators capable of unequivocally characterizing structural and functional responses

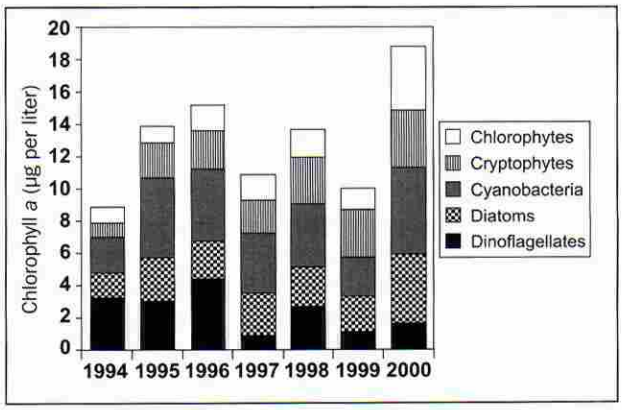

Figure 7. Yearly means of surface chlorophyll a *concentrations (micrograms per liter) contributed by chlorophytes, cryptophytes, cyanobacteria, diatoms, and dinoflagellates at Station 120 in the Neuse River Estuary.*

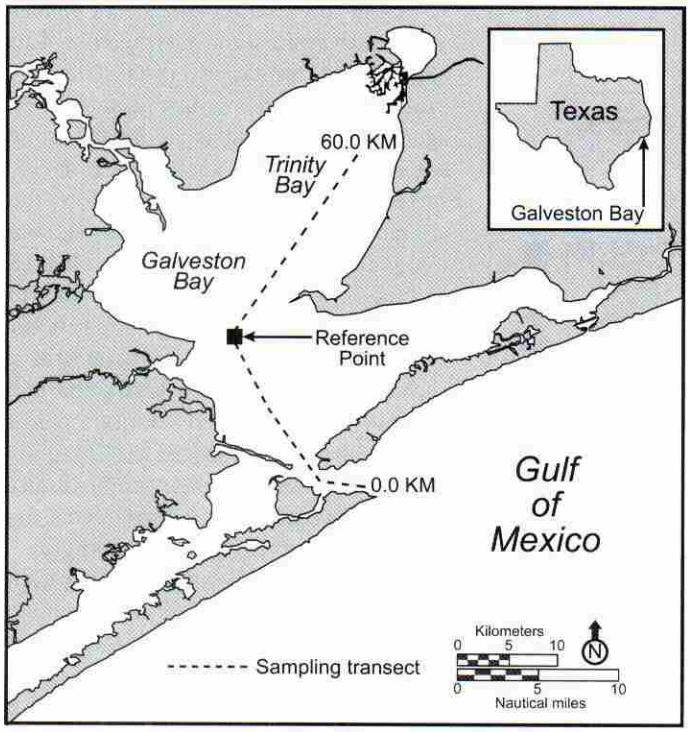

Figure 8. Map of Galveston Bay, Texas. The dashed line down the center of the bay indicates the sampling transect (from 0.0 to 60.0 kilometers) used to construct the spatiotemporal contour plots in figures 9 and 10. The reference point shows the location of several large commercial oyster reefs in the bay where pink oysters have been collected.

of PFGs. Diagnostic photopigments offer a chemotaxonomic tool for rapidly identifying and quantifying the relative composition, biogeochemical and trophic roles, and responses to diverse anthropogenic and natural perturbations of PFGs.

Researchers are making progress in applying these techniques on ecosystem and regional scales, where human and climatic perturbations often coincide and interact. Deployment of diagnostic photopigment analyses on platforms, including moorings and "ships of opportunity" such as ferries and commercial vessels, will greatly enhance researchers' ability to clarify the impacts of specific forcing features on primary producers. In a recent study, these techniques have been applied on the fleet of ferries traversing the Pamlico Sound and its tributaries (*www.ferrymon.org;* Buzzelli et al. 2003). Automated sampling during ferry transits was combined with diagnostic photopigment analyses to quantify spatial and temporal patterns in the phytoplankton community and to map and track PFG and individual taxa blooms. Many estuarine and coastal blooms of PFGs are dominated by one or two species in each functional group (Harding and Perry 1997, Pinckney et al. 2001). Under these circumstances, diagnostic pigment analyses can provide a means of delineating and quantifying specific blooms. Furthermore, diagnostic photopigment analyses may serve dual functions that are not easily accomplished by either microscopic, molecular, or immunological techniques, in that the pigment analyses can provide a broadly applicable calibration source for remote sensing of phytoplankton, facilitating "scaling up" for coastal water-quality assessments.

Diagnostic photopigment analysis can be used to examine the effects of both short-term acute and longer-term chronic effects of environmental perturbations on PFG composition and function. For example, impacts of short-term (i.e., hours to days) pulsed nutrient loads on PFGs have been quantified by using HPLC as a phytoplankton growth and compositional response parameter in nutrient addition bioassays (Paerl et al. 1995, 1998, Pinckney et al. 2001). Results from these bioassays have been used to develop predictive models aimed at clarifying nutrient drivers of phytoplankton community shifts in response to nutrient pulses, such as wastewater spills, storm-based runoff, and agricultural and industrial discharges (Pinckney et al. 1999, 2001).

Diagnostic photopigments are also capable of quantifying longer-term (i.e., months to years) patterns in PFG succession in response to changes in hydrologic and nutrient loading, salinity regimes, and grazing (Pinckney et al. 1998, 2001, Harding et al. 2002). They have been particularly useful in examining long-term phytoplankton community shifts in response to climatic perturbations accompanied by seasonal to multiannual changes in hydrologic and nutrient regimes, such as those following the spate of hurricanes that struck coastal North Carolina in the late 1990s (Paerl et al. 2001). Examining changes in relative

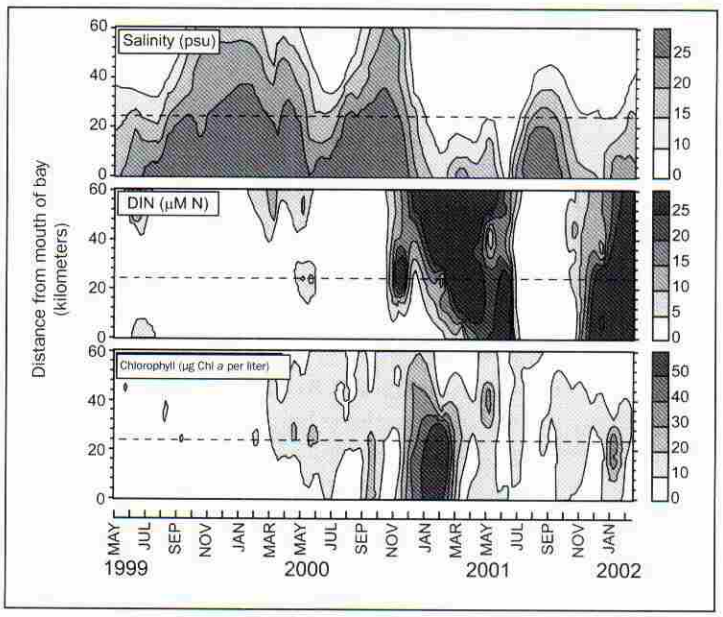

Figure 9. Spatiotemporal contour plots of salinity (practical salinity units, or psu), total dissolved inorganic nitrogen (DIN, micromoles), and total chlorophyll* a *(phytoplankton biomass, micrograms per liter) along the transect shown in figure 8. The horizontal dashed line indicates the location of the reference point shown in figure 8.

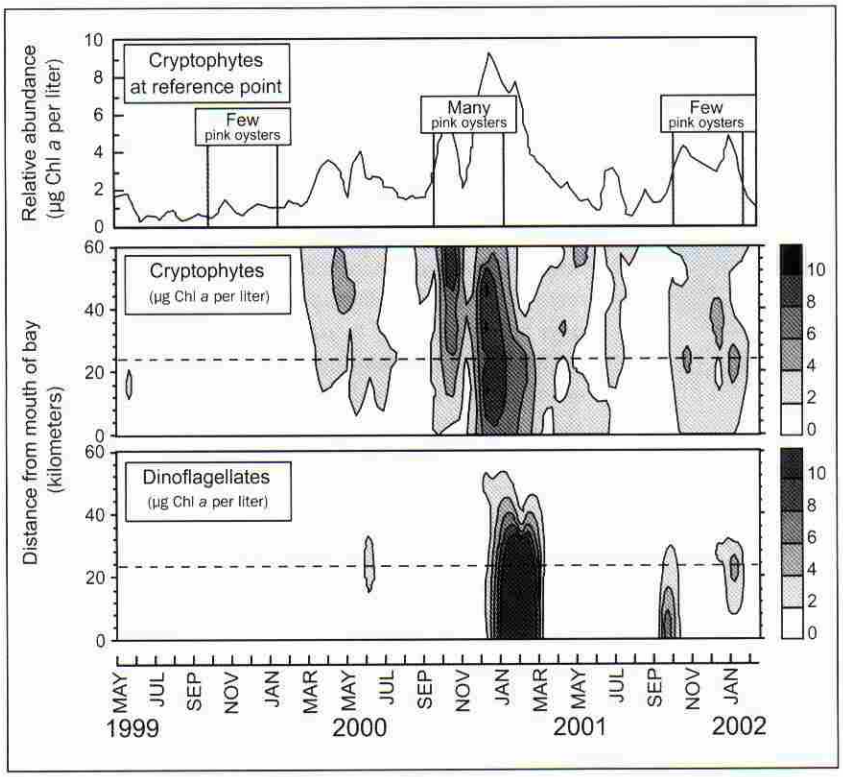

Figure 10. Spatiotemporal contour plots of the relative abundance of cryptophytes and dinoflagellates along the transect shown in figure 8. The horizontal dashed line in the lower two panels indicates the location of the reference point shown in figure 8. The graph in the top panel illustrates the relative abundance of cryptophytes (micrograms of chlorophyll a per liter) at the reference point in figure 8, the time period when pink oysters occur, and the prevalence of pink oysters from May 1999 through January 2002.

abundances of PFGs after these hurricanes proved quite useful in predicting larger-scale biogeochemical and trophic changes in estuarine and coastal systems (Paerl et al. 2001). Because sample size is relatively small, preparation is simple and rapid, and the pigment-based indicators can be incorporated in a wide spectrum of programs for monitoring estuarine and coastal water quality and habitat using manual and automated sampling techniques. HPLC-based PFG characterizations in long-term water quality monitoring programs such as the Chesapeake Bay Program *(www.chesapeakebay.net/)* and the Neuse River Estuary Modeling and Monitoring Program *(www.marine.unc.edu/neuse/modmon)* have already provided timely and insightful large-scale, interseasonal, and interannual comparisons of phytoplankton community compositional changes in response to varying hydrologic events (i.e., droughts, floods), temperature, irradiance, and nutrient loading regimes in different years. PFG-level taxonomic indicators complement microscopic, molecular, and immunological techniques applied at the species level, enabling researchers and managers to examine and evaluate the responses of the phytoplankton community over a broad spectrum of scales relevant to coastal eutrophication dynamics.

Despite the benefits of diagnostic pigment analyses, there are some interpretational and applicational limitations, pitfalls, and precautions. From a taxonomic perspective, diagnostic photopigments are largely group-specific and, in a few cases (e.g., *K. brevis),* species-specific indicators. Therefore, they are most suitable and effective for showing trends at a taxonomic level, such as hypoxia and food web alterations, that may indicate broad-based shifts in ecosystem structure and function. More specific characteristics, such as toxicity, poor palatability, and indigestibility, are often more readily distinguishable at the species level. In this regard, group-level chemotaxonomic indicators, such as diagnostic photopigments, may be most effective and meaningful if accompanied by species-specific microscopic or molecular indicators of problematic or enigmatic species that exhibit these highly individualistic but important attributes. While the latter techniques have the benefit of potentially being species or strain specific, they are also far more laborious and not as readily adaptable to large-scale, spatially and temporally intensive monitoring and assessment programs. However, recent advances in automated sampling and processing of environmental samples for molecular analysis (Scholin et al. 2002) provide hope that both broad-based and specific phytoplankton characterization techniques may soon accompany each other in such programs.

While offering a broad suite of diagnostic pigments, HPLC-based techniques require more sophisticated and expensive equipment than that required for simpler Chl *a* measurements. Researchers have to weigh the costs and time of running samples (only a few minutes for Chl *a* versus at least 20 minutes for HPLC) against informational needs. If a simple assessment of total phytoplankton biomass is needed, regardless of its composition, then Chl *a* (determined either fluorometrically or spectrophotometrically) is the most desirable indicator. However, to look into the "green box" to examine phytoplankton compositional dynamics, HPLC is the method of choice. HPLC analyses require more training in data analysis and instrumentation maintenance; however, it has been our experience that a technician can become proficient in these techniques in a matter of a few weeks when working alongside experienced individuals (Millie et al. 1993, Pinckney et al. 2001).

Complementary use of HPLC, fluorometric, spectrophotometric, microscopic, molecular, and immunological techniques in highly dynamic estuarine and coastal environments offers a qualitative and quantitative suite of indicators for diverse ecological applications. For example,

using diagnostic photopigments in conjunction with taxon-specific molecular identification techniques facilitates the assignment of functional specificity to basic measurements of phototrophic biomass (Chl *a*) that are routinely made worldwide. Piggybacking these techniques on existing sampling protocols will require relatively minor modifications and costs. Moreover, results from these techniques can be linked to meteorologic and hydrologic driver assessments made over larger scales by aircraft- and satellite-based remote sensing and by circulation, sediment, and nutrient transport studies and modeling. In the long term and on a regional level, the added dimensionality and benefits gained by combining these approaches will far outweigh the initial investment.

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2004 SOCIETY FOR CONSERVATION BIOLOGY (SCB)
ANNUAL MEETING

SCB's 18th Annual Meeting, July 30-Aug 2, 2004, will be hosted by the Center for Environmental Research and Conservation (CERC) at the Earth Institute at Columbia University in New York City. CERC is a consortium of Columbia University, the American Museum of Natural History, The New York Botanical Garden, Wildlife Conservation Society, and Wildlife Trust.

The meeting theme is "Conservation in an Urbanizing World." This year, for the first time in history, more of the world's population lives in urban rather than non-urban settings, posing significant conservation challenges and changing patterns of consumption, trade and ecosystem use. The conference will consider this theme and other emerging topics through symposia, workshops, organized discussions, contributed oral presentations and posters.

You are invited to submit proposals for symposia, workshops and organized discussions by October 31, 2003 to 2004@conservationbiology.org. For guidelines, previous topics, deadlines and updates, please check the meeting website, www.conbio.org/2004. Details on submissions for Abstracts for Oral Presentations and Posters will be posted in October. Participant registration begins in November 2003.

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