

PHYTOPLANKTON INDICATORS OF ECOLOGICAL CHANGE IN THE EUTROPHYING PAMLICO SOUND SYSTEM, NORTH CAROLINA

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Abstract. Nutrient enrichment and eutrophication of estuarine and coastal waters are accelerating, and there is a need to develop rapidly detectable and quantifiable indicators of these changes. Coastal systems are also impacted by climatic perturbations, including droughts, storms, and floods, the frequencies of which may be increasing. Phytoplankton are excellent indicators of ecological change. They are relatively easy to detect, identify, and quantify; they conduct a large share of primary production; and they are sensitive to diverse environmental stressors. In this study, phytoplankton total biomass, as chlorophyll *a*, and group-specific chemotaxonomic indicators (including chlorophylls and carotenoids) were used to characterize community responses to human (nutrient) and climatic (hydrologic) perturbations in the Neuse River Estuary–Pamlico Sound, North Carolina, USA. This estuarine–coastal continuum is experiencing anthropogenic nutrient enrichment and, since 1996, a rise in hurricane frequency. Freshwater input and flushing strongly interacted with supplies of the limiting nutrient nitrogen (N) to determine the location, magnitude, and composition of phytoplankton biomass along this continuum. Elevated flow (high flushing) following hurricanes favored dominance by the fast-growing chlorophytes and cryptophytes. Diatoms tended to dominate under moderate flow, while dinoflagellates and cyanobacteria increased in dominance when low flow prevailed in winter/spring and summer/fall, respectively. Depending on seasonal hydrologic cycles and episodic (hurricane) conditions, phytoplankton community structure differed substantially. These changes impact eutrophication, food web, biogeochemical (e.g., hypoxia), and habitat conditions in this and other coastal ecosystems currently experiencing changes in nutrient inputs and climatic events. Phytoplankton-based indicators are adaptable to unattended monitoring platforms (e.g., ferries) that can be coupled to remote sensing and modeling efforts, in order to evaluate and help manage ecological change at ecosystem and regional scales.

Key words: climate; estuaries; eutrophication; hydrology; indicators; North Carolina, USA; nutrients; photopigments; phytoplankton; water quality.

INTRODUCTION

Nearly three-quarters of the world's human population resides in coastal river basins, which has led to an unprecedented increase of nutrients associated with coastal development (Vitousek et al. 1997). While nutrient over-enrichment and eutrophication of downstream estuarine and coastal ecosystems is accelerating (Nixon 1995, NRC 2000, Boesch et al. 2001), there is a paucity of information on the manner in which the structure and function of aquatic communities mediating productivity and cycling of major elements (i.e., C, N, O, P) in these ecosystems are altered by these activities. Understanding how anthropogenically induced change affects estuarine- and coastal-ecosystem biodiversity, water quality, fisheries habitat, and resources is a major research and management challenge.

Nutrient (especially nitrogen) over-enrichment is a key driver of coastal ecological change (Nixon 1995, Paerl 1997, Boesch et al. 2001), and nitrogen (N) discharge to coastal waters is directly proportional to human population density of their watersheds (Peierls et al. 1991, Vitousek et al. 1997). Resultant eutrophication has significantly altered coastal productivity, nutrient (C, N, P, Si) cycling, water quality, biodiversity, and overall ecosystem health (Smetacek et al. 1991, Vollenweider 1992, Conley 2000, NRC 2000, Cloern 2001, Rabalais and Turner 2001). Short- and longer-term eutrophication trends have been documented by various indicators, including: sediment paleoecological indices (Brush 1986, Cooper 1995, Bianchi et al. 2000), loss of water clarity (Harding and Perry 1997, Goldman 2000), increased frequencies and expansion of low-oxygen (hypoxic) bottom waters (Officer et al. 1984, Rabalais and Turner 2001) accompanied by a shift from aerobic to anaerobic metabolism (Paerl et al. 1998, Boynton and Kemp 2000), altered stoichiometry and shifts in nutrient limitation (Conley 2000, Rabalais and Turner 2001), and increased harmful algal bloom activity (Paerl 1988, 1997, Hallegraeff 1993, Richardson 1997).

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Estuarine and coastal (jointly termed coastal) ecosystems are also affected by climatic change and associated perturbations, including droughts, hurricanes, and floods (Paerl et al. 2001, Peierls et al. 2003). These events appear to be increasing in frequency and magnitude (Goldenberg et al. 2001, Webster et al. 2005). It is suspected that these perturbations affect primary production and higher-trophic-level responses by modifying both hydrologic and nutrient regimes of coastal systems (Odum et al. 1995, Peierls et al. 2003). There is a need for rapid, unequivocal, quantitative detection and evaluation of the ecological impacts of these interactive stressors on coastal-water quality.

Phytoplankton dominate primary production in most estuarine and coastal waters (Nixon 1986, Paerl et al. 1998). They are highly sensitive indicators of nutrient-controlled, and hence nutrient-enhanced, production and physical forcing (i.e., vertical mixing, changes in flushing and residence time, and altered optical properties) of these systems, since their growth is rapid (doubling times as low as 0.5 d^{-1}), growth responses to nutrients occur at nanomole to micromole concentrations, and their photosynthetic responses can be measured by highly sensitive techniques. As such, phytoplankton are relevant, readily detectable indicators of biogeochemical and ecological change.

In recent years, a suite of easily deployed, broadly applicable phytoplankton-based water quality indicators has been developed. These include production, growth rate, and community compositional indicators (Paerl et al. 2002, 2005, 2006). In particular, the use of diagnostic (of various microalgal groups) photopigments as quantitative indicators of phytoplankton taxonomic group biomass has gained popularity and use in water quality monitoring and ecological assessment programs (Millie et al. 1993, Mackey et al. 1996, Pinckney et al. 2001). This technique is complementary to standard fluorescence and spectrophotometric absorbance determinations of chlorophyll *a*, microscopic counts, cell-specific indicators such as microautoradiography and fluorescence in situ hybridization (FISH) (Paerl et al. 2006).

Using these diagnostic indicators, we examined how nutrient supplies interact with climatic (hydrologic) and other physical forcings, including freshwater discharge, temperature, and irradiance, to determine the amount, distribution, and composition of phytoplankton communities in the Neuse River Estuary–Pamlico Sound (NRE–PS). This freshwater–marine continuum is representative of the greater Pamlico Sound system, North America's largest lagoonal estuarine ecosystem and one of its most important fisheries nursery habitats (Copeland and Gray 1991). Previous research has shown N to be the nutrient controlling or limiting primary production throughout the NRE–PS (Rudek et al. 1991, Boyer et al. 1994, Paerl et al. 1995, Piehler et al. 2004). Phytoplankton account for at least 80% of the primary production in this system (Paerl et al. 1998). Hence, N loading and other environmental impacts on the phytoplankton community are likely to

have major ramifications for higher-trophic-level, habitat, and ecosystem processes.

METHODS AND MATERIALS

Site characteristics: The Neuse River Estuary–Pamlico Sound system

The Pamlico Sound (PS) receives input from five watersheds (the Neuse, Pamlico, Chowan, and Roanoke Rivers and the Albemarle Sound) and drains to the Atlantic Ocean via three narrow passages (Oregon, Hatteras, and Ocracoke Inlets; Fig. 1). This results in a relatively long (approximately one-year) water retention time (Pietrafesa et al. 1996). The PS's shallow riverine tributaries also experience comparatively long residence times, e.g., one to over three months for the Neuse River Estuary (Luettich et al. 2000). Consequently, allochthonous and autochthonous inputs of nutrients and other pollutants readily accumulate in this system. These conditions are conducive to effective nutrient retention and cycling (Christian et al. 1991), making the system sensitive and highly responsive to external nutrient loading (Boyer et al. 1994, Paerl et al. 2001, Peierls et al. 2003).

The Neuse River Estuary (NRE) is the largest of the four major riverine tributaries of the PS. It is downstream of rapidly expanding agricultural (hog, poultry, and rowcrop operations), urban (Raleigh-Durham-Research Triangle), and industrial activities in North Carolina coastal watersheds. Excessive nutrient (especially nonpoint source N) discharge associated with such expansion has promoted eutrophication and water quality degradation (Paerl et al. 1990, Rudek et al. 1991, Stow et al. 2001). Symptoms include periodic nuisance algal blooms, hypoxia, anoxia, toxicity, disease, and potentially mass mortalities of finfish and shellfish (Copeland and Gray 1991, Paerl et al. 1995, 1998, Burkholder and Glasgow 1997, Lenihan and Peterson 1998). In response to scientific evidence of N-driven eutrophication and public pressure to reduce its unwanted symptoms, the North Carolina General Assembly mandated a 30% reduction in N loading to the NRE to be in place by 2006 (NCDENR-DWQ 2001). In addition, the U.S. EPA has imposed a total maximum daily load (TMDL) for N (U.S. EPA 1997) for the NRE, using the State's 30% N reduction strategy. Phytoplankton biomass, as chlorophyll *a* ($40 \mu\text{g chl } a/\text{L}$ is the "acceptable" level) is the criterion for attainment of the TMDL (NCDENR-DWQ 2001). This large-scale N manipulation and earlier (mid-1980s) upstream reductions in P loading have provided opportunities to examine the effects of nutrient management using phytoplankton-based indicators (cf. Paerl et al. 2004).

This system has also been under the influence of natural perturbations, including droughts, hurricanes, and flooding, the frequency and intensity of which have increased markedly since the mid-1990s (Paerl 2005, Paerl et al. 2005). The recent upsurge in hurricanes began in 1996, with the arrival of Hurricane Bertha in July. This relatively low-rainfall storm was followed by

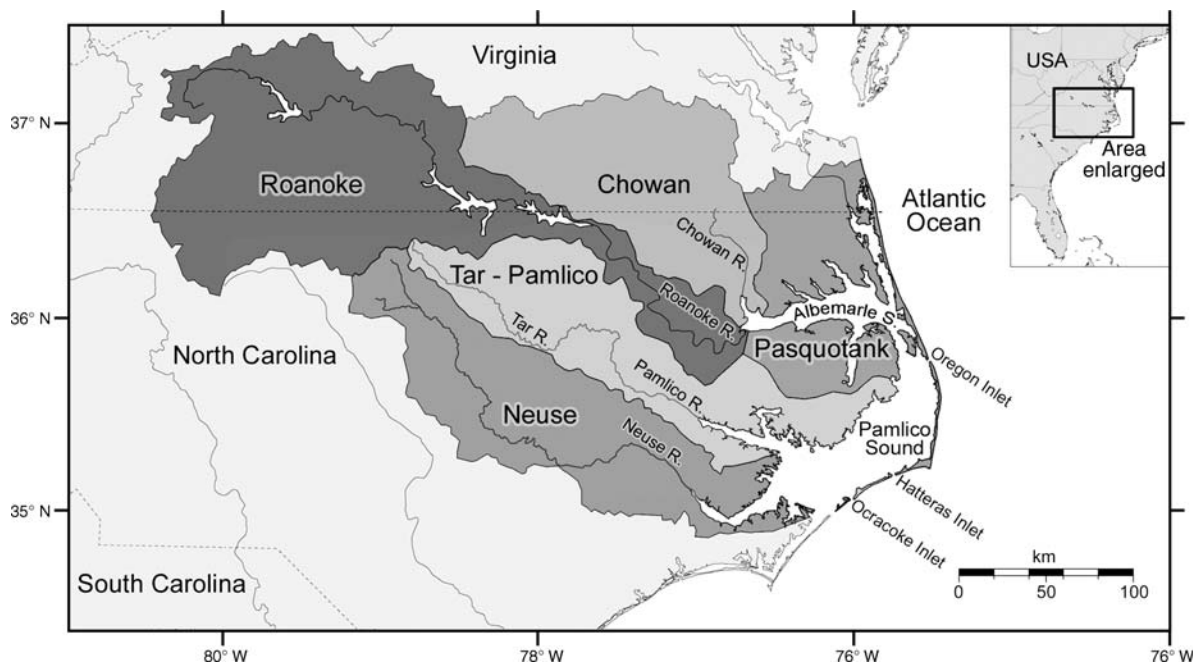


FIG. 1. North Carolina's Pamlico Sound system, including its sub-estuarine tributaries (Chowan, Roanoke, Tar-Pamlico, and Neuse Rivers) and their watersheds.

Fran, a large, category 2 hurricane that delivered >30 cm of rainfall and caused extensive flooding throughout the NRE watershed. Fran's floodwaters delivered very high inorganic-nutrient and organic-matter loads to the NRE, causing severe oxygen depletion and triggering fish kills in the estuary (Paerl et al. 1998). Category 2 Hurricane Bonnie crossed the PS in late August 1998, after moving up the coast from near Wilmington, where it first made landfall. This was a fast-moving, low-rainfall storm. In stark contrast, during the fall of 1999, Hurricanes Dennis, Floyd, and Irene inundated coastal North Carolina with up to 1 m of rainfall, causing extensive flooding throughout the watershed of the PS. During the ensuing six weeks, sediment- and nutrient-laden floodwaters totally freshened the NRE and reduced the PS salinity by 70%. These storms as well as Fran (1996) delivered enough N to equal the annual N load normally entering this N-sensitive system (Paerl et al. 2001) (Fig. 2). Biogeochemical and ecological effects included hypoxic (<4 mg O₂/L) bottom waters, major changes in nutrient cycling, a threefold increase in algal biomass, persistent algal blooms, altered fish distributions and catches, and an increase in fish disease (Paerl et al. 2001, Adams et al. 2003, Peierls et al. 2003, Tester et al. 2003).

Recently, category 2 Hurricane Isabel crossed the Pamlico Sound in mid-September 2003. The storm surges and high waves associated with Isabel caused a breach in the Outer Banks near Cape Hatteras. Despite the violent winds, rainfall amounts from Hurricane Isabel were relatively small (<6 cm in coastal North Carolina; North Carolina Climatology Office, North

Carolina State University, Raleigh, North Carolina, USA), in part because it was a fast-moving storm. As a result, freshwater discharge and nutrient (N) load were low for Isabel compared to the 1999 hurricanes and more comparable to effects of localized summer/fall thunderstorms (Fig. 2). Most recently, in September 2005, Hurricane Ophelia grazed the Outer Banks. Ophelia was another relatively dry, low-N-input storm (data not shown).

Like many coastal systems, the PS is strongly influenced by both human (nutrient and other pollutant) and hydrologic perturbations, including water supply diversions and manifestations of climate change (droughts, hurricanes, floods). These interactive perturbations have intensified during the past decade, reflecting a recent rise in Atlantic hurricane activity and increased development in its watershed.

Water column hydrographic measurements

In the NRE, water column hydrographic data have been collected from fixed sampling locations at two-week intervals from 1994 to the present as part of the NRE modeling and monitoring program ModMon (Fig. 3; data available online).² The numbers and locations of sampling stations have varied slightly through time. In the PS, hydrographic data have been collected from 10 locations since late 1999 (Peierls et al. 2003) (Fig. 3). Vertical profiles of temperature, salinity, dissolved oxygen (DO), conductivity, chlorophyll *a* fluorescence,

² www.marine.unc.edu/neuse/modmon

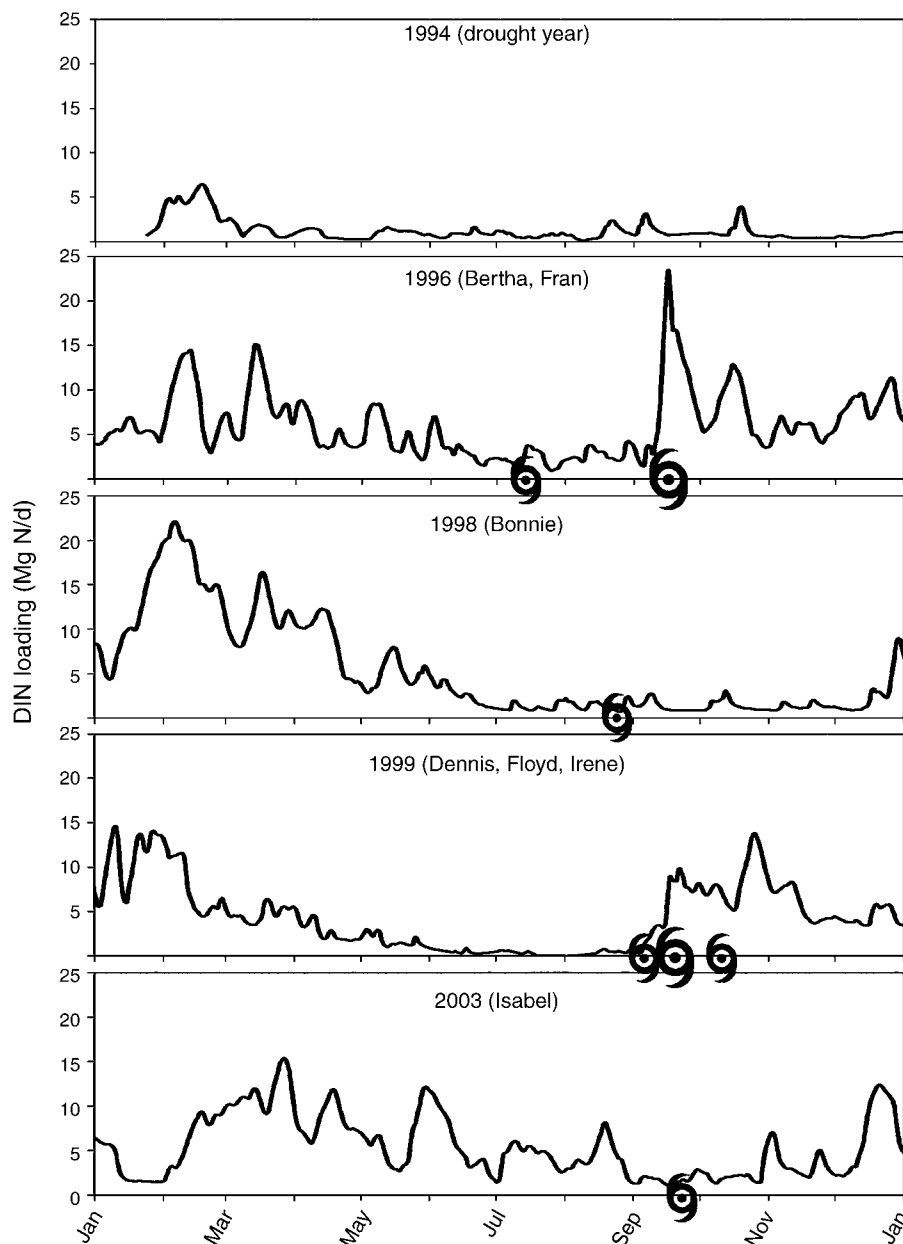


FIG. 2. Dissolved inorganic nitrogen (DIN) loading to the Neuse River Estuary for four hydrologically different years, including those years in which hurricanes (named) struck eastern North Carolina. The DIN loading was calculated by multiplying freshwater discharge from the Neuse River (at the U.S. Geological Service gauging station at Kinston, 30 km upstream from the head of the estuary) by DIN (ammonium, nitrate/nitrite) concentrations. Times of landfall for individual hurricanes are indicated with hurricane symbols.

turbidity, depth, and pH were measured with a YSI 6600 multiparameter water quality monitor coupled to a YSI 650 MDS logger (Yellow Springs Instruments, Yellow Springs, Ohio, USA). Additional hydrographic and water quality data were collected by the ferry-based water quality program FerryMon (data *available online*)³ and two autonomous vertical profilers (AVPs)

located in the NRE (Fig. 3). The North Carolina State Climate Office (Raleigh, North Carolina) provided daily precipitation and tributary discharge data from station Kinston 5 SE located ~30 km upstream from the head of the estuary (35.22° N, 77.53° W).

Nutrient analyses

Nutrient analyses were conducted on water samples collected by the ModMon program at 0.5 m below the

³ www.ferrymon.org

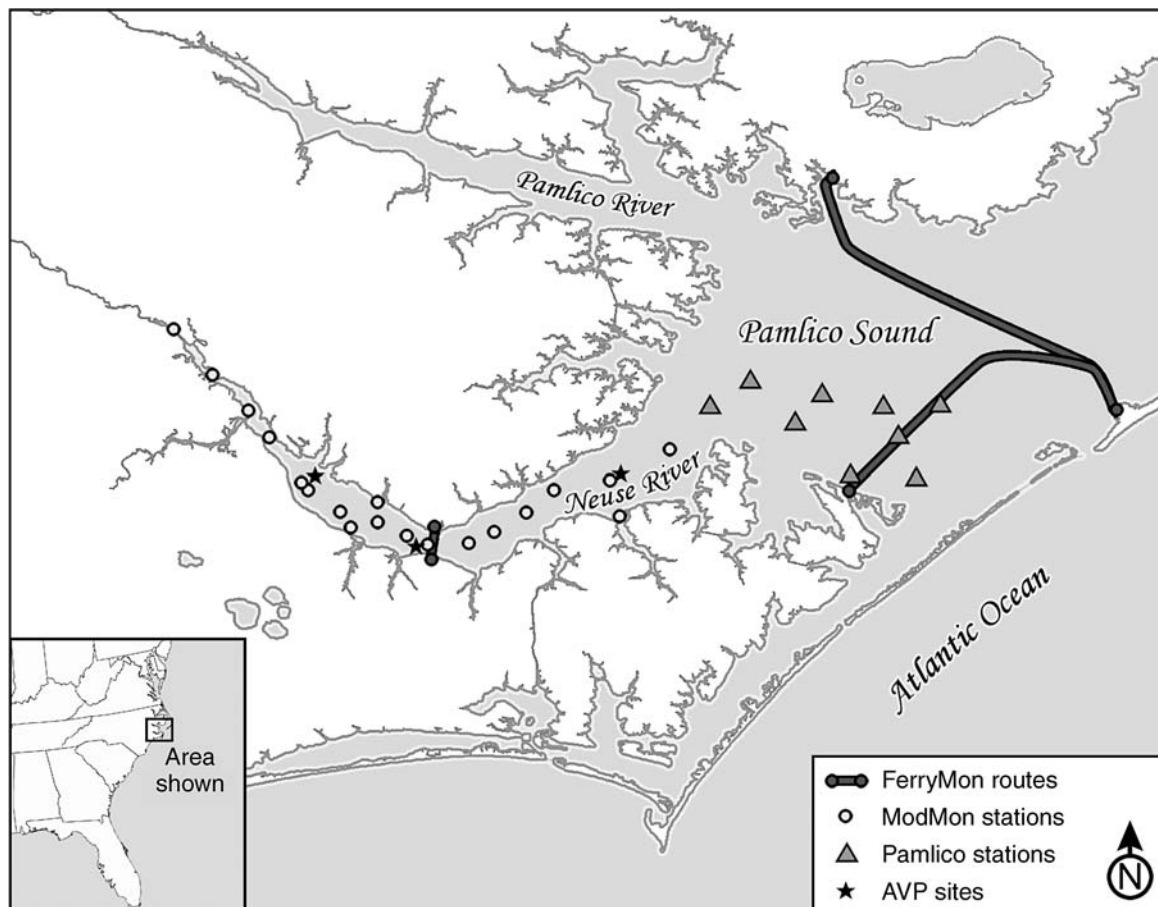


FIG. 3. Map of the Neuse River Estuary and western Pamlico Sound, showing the sampling locations for the Neuse River modeling and monitoring program (ModMon), the ferry-based water quality monitoring program (FerryMon), and autonomous vertical profiler (AVP) locations. These programs were the sources of data for this study.

surface and 0.5 m above the sediments, using a horizontal PVC Van Dorn sampler, and automatically, using a carousel collector at 0.5 below the surface by FerryMon. Water samples for dissolved inorganic-nutrient analyses were gently filtered through 25 mm diameter Whatman GF/F filters and analyzed in the laboratory. Nitrate + nitrite ($\text{NO}_3^- + \text{NO}_2^-$), ammonium (NH_4^+), orthophosphate (PO_4^{3-}), and silicate (SiO_3^{2-}) concentrations were determined with a Lachat Quick-Chem 8000 auto-analyzer (Lachat, Milwaukee, Wisconsin, USA), using standard protocols (Lachat Quik-Chem methods 31-107-04-1-C, 31-107-06-1-A, and 31-115-01-3-C, respectively).

Phytoplankton biomass

As an indicator of total phytoplankton community biomass, chlorophyll *a* (chl *a*) concentrations have been measured once every two weeks as part of the ModMon transects of stations extending from the upper freshwater region of the NRE to the entrance of PS, daily as part of the FerryMon program, and during specific experimental work since 1994. Near-surface and near-

bottom grab samples were analyzed for chl *a* by filtering 100 mL of NRE water onto Whatman glass fiber filters (GFF, 0.7- μm porosity). Filters were sonicated in 90% acetone, extracted overnight, and analyzed fluorometrically for chl *a* (Paerl et al. 1995).

Group-specific diagnostic photopigment analyses

In addition to chl *a*, which accounts for total phytoplankton community biomass, chemosystematic chlorophyll and carotenoid photopigments characteristic of specific taxonomic groups (i.e., diatoms, chlorophytes, dinoflagellates, cyanobacteria, cryptomonads; see Plate 1) have proved useful as measures of taxa-specific biomass (Mackey et al. 1996, Jeffrey et al. 1997). Near-surface and near-bottom samples from biweekly ModMon runs along the NRE–PS continuum were gently filtered (200–500 mL, depending on phytoplankton density) on Whatman GFF filters. Filters were then sonicated in 100% high-performance liquid chromatography (HPLC)-grade acetone and extracted overnight, and the extracts were analyzed by HPLC coupled to

photodiode array spectrophotometry (PDAS) (Wright et al. 1991, Millie et al. 1993).

A statistical procedure, ChemTax (Mackey et al. 1996), which partitions chl *a* into the major algal groups based on diagnostic carotenoids, was used to determine the relative and absolute contributions of each group. The software CHEMTAX was used (Mackey et al. 1998). In the NRE, key photopigment markers include chlorophyll *b* and lutein (chlorophytes), zeaxanthin, myxoxanthophyll and echinenone (cyanobacteria), fucoxanthin (diatoms), peridinin (dinoflagellates), and alloxanthin (cryptomonads) (Paerl et al. 2003). Photopigment composition is generally significantly (linearly) correlated with species cell counts or biovolume estimates (Tester et al. 1995, Wright et al. 1996), although there are some relatively rare “outlier” phytoplankton species that may contain pigments that lie outside of their taxonomic grouping (e.g., euglenoids). Therefore, care was taken to periodically make microscopic checks to ensure that the appropriate phytoplankton groups were identified and quantified (Lewitus et al. 2005). Prior studies have shown that examining phytoplankton community dynamics and successional changes at the taxonomic-group level often provides excellent insight into the environmental controls of shifts in productivity, biogeochemical fluxes, and food web dynamics (Cottingham and Carpenter 1998, Pinckney et al. 2001, Paerl et al. 2003, 2006).

RESULTS AND DISCUSSION

Distribution of phytoplankton biomass along the estuarine continuum

Seasonal and multi-annual examinations of phytoplankton biomass, determined as chlorophyll *a* concentrations, indicated that biomass was heterogeneously distributed both with depth and along the NRE–PS continuum. Frequently, chl *a* showed strong vertical gradients during salinity-stratified periods, with highest concentrations appearing near the surface or just above the pycnocline (Paerl et al. 1995, Valdes et al. 2006; N. S. Hall, *unpublished manuscript*). Longitudinally, phytoplankton biomass frequently accumulated as distinct peaks or chl *a* maxima (“chl *a* max”), which most often occurred midway down the NRE, beginning where the river begins to widen and diminishing where the river bends to the northeast (see Fig. 3). The longitudinal distribution of chl *a* concentrations is strongly influenced by the interacting effects of freshwater discharge, nutrient availability, and the morphology of the NRE. Freshwater discharge controls longitudinal transport and water residence time in the NRE and as such delivers nutrients and simultaneously influences the accumulation and transport of biomass through variable flushing rates. When flow was extremely high, flushing rates exceeded the ability of any phytoplankton groups to accumulate biomass and form blooms in the estuary. This scenario typically occurred during relatively high rainfall during late winter and spring months, when

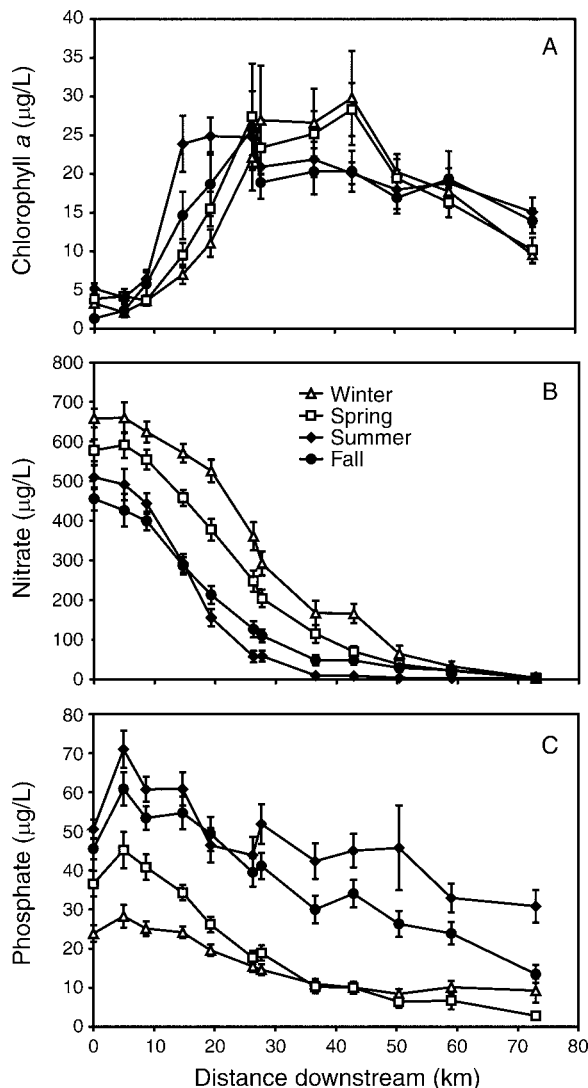


FIG. 4. Seasonal means (\pm SE) of (A) chlorophyll *a*, (B) nitrate, and (C) phosphate concentrations measured at each sampling station along the length of the Neuse River Estuary for all years from 1994 to 2003. Distance downstream refers to the distance in kilometers of the sampling station from the most upstream sampling location at Streets Ferry Bridge.

elevated runoff prevented accumulation of chl *a* in the upper NRE (Fig. 4A; i.e., flushing greatly exceeded growth rates). During moderate-to-low-flow conditions, which predominated during the summer, chl *a* accumulated further upstream and along the axis of the estuary where the residence time is long enough to allow growth rates to exceed flushing or “washout” (Fig. 4A). Depending on the actual flushing rates and season, specific groups dominated phytoplankton biomass and the chl *a* max.

Typically, the chl *a* max formed where lateral increases (gradients) in residence time coincided with adequate supplies of growth-limiting nutrient(s). These are often correlated in the NRE, because the actively

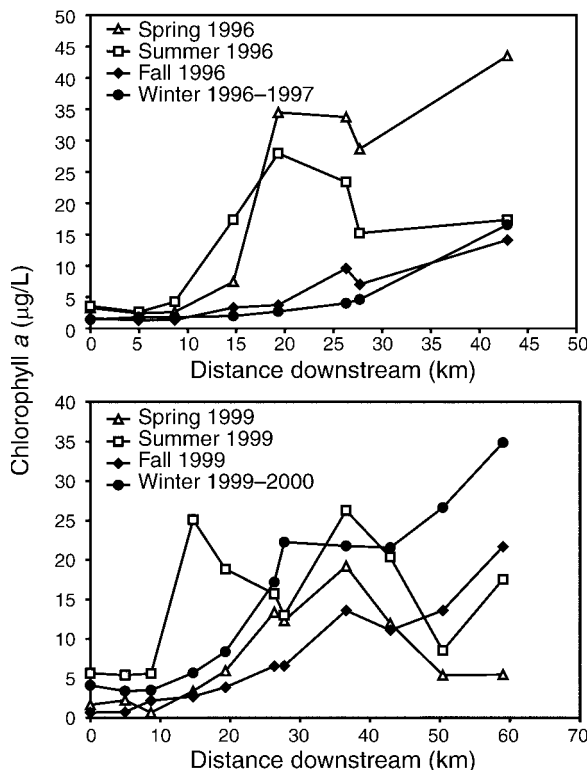


FIG. 5. Seasonal means of chlorophyll *a* concentrations along the length of the Neuse River Estuary during two major hurricane years: 1996 (top panel) and 1999 (bottom panel).

growing phytoplankton populations in the chl *a* max are responsible for removing limiting nutrients, thereby establishing a co-occurring nutrient gradient. This can be demonstrated for the dissolved inorganic forms of the most limiting nutrient nitrogen (N), specifically nitrate, the N form that most directly reflects externally supplied (watershed) or “new” N entering the estuary. This relationship is often most profound in the upstream oligohaline (>10 psu) region of the NRE, where under favorable spring/summer flow rates the chl *a* max coincides with a steep decline in NO_3^- concentrations (Fig. 4B). Ammonium (NH_4^+) concentrations (data not shown) tend to be low throughout the estuary year around, hence no strong gradients are apparent. On occasions, brief spikes in NH_4^+ can at times be seen in the mid-region of the estuary, resulting from periods of hypoxia; however, these spikes rapidly dissipate due to phytoplankton uptake (Paerl et al. 1995, 1998).

Seasonally, the chl *a* max tends to migrate upstream in the summer as dry conditions reduce freshwater discharge (and flushing) and slow the delivery of “new” N (Fig. 4A). This pattern is interrupted by large storm events, including tropical storms and hurricanes, which can suddenly and dramatically increase discharge, flow, and flushing of the estuary. This scenario was observed following Hurricanes Fran (1996) and the three hurricanes of 1999 (Dennis, Floyd, and Irene). In both cases, high flow rates following these high-rainfall events

caused the entire NRE to turn fresh and rapid flushing prevented any significant buildup of chl *a* in the estuary. Following the 1999 hurricanes, chl *a* responses to nutrient loads were largely confined to the downstream PS, where longer residence times allow the phytoplankton community to effectively utilize nutrients for growth and biomass accumulation (Peierls et al. 2003) (Fig. 5).

While NO_3^- concentrations show a steep downward gradient concurrent with the chl *a* max, dissolved inorganic phosphorus, or orthophosphate (PO_4^{3-}), does not (Fig. 4B, C). This indicates that P supplies are not limiting, relative to N, and confirms earlier studies that N is the most limiting nutrient for phytoplankton growth in the NRE (Paerl et al. 1990, 1995, Christian et al. 1991, Rudek et al. 1991). Furthermore, the sediments of the NRE are highly enriched in P (Christian et al. 1991, Paerl et al. 1995). Effective sediment–water column recycling of P most likely prevents P limitation in this and other shallow sub-estuaries of the Pamlico Sound system (Hobbie et al. 1972, Hobbie and Smith 1975). While there is evidence that P reductions have reduced chl *a* concentrations in the P-limited freshwater rivers draining to these estuaries (Paerl et al. 2004), the downstream estuarine waters have remained N-limited. If anything, aggressive upstream P reductions may have exacerbated downstream eutrophication by having reduced the biological filter (chl *a* max) for N removal in the upstream riverine components of these ecosystems.

The location and magnitude of the chl *a* max are useful for assessing the interactive effects and influences of nutrient (largely N) loading and hydrologic discharge on phytoplankton production patterns and trends in the NRE. The size of the chl *a* max can be used as an indicator of the magnitude of new production in response to nutrient loading when more predictable seasonal hydrologic cycles are encountered. Under these conditions, it is a useful tool for assessing long-term effects and benefits of N reduction strategies aimed at reversing eutrophication in this and other seasonally hydrologically variable estuaries. However, when large storms impact this system, the location, magnitude, and longitudinal extent of the chl *a* max are strongly affected. When corrected for and integrated over the volume and length of the estuary, the storm-influenced (displaced downstream) chl *a* max can contain from two to over four times as much phytoplankton biomass than more predictable seasonal peaks. This indicates that elevated N loads associated with storm discharge are effectively utilized and translated into new production along the continuum.

The long-term (1994–2004) phytoplankton biomass and community composition record for the NRE shows highly repeatable seasonal patterns during hurricane-free years as well episodic shifts in composition following large changes in freshwater input. During this time, the NRE was affected by droughts, and since 1996, an increase in the frequency and magnitude of tropical storms and hurricanes. During droughts, like those

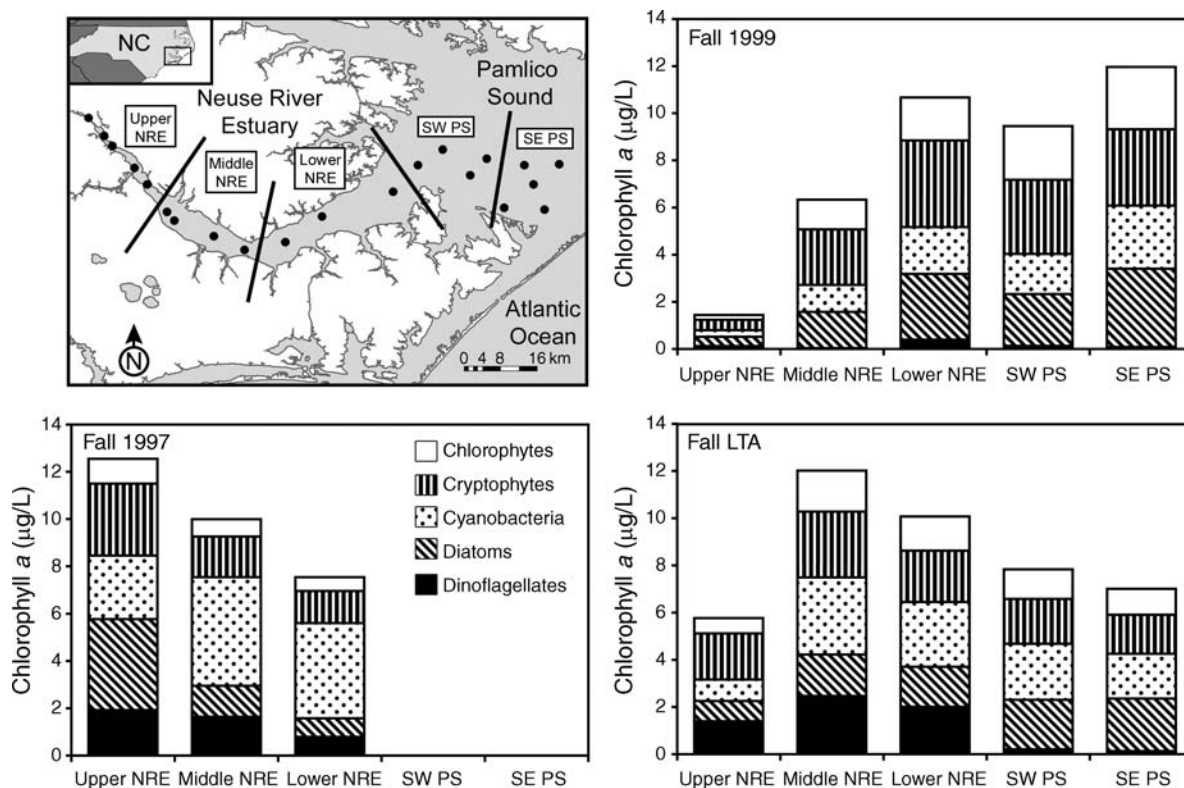


FIG. 6. Mean total and taxonomic group-specific phytoplankton biomass (as chlorophyll *a*) in each of the five regions of the Neuse River Estuary–Pamlico Sound (NRE–PS, including SW [southwestern] and SE [southeastern] PS) system during a drought period (fall 1997) and a period characterized by frequent and intense hurricane activity (fall 1999). These patterns were compared to the long-term average (LTA) phytoplankton community structure during fall months (September through November, 1994–2003) in each of the five regions. Values were determined from CHEMTAX analysis of diagnostic photopigment concentrations measured from surface samples collected at fixed sampling locations (black dots in the uppermost panel). Pamlico Sound regions were not sampled in fall 1997.

observed in summer and fall of 1997 in the NRE, the chl *a* max was consistently observed in the upper NRE region. Dissolved inorganic N (especially NO_3^-) gradients proved to be quite steep in this persistent and stationary drought-driven chl *a* max. This led to strong mid- and downstream N limitation, accompanied by consistently low chl *a* concentrations (Fig. 6). During the 1997 drought period, total phytoplankton biomass (as chl *a*) in the upper NRE was double the fall long-term average (fall LTA) for this region. The opposite pattern was observed during the fall 1999 hurricane period when total phytoplankton biomass was significantly reduced in the upper NRE region (by more than a third when compared to the fall LTA) and incrementally increased along the length of the NRE, remaining elevated within the two PS regions. These results illustrate that drought conditions tend to favor the formation of phytoplankton maxima upstream, while elevated rainfall and freshwater discharge displaced the maxima downstream. The average spatial distribution of total phytoplankton biomass during the fall (fall LTA) illustrates that on average, the greatest abundance of phytoplankton occurs in the middle and lower portions of the NRE (Figs. 5 and 6).

Assessing phytoplankton community composition dynamics along the NRE–PS continuum

When evaluated by HPLC–CHEMTAX, phytoplankton composition was strongly impacted by the changes in hydrology in the NRE–PS. During the fall 1997 drought, diatoms were the most abundant phytoplankton taxonomic group in the upper NRE, while cyanobacteria were dominant in the middle and lower NRE regions (Fig. 6). In contrast, on average (fall LTA), cryptophytes were the most abundant group in the upper NRE, while all five phytoplankton taxonomic groups were on average (fall LTA) present in about equal proportions (~20%) in the middle and lower NRE regions. During the fall 1999 hurricane season, dinoflagellates, which generally comprise ~20% of the phytoplankton in the three NRE regions (Fig. 6), were significantly reduced in abundance during this elevated flushing (reduced water residence time) condition, especially in the upper and middle NRE regions (Fig. 6). Phytoplankton community structure during the fall 1999 hurricane period was primarily comprised of diatoms, chlorophytes, and cryptophytes. Elevated runoff, which continued for at least five months

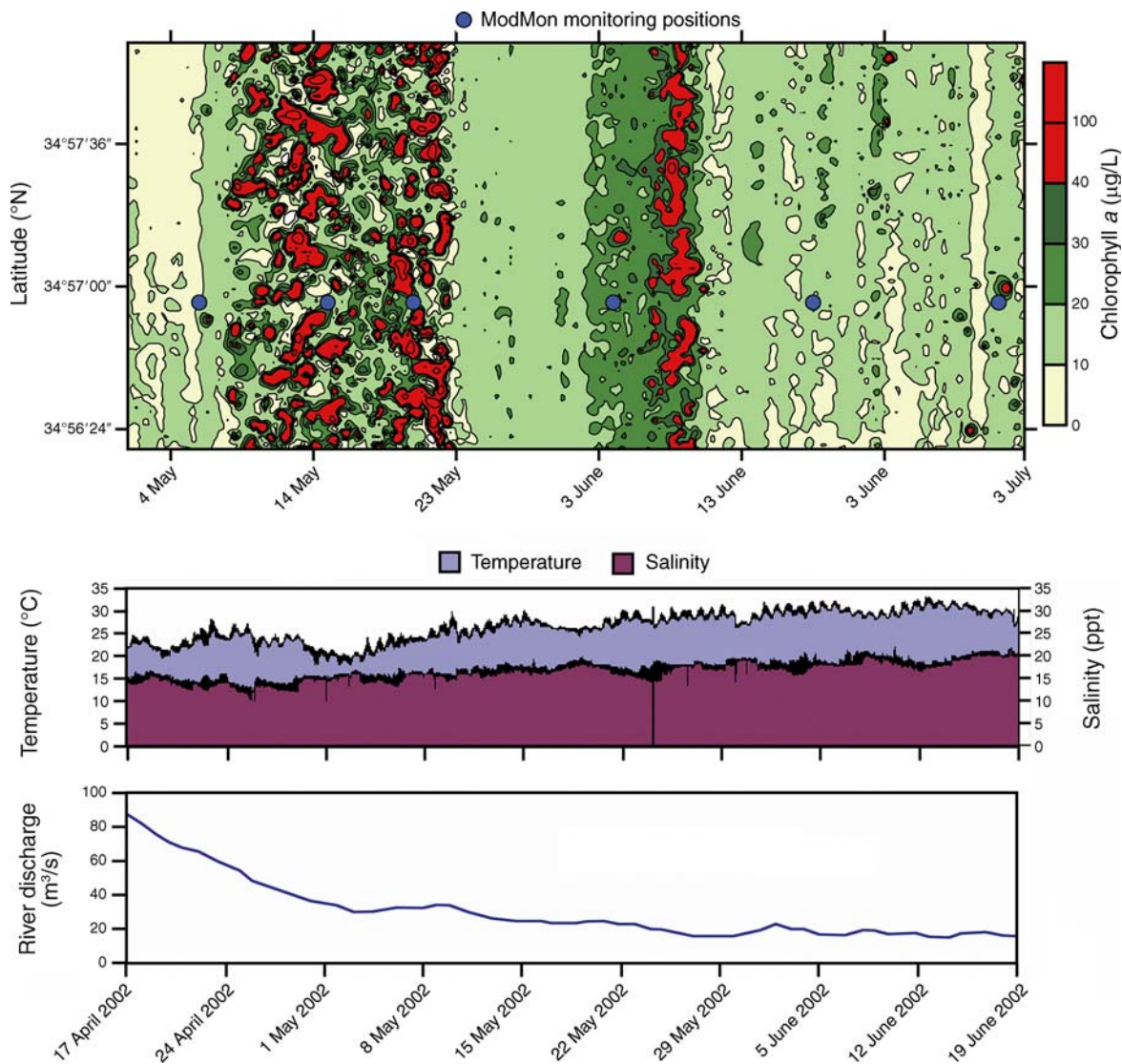


FIG. 7. Example of phytoplankton community dynamics and associated physicochemical “driver” data collected by the FerryMon program. The upper graph shows chlorophyll *a* concentration data (using a fluorescence probe mounted on a YSI 6600 multiparameter sonde, calibrated with extracted/fluorescence measurements [Yellow Springs Instruments, Yellow Springs, Ohio, USA]) collected over a 2.5-month period on the Neuse River Estuary (NRE) crossing. Shown are time (x-axis) and GPS-based locations (y-axis) of data. Chlorophyll *a* data was based on 20 ferry crossings per day (~15 data points per transect) that the ferry makes between Cherry Point and Minnesott Beach on the NRE over the 2.5-month period. The blue dots show the time and location of ModMon data collection at a site that intersects with the FerryMon crossing. On some occasions ModMon captures data similar to FerryMon, while at other times, ModMon is not able to capture the variability that FerryMon is able to detect. These data indicate that phytoplankton populations occur as distinct patches as they are transported in the estuary. The frequency, magnitude, and location of patches vary with time and space and appear to be related to variable flow regimes (freshwater discharge is shown in the lower frame). They may also be partially controlled by temperature and salinity (middle frame), parameters known to influence phytoplankton community growth rates and structure.

following the 1999 hurricanes, promoted prolonged periods of flushing (short residence time) and reduced salinity conditions throughout the NRE going well into mid-2000. This created conditions favorable for fast-growing, low-salinity-adapted chlorophytes and cryptophytes, which dominated the upper and middle NRE, starting in fall of 1999 and lasting throughout spring and early summer of 2000 (Fig. 6) (Paerl et al. 2005).

Conversely, relatively slow-growing dinoflagellates, which regularly form late-winter blooms (e.g., *Heterocapsa triquetra*) in years during which flow is low to moderate (i.e., years free of high-rainfall hurricanes) (Paerl et al. 1995), were absent in winter/spring periods when extensive flooding and freshwater runoff followed the 1999 and 1996 large hurricanes (Valdes et al. 2006). In the hurricane-free decade prior to 1996, these blooms

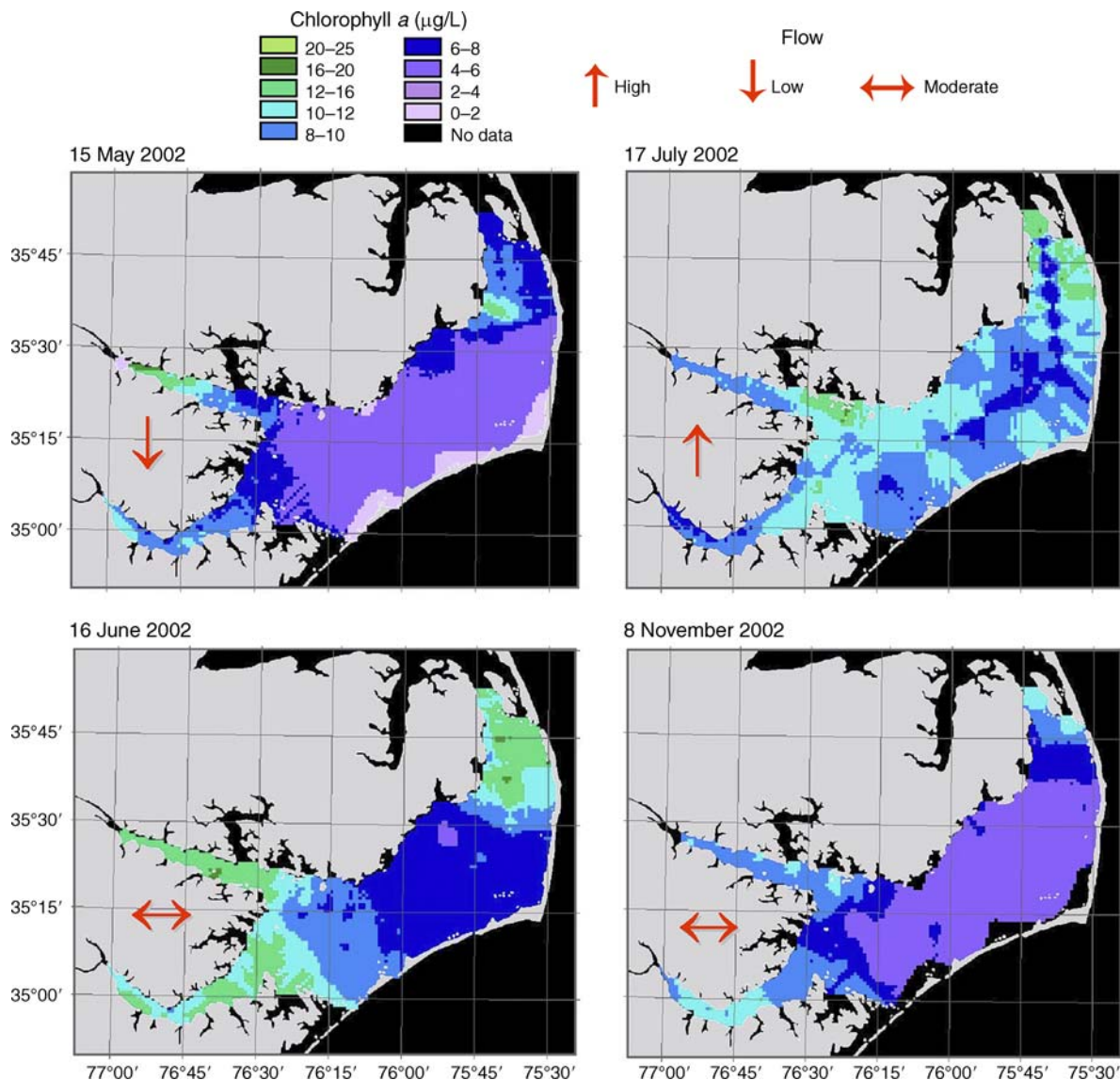


Fig. 8. Spatial relationships between remote-sensed phytoplankton biomass, as chlorophyll *a*, and freshwater discharge (flow arrows) to the Pamlico Sound system. Surface water chlorophyll *a* concentrations were estimated using an aircraft-based sea-viewing wide field-of-view sensor (SeaWiFS) remote sensing system, calibrated by FerryMon-based chlorophyll *a* data. Under relatively low-flow, long residence time conditions, phytoplankton biomass is concentrated in the upstream reaches of the estuaries (i.e., Neuse and Pamlico River Estuaries). Under moderate flow, phytoplankton biomass maxima extend farther downstream. Under high flow (short residence time), phytoplankton biomass maxima are shifted farther downstream into Pamlico Sound.

were a highly predictable feature of the NRE (Paerl et al. 1995). Interestingly, these blooms are now making a comeback since 2000. We speculate that this bloom resurgence is largely a response to the absence of high-rainfall hurricanes in the NRE watershed.

Estuarine phytoplankton taxonomic groups exhibit variable optimal growth rates (doubling times) (Redalje 1993, Pinckney et al. 2001). The net effect of variable growth rates and changeable hydrologic regimes in the NRE–PS continuum is that phytoplankton composition is strongly influenced by large hydrologic perturbations, whether they be droughts or hurricanes. While the most

profound examples of this were the shifts observed in response to the lengthy periods of freshwater discharge following the 1996 and 1999 hurricanes, the drought periods in 1997 and the summer preceding the 1999 hurricanes are additional, albeit less spectacular, examples. These periods tended to favor slower growing, relatively high-salinity-tolerant-phytoplankton groups that, by virtue of their slow growth rates are able to grow on lower nutrient supply regimes. These are typified by the picoplanktonic cyanobacteria, which exhibited an increasing and persistent degree of dominance during summer low-flow drought conditions.

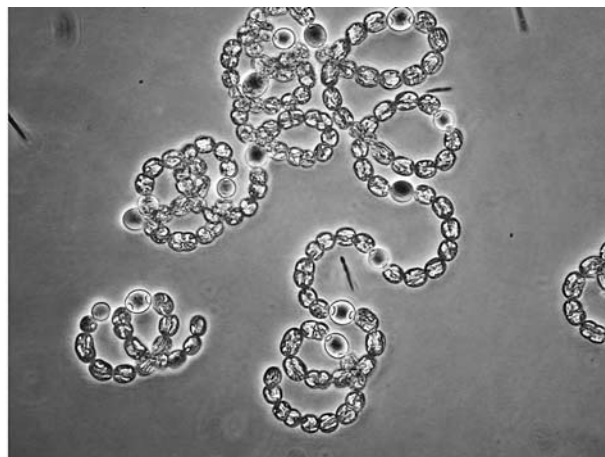
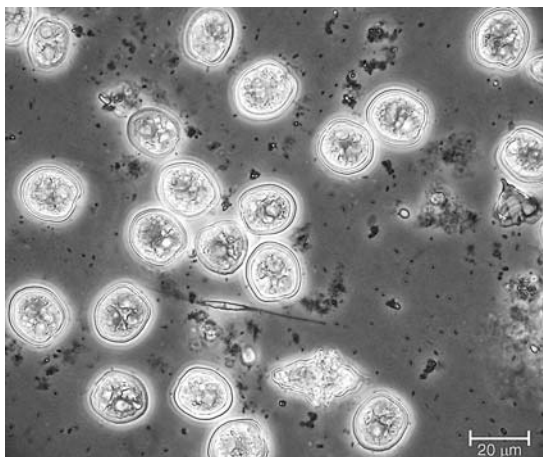


PLATE 1. (Left) A dinoflagellate bloom in the Neuse River Estuary, in response to nutrient inputs associated with runoff from tropical storm Ernesto, which made landfall in eastern North Carolina (USA) in mid-September 2006. The bloom was dominated by *Prorocentrum minimum* (round cells), with another dinoflagellate *Heterocapsa triquetra* ("top" shaped cell on its side) as subdominant. (Right) Filamentous, bloom-forming blue-green algae (cyanobacterium) *Anabaena circinalis* from the nutrient-enriched, upstream, freshwater portion of the Neuse River Estuary. This cyanobacterium has specialized cells, called heterocysts (the round, clear cells with bright "polar plugs" at each end of the cell), in which nitrogen fixation takes place. Nitrogen fixation is the biochemical process enabling cyanobacteria like *Anabaena* to convert atmospheric nitrogen gas to the biologically utilizable form of nitrogen, ammonia. This enables *Anabaena* to thrive in nitrogen-depleted waters. Because these organisms supply their own nitrogen, by nitrogen fixation, phosphorus is often the nutrient that controls the growth and expansion of such nuisance blooms. Photo credits: H. Paerl.

CONCLUSIONS AND RECOMMENDATIONS

Photopigment analyses of phytoplankton biomass and community composition have shown that sudden and large changes in hydrologic conditions associated with tropical storms and hurricanes are important, and sometimes overriding, drivers of phytoplankton community structure and function in estuarine and coastal ecosystems. This is especially true for microtidal, relatively long residence time, lagoonal systems like the NRE-PS, where the full sequence of phytoplankton community nutrient utilization, growth, and successional responses falls within the time scales of hydrologic and nutrient-loading drivers. It follows that when formulating nutrient (in this case N) input constraints designed to control eutrophication, hydrologic variability should play an integral role. For example, nutrient eutrophication models that rely on the interactions of nutrient supply and phytoplankton growth kinetics to predict phytoplankton group responses to nutrient-loading events must also take freshwater input, flushing, and water residence time into consideration. Predictability of phytoplankton group successional patterns derived from competition for nutrients in a variable flow environment will strongly rely on incorporating nutrient uptake/utilization kinetics and flushing or "washout," as well as seasonality in models that realistically depict interactive phytoplankton community-structuring features.

Spatiotemporal analysis of phytoplankton community responses to physical and chemical drivers associated with human and climatic perturbations has shown the importance of examining these responses along the entire freshwater-marine continuum. Depending on

freshwater runoff associated with seasonal precipitation patterns, acute and sometimes catastrophic storms and droughts, a bulk of the phytoplankton production and successional responses may be confined to either narrow, well-defined chl *a* max zones or more diffuse zones along this continuum. Capturing and quantifying phytoplankton community responses along this continuum requires sensitive process-level indicators and appropriate spatiotemporal sampling strategies. Phytoplankton responses to environmental drivers are often episodic, that is they can form, proliferate, and/or dissipate over a timescale of a few days to several weeks. These responses, as blooms, account for significant fractions of seasonal or annual production. They are also causative agents for hypoxic events, fish kills, and other advanced symptoms of eutrophication, yet may be missed by biweekly or monthly sampling programs, which tend to be the norm for a wide range of estuarine ecosystems (cf. U.S. EPA 1998, 2001, Bricker et al. 1999). As such, there is a widespread need for spatially and temporally intensive sampling and analysis of the causative factors, as well as locations, manifestations, and fates of phytoplankton blooms. Appropriate spatial and temporal coverage and comprehensiveness of the indicators discussed here can be ensured by deploying them on intensive monitoring programs and unattended platforms, including moorings, channel markers, docks, and vessels that cover repeatable routes in estuarine and coastal waters.

One example is the use of the North Carolina Department of Transportation (DOT) ferries for automated water quality monitoring of the NRE-PS system,

FerryMon. FerryMon was initiated as part of the State's response to the flooding, nutrient, and sediment discharge following the three 1999 hurricanes. FerryMon uses three ferries as data-gathering "ships of opportunity" that transit the NRE and PS continuously from 05:00 until midnight daily (see Fig. 3 for routes). Since 2000, FerryMon has provided unattended, near-real-time spatially and temporally intensive data on key environmental parameters pertinent to the control of phytoplankton production and composition. These include physical (temperature, turbidity), chemical (dissolved oxygen, salinity, pH, nutrients), and biological (chl *a* and diagnostic photopigments) data. It is establishing a baseline for assessing the relative impacts of storm-related ecological change on the event and longer term scales. The program provides large data sets (~80 000 data points per year) that can produce maps of chl *a* and algal blooms with a high degree of statistical confidence. This information is used to provide a database for calibrating and verifying process-level and probabilistic models for predicting nutrient productivity–algal bloom relationships (Borsuk et al. 2001, 2003, Bowen and Hieronymous 2003).

Additional examples of FerryMon's recent applications for examining and distinguishing physicochemical drivers of phytoplankton community dynamics include: (1) the use of the Neuse River Ferry data to assess TMDL compliance, based on the State's 40 µg/L acceptable chlorophyll *a* level (Fig. 7), (2) assessing the effects of recent hurricanes such as Isabel (September 2003) and Ophelia (September 2005) on PS water quality (Paerl 2005), and (3) the use of chl *a* data for calibration of aircraft- and satellite-based remote sensing, allowing investigators to enlarge studies of nutrient and hydrologic forcing of phytoplankton production for the entire Pamlico Sound system (Fig. 8).

All three examples illustrate the strong interactive effects of nutrient and hydrologic forcing in this system, based on the use of phytoplankton as an overall response indicator. Current and future nutrient management strategies aimed at controlling eutrophication of coastal systems periodically influenced by tropical storms and hurricanes should develop and adopt criteria and thresholds for gauging success that factor in hydrologic variability, especially on the episodic scale. In North Carolina, this involves adaptive nutrient management strategies that consider the timing of nutrient (fertilizer) applications and wastewater nutrient releases, no-till agricultural practices, improved stormwater and agricultural (animal) waste containment/treatment, and greater coupling and integration of groundwater and atmospheric nutrient sources with estuarine and coastal nutrient budgets and algal bloom dynamics. The overall lesson learned is that, in order to be most meaningful and effective, nutrient productivity and resultant eutrophication management strategies must address the entire airshed/watershed-to-coastal-water continuum.

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