

Phytoplankton Community Indicators of Short- and Long-term Ecological Change in the Anthropogenically and Climatically Impacted Neuse River Estuary, North Carolina, USA

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Abstract Estuarine and coastal systems represent a challenge when it comes to determining the causes of ecological change because human and natural perturbations often interact. Phytoplankton biomass (chlorophyll *a*) and group-specific photopigment indicators were examined from 1994 to 2007 to assess community responses to nutrient and climatic perturbations in the Neuse River Estuary, NC. This system experienced nutrient enrichment and hydrologic variability, including droughts, and an increase in hurricanes. Freshwater input strongly interacted with supplies of the limiting nutrient nitrogen (N) and temperature to determine the location, magnitude, and composition of phytoplankton biomass. Multi-annual, seasonal, and episodic hydrologic perturbations, including changes in the frequency and intensity of tropical storms, hurricanes and droughts, caused significant shifts in phytoplankton community structure. Climatic oscillations can at times overwhelm anthropogenic nutrient inputs in terms of controlling algal bloom thresholds, duration, and spatial extent. Eutrophication models should incorporate climatically driven changes to better

predict phytoplankton community responses to nutrient inputs and other anthropogenic perturbations.

Keywords Phytoplankton · Estuary · Ecological change · Climate · Nutrients · Photopigments

Introduction

In estuarine and coastal systems worldwide, human activity puts considerable stress on ecosystem functioning. At least half the world's population resides in the coastal zone (Vitousek et al. 1997), and this influence continues to grow. Human development in coastal river basins has greatly increased nutrient, sediment, and other pollutant loads to these waters (Nixon 1995; Paerl 1997; Peierls et al. 1991), resulting in deterioration of water quality, losses of fisheries habitat and resources, and an overall decline in ecological condition (Boesch et al. 2001; Bricker et al. 1999; National Research Council 2000; Scavia and Bricker 2006).

Estuarine and coastal systems represent a formidable challenge when it comes to determining status and trends in water quality and ecological condition. These systems are highly dynamic from hydrologic, nutrient cycling, and biotic resource perspectives. Hydrologically, freshwater runoff interacts with tidal saltwater exchange and variable winds, leading to complex circulation and mixing patterns. These patterns, shaped by climatic forcing features (i.e., temperature, rainfall, winds, etc.) that vary over multiple time and space scales, strongly influence the chemical and biological characteristics and responses of these ecosystems to environmental changes and perturbations. Furthermore, human and natural perturbations often interact. For exam-

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ple, accelerating nutrient and sediment inputs can accompany climatic perturbations such as storms and floods, which in the case of tropical storm and hurricane activity, are increasing (Emanuel 2005; Goldenberg et al. 2001; Holland and Webster 2007; Webster et al. 2005).

Given the importance and dynamic nature of estuarine ecosystems, there is an urgent need to develop sensitive and broadly applicable indicators for detecting water quality changes and overall ecological condition and changes therein. Phytoplankton, including prokaryotic cyanobacteria and eukaryotic algal groups, conduct a bulk of primary production supporting food webs and play a central role in carbon, nutrient, and oxygen cycling in estuaries. Because phytoplankton have fast growth rates (i.e., doubling times of a day or less) and can rapidly respond to a wide range of environmental perturbations, they represent a sensitive and important indicator for detecting ecological change in estuaries. Lastly, changes in phytoplankton community structure and activity often precede larger-scale, longer-term changes in ecosystem function, including shifts in nutrient cycles, food webs, and fisheries (Paerl and Peierls 2008).

The Neuse River Estuary (NRE) is a major sub-estuary of the USA's second largest estuarine complex, the Pamlico Sound (North Carolina), a major fisheries nursery for the mid-Atlantic and Southeastern US coasts. The NRE exemplifies an estuary that is increasingly affected by human (nutrient loading) and climatic (hurricanes, droughts, floods) perturbations. We explored the use of diagnostic phytoplankton group-level photopigments to assess the impact of climatic perturbations acting on multiple timescales. Using a long-term (since 1994) data set from the bi-weekly Neuse River Estuary Modeling and Monitoring Program (ModMon), and data from a more recently established (since 2000) ferry-based autonomous monitoring program (FerryMon), we report on the impacts of climate variability spanning multiple timescales on NRE phytoplankton dynamics. Evidence presented here indicates that climatic changes and oscillations can at times overwhelm nutrient inputs in terms of controlling algal bloom thresholds, their duration, and spatial extent. In short, our results indicate that, to fully manage nutrient inputs and eutrophication, parallel (to anthropogenic factors) climatic changes need to be taken into consideration.

Background: Phytoplankton Dynamics of the Neuse River Estuary

Previous work in the NRE has set the stage for this study by demonstrating linkages of nutrient inputs to phytoplankton production and trends in spatio-temporal distributions of major phytoplankton taxa (Paerl et al. 2007; Pinckney et al. 1998, 1999; Valdes-Weaver et al. 2006). These historical data define a baseline from which comparisons can be made to assess trends in phytoplankton community spatial and

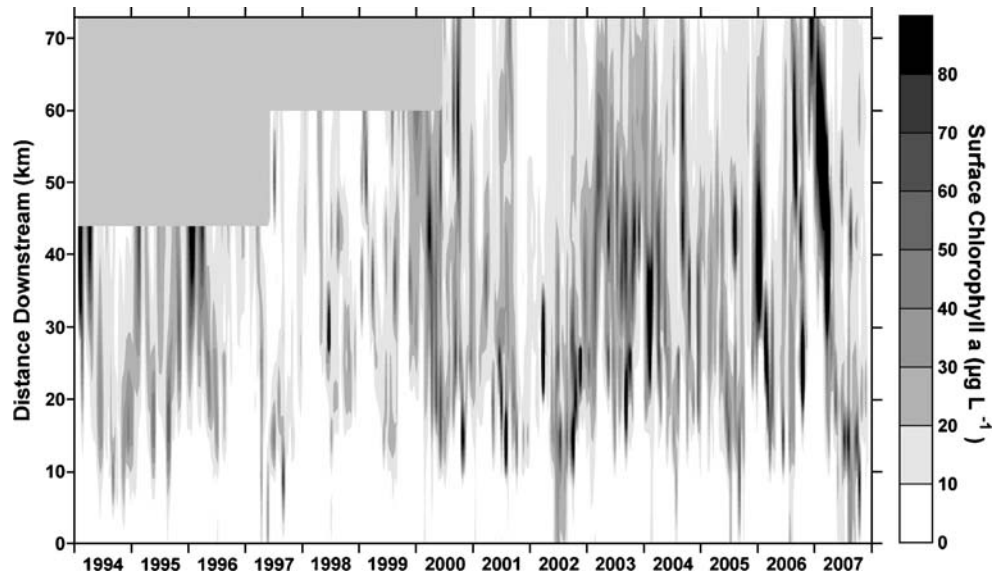
temporal distributions and abundance in response to human and climatic perturbations and longer-term changes.

Along much of its fresh to saltwater continuum, the NRE is nutrient sensitive (largely N limited) throughout much of the year, with sequential phytoplankton blooms occurring from late winter through fall (Paerl et al. 1995, 2006a; Rudek et al. 1991; Valdes-Weaver et al. 2006). These blooms reflect both nutrient enrichment and favorable hydrologic conditions, i.e., variable freshwater discharge and flushing/residence times suitable for development of blooms (Paerl et al. 2007; Valdes-Weaver et al. 2006). Water residence time varies temporally (depending on freshwater discharge) and spatially along the length of the continuum (Luettich et al. 2000). In the mesohaline segment of the NRE, where the estuary broadens, residence time increases dramatically and persistent summer salinity stratification occurs (Buzzelli et al. 2002; Luettich et al. 2000). It is also most sensitive to symptoms of nutrient over-enrichment, most notably periodic phytoplankton blooms, with chlorophyll *a* (Chl *a*) levels at times exceeding $100 \mu\text{g l}^{-1}$ (Paerl et al. 1995, 2006a).

Seasonal discharge patterns and episodic pulses of freshwater input strongly influence bloom patterns. The locations of Chl *a* maxima tend to reflect the magnitude of freshwater runoff. For example, the three hurricanes of 1999 delivered the largest amount of discharge to the NRE and Tropical Storm Ernesto proved to also be a very high discharge event. The Chl *a* maxima that formed in the estuary following these events were further downstream than peaks following other wet but not as high discharge periods (e.g., spring–summer 2003) (Fig. 1). During drought periods (such as 2002 and 2007), the Chl *a* maxima tend to be located much further upstream than normal (Fig. 1).

In terms of phytoplankton community composition, the NRE frequently exhibits late winter–early spring dinoflagellate blooms comprised of *Prorocentrum minimum*, *Heterocapsa triquetra*, and *Heterocapsa rotundata* that have been attributed to enhanced N loading (Mallin et al. 1993; Pinckney et al. 1998; Rudek et al. 1991). Dinoflagellate blooms can also occur in fall, especially during sunny, calm periods (Hall et al. 2008). Cryptophyte blooms occur sporadically throughout the year in response to pulses of riverine nutrient loading (Pinckney et al. 1998). Diatoms are most numerous during spring–summer (May to July), but they rarely attain bloom proportions (Pinckney et al. 1998). In the upper, freshwater riverine portion, increases in water residence time in summer, coupled to residual spring N loading, have, in certain years, led to periodic surface-dwelling cyanobacterial (*Microcystis*) blooms (Christian et al. 1986; Paerl 1987). Chlorophytes are a significant but infrequently dominant fraction of the phytoplankton. Occasional blooms occur during the spring and summer (Pinckney et al. 1998), and often “track” nutrient-laden freshets following large storm events (Paerl et al. 2007).

Fig. 1 Distribution of surface water Chl *a* concentrations along the axis of the Neuse River Estuary from 1994 and 2007. Data are from bi-weekly samples collected as part of the ModMon project (see Valdes-Weaver et al. 2006 for details). Contours generated from gridded data using kriging interpolation method. *Hatched area* indicates time and places without data



Materials and Methods

Study Site and Observational Programs

The NRE is a shallow, microtidal sub-estuary of the Pamlico Sound system (Fig. 2; Table 1). The NRE is downstream of rapidly expanding agricultural (animal and row crop operations), urban, and industrial activities in its watershed. Anthropogenic N and P loadings to the NRE have approximately doubled in the past four decades

(Stanley 1988; Stow et al. 2001). Urban regions have sprawled in the upper NRE watershed, while industrial-scale hog farms in the coastal plain region have led to an increase in hogs from less than 1 million in 1989 to more than 12 million today. Excessive nutrient discharge (~80% non-point source) associated with expanded human activities has promoted eutrophication in the NRE (Burkholder et al. 2006; North Carolina Department of Environment and Natural Resources 2001; Paerl et al. 1995, 1998).

Fig. 2 Map of the Neuse River Estuary and greater Pamlico Sound system, showing Mod-Mon sampling sites, the Neuse River Estuary FerryMon crossing, and the representative zone of summer hypoxia

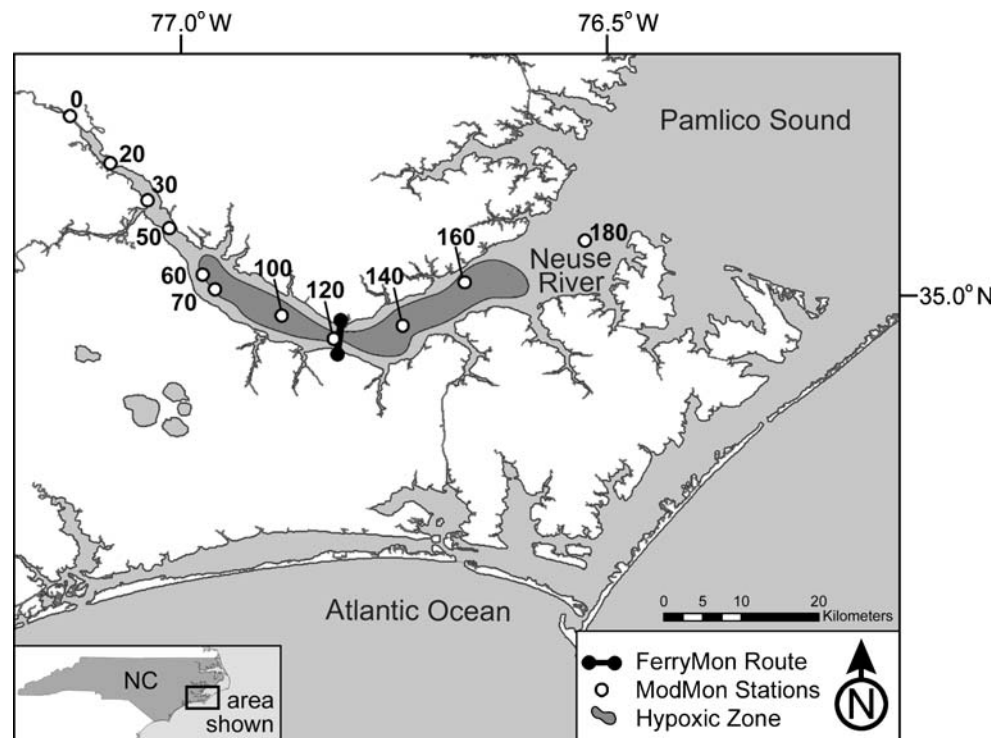


Table 1 Physical characteristics of the Neuse River Estuary

Characteristic	Neuse River Estuary
Watershed size (km ²)	16,108
Surface area (km ²)	455
Average depth (m)	2.7
Discharge (m ³ s ⁻¹)	50–1,000
Freshwater flushing time (days)	7–200
Summer hypoxic area (% of bottom)	20–>70

In 1999, the US EPA and the North Carolina Department of Environment and Natural Resources implemented a total maximum daily load (TMDL) for nitrogen inputs to the NRE, which targeted the estuary for a mandated 30% nitrogen load reduction (based on 1990–1995 mean total annual N loads; North Carolina Department of Environment and Natural Resources 2001). However, despite the mandated human nutrient-loading reductions, symptoms of eutrophication persist, in part, due to natural climatic forcing. Since the mid-1990s, increased nutrient loading has accompanied a dramatic increase in hurricanes as well as several extensive rainy periods (Bales 2003; Burkholder et al. 2004; Paerl et al. 1998, 2001, 2006a, b; Peierls et al. 2003). These events have been linked to increases in hypoxic bottom waters (Buzzelli et al. 2002; Paerl et al. 2001, 2006b), altered nutrient inputs and cycling (Peierls et al. 2003), shifts in salinity and nutrient loading (Paerl 2006a, b), with resultant changes in phytoplankton structure and function, as well as impacts at invertebrate and fish consumers levels (Burkholder et al. 2004; Eby and Crowder 2002; Paerl et al. 2006a, b).

Water quality of the NRE has been assessed by two spatially and temporally intensive long-term monitoring programs, ModMon and FerryMon (Fig. 2). These programs include an extensive array of measurements (Table 2). ModMon is a collaborative University–State of North Carolina (North Carolina Department of Environment and Natural Resources, Division of Water Quality; NC DENR–DWQ) program ([www.unc.edu/ims/neuse/modmon/index](http://www.unc.edu/ims/neuse/modmon/index.htm)

[htm](http://www.unc.edu/ims/neuse/modmon/index.htm)), which has operated since 1994. It consists of bi-weekly visits to 11 mid-river stations in the NRE (Fig. 2; Table 2) for vertical profiling and collection of near-surface and near-bottom water. FerryMon (www.ferrymon.org) consists of NC Department of Transportation ferries equipped with automated water quality monitoring systems (Buzzelli et al. 2003; Ensign and Paerl 2006). The Neuse River ferry makes 34 crossings per day between Cherry Point and Minnesott Beach, a mid-estuarine location in the high productivity, mesohaline region of the NRE (Fig. 2). Measurements are made with the automated systems (Table 2) and data transmitted via the internet to a shore-based data server. In addition, ISCO samplers collect discrete samples at regular programmed intervals. FerryMon serves as a source of space–time intensive information on phytoplankton community biomass (Chl *a*; using a flow-thru YSI sensor that is calibrated by extracted Chl *a* measurements) and composition (HPLC-based Chl *a* and diagnostic photopigments—Paerl et al. 2003). Data from the U.S. Geological Survey (USGS) real time water quality instrumentation (station 0209265810; <http://waterdata.usgs.gov/nc/nwis>) were used to fill gaps in the FerryMon salinity and temperature data. This station is co-located with ModMon station 120 and situated midway along the ferry transect.

Analytical Methods

For Chl *a* (phytoplankton biomass) and inorganic nitrogen (nitrate+nitrite and ammonium) analyses, ModMon samples were collected from the surface and near the bottom, while FerryMon collected only near-surface samples. All samples were analyzed for Chl *a* by extracting material collected on Whatman GF/F glass fiber filters (0.7 μm nominal pore size) overnight in 90% acetone. Prior to August 1999, extracts were analyzed spectrophotometrically using the trichromatic technique, while a fluorometric technique was used after that date. Concentrations were corrected ($\text{Chl } a_{\text{fluor}} = 1.81 \times [\text{Chl } a_{\text{spec}}]^{0.935}$) based on an empirically derived relationship between the two analyses and HPLC-analyzed Chl *a* concentrations (see below).

Table 2 Comparison of the sampling programs that serve as data sources for this study

Program	Location	Key measurements	Resolution	
			Spatial	Temporal
ModMon	Along axis (73 km length)	Vertical profiles of temperature, salinity, pH, O ₂ , chlorophyll fluorescence, and turbidity Surface and near-bottom inorganic nutrients, Chl <i>a</i> , HPLC pigments, preserved phytoplankton, and microzooplankton	2–14 km between sites 0.5 m depth resolution for profiles	Bi-weekly cruises
FerryMon	Across channel (5 km width)	Horizontal transects of temperature, salinity, pH, chlorophyll fluorescence, and turbidity Surface inorganic nutrients, Chl <i>a</i> , HPLC pigments	~700 m between data collection	~34 transects day ⁻¹

Inorganic nitrogen concentrations were measured using flow injection analysis on glass fiber filtrate (Whatman GF/F). For details of these analyses, see Paerl et al. (1998) and Peierls et al. (2003). Instantaneous nitrogen loading was calculated as the product of nitrogen concentration in $\mu\text{g l}^{-1}$ and daily mean flow in $\text{m}^3 \text{s}^{-1}$ at Kinston, NC (USGS station 02089500 <http://waterdata.usgs.gov/nc/nwis>).

In addition to Chl *a*, which accounts for total phytoplankton community biomass, photopigments characteristic of the specific taxonomic groups were measured (Jeffrey et al. 1999; Mackey et al. 1996; Millie et al. 1993). Near surface samples from ModMon and from discrete samples collected by FerryMon were gently filtered (200–500 ml, depending on phytoplankton density) on Whatman GF/F filters. For size fractionation purposes, ~300–500 ml of water was first filtered onto 3 μm porosity Nuclepore filters and the resulting filtrate was then filtered onto Whatman GF/F filters. Filters were then sonicated in 100% HPLC grade acetone, extracted overnight in darkness at 4°C, and the extracts analyzed by high performance liquid chromatography (HPLC) photodiode array spectrophotometry (Millie et al. 1993; Van Heukelem et al. 1994; Wright et al. 1991).

Statistical Methods

Trend analyses for nutrient loading and Chl *a* were performed using the non-parametric seasonal Kendall test for uncensored data as implemented in the S-PLUS (Insightful Corp.) version ([water.usgs.gov/software/S-](http://water.usgs.gov/software/S-PLUS/)

PLUS/) of ESTREND (Schertz et al. 1991; Slack et al. 2003). The analyses used 12 seasons per year (i.e., months) and a significance level (α) of 0.05. Concentrations reported as below detection limits were coded as one half the method detection limit. Less than 5% of values were reported as below detection.

Cyanobacterial biomass as estimated by zeaxanthin concentration was regressed on the physical parameters temperature, salinity, and river discharge, and riverine DIN loading using a stepwise multiple linear regression ('stepwise fit', Matlab version 7.0 R14, The Mathworks, Inc. Natick, MA, USA). A square root transformation was applied to zeaxanthin and assumptions of normality and homoscedasticity of the model residuals were confirmed using Lilliefors (Lilliefors 1967) and Breusch–Pagan (Breusch and Pagan 1979) tests, respectively.

Results and Discussion

Hydrologic Variability as a Driver of Nutrient Dynamics

During the study period, the NRE was both chronically and acutely impacted by climatic and subsequent hydrologic variability. The hydrologic variability associated with the various large storms, droughts, and high discharge periods is shown as freshwater discharge at a USGS gauging station (Kinston, NC), ~60 km upstream from the head of the estuary (Fig. 3).

Fig. 3 Neuse River daily mean streamflow data measured at the USGS gauge at Kinston, NC (station 02089500). Plotted is a smoothed line through the data using a locally weighted least squared (Loess) regression technique. Major tropical cyclones impacting the system are indicated

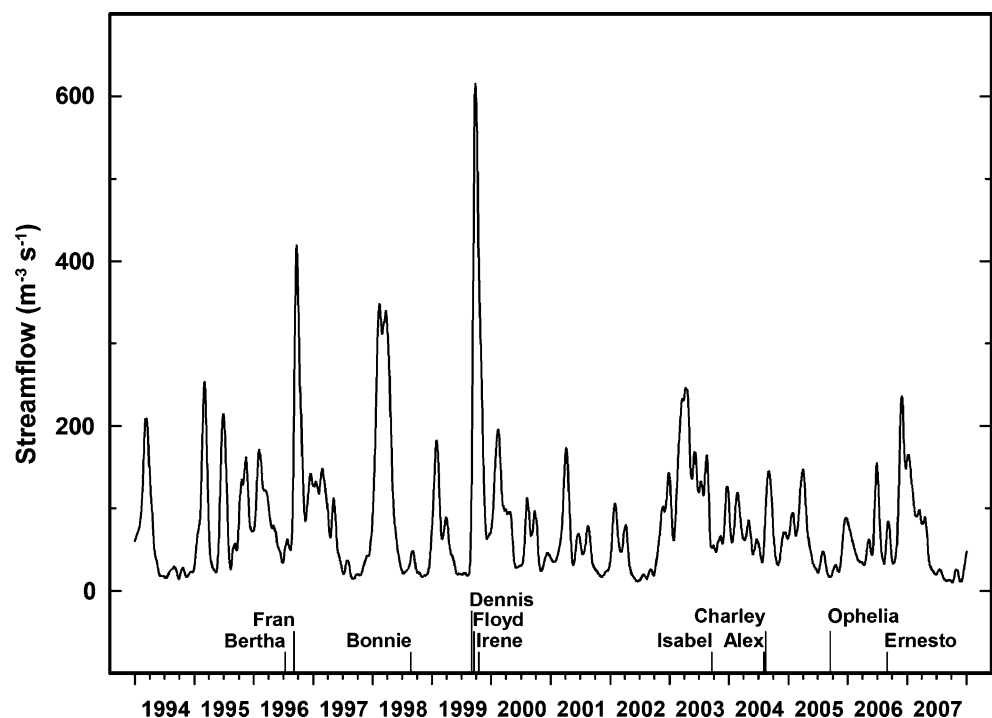
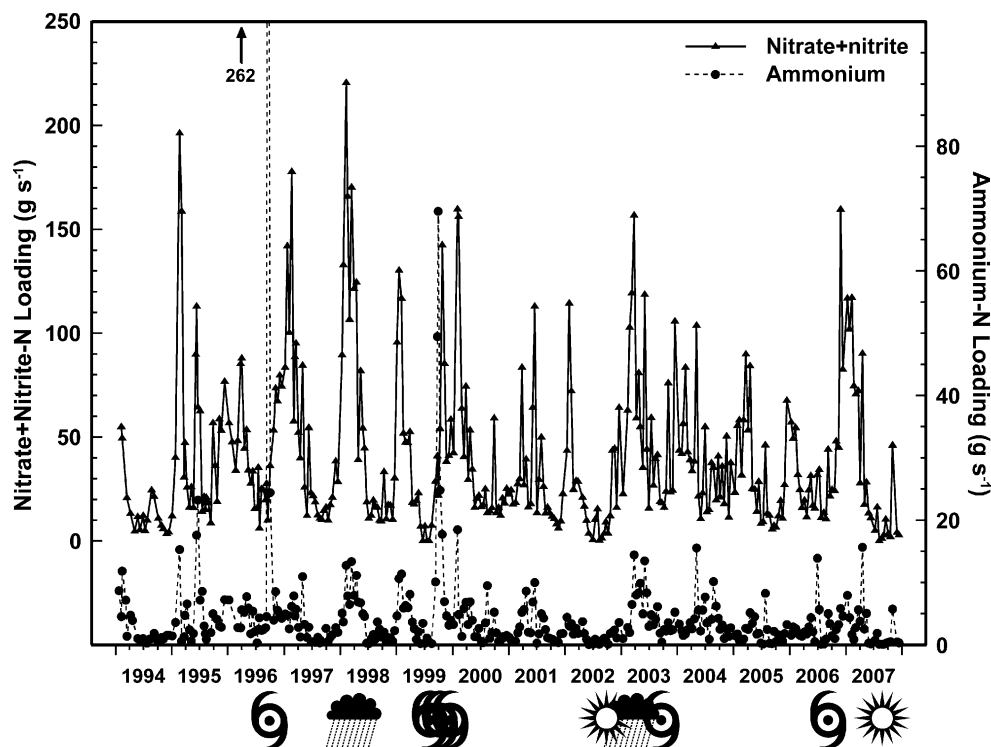


Fig. 4 Instantaneous loading of the dissolved inorganic N forms nitrate+nitrite and ammonium during the 1994–2007 sampling period. Bi-weekly values were calculated as the product of N concentration at station 0 and streamflow on the same date at Kinston, NC. Major storm events, high rainfall periods, and droughts are indicated with icons



Hydrologic variability strongly impacted nutrient inputs to the NRE (Fig. 4), shown here as instantaneous loading of dissolved inorganic nitrogen (DIN) at the head of the estuary at the Streets Ferry Bridge location (station 0, Fig. 2). Hurricanes Fran in September 1996, Dennis and Floyd in 1999, Tropical Storm Ernesto as well as the very wet spring–summers of 1998 and 2003 led to increased DIN loading. Conversely, extreme droughts, including the summer of 1999, proved to be periods of lowest discharge and nutrient loading. Externally supplied “new” DIN inputs were dominated by nitrate, which agrees well with previous studies conducted over shorter time intervals (Christian et al. 1991, 2004; Paerl et al. 1995, 2007).

Large spikes in ammonium loading were observed following the two largest (discharge wise) hurricanes, Fran in 1996 and Floyd in 1999. ModMon monitoring of water quality following these storms showed hypoxic ($<4 \text{ mg O}_2 \text{ l}^{-1}$) and anoxic ($<2 \text{ mg O}_2 \text{ l}^{-1}$) water being delivered from the watershed. These reduced conditions helped explain the preponderance of ammonium in the DIN load at these times. Otherwise, ammonium proved to be a relatively minor fraction of the external DIN load. Ammonium concentration spikes have been observed further downstream in the NRE; however, these spikes are strongly correlated to periods of bottom water hypoxia and anoxia indicating that they are internal DIN release events (Christian et al. 1991; Paerl et al. 1995, 2007). Overall, no significant long-term trends in nitrate and ammonium loading were detected (Fig. 4).

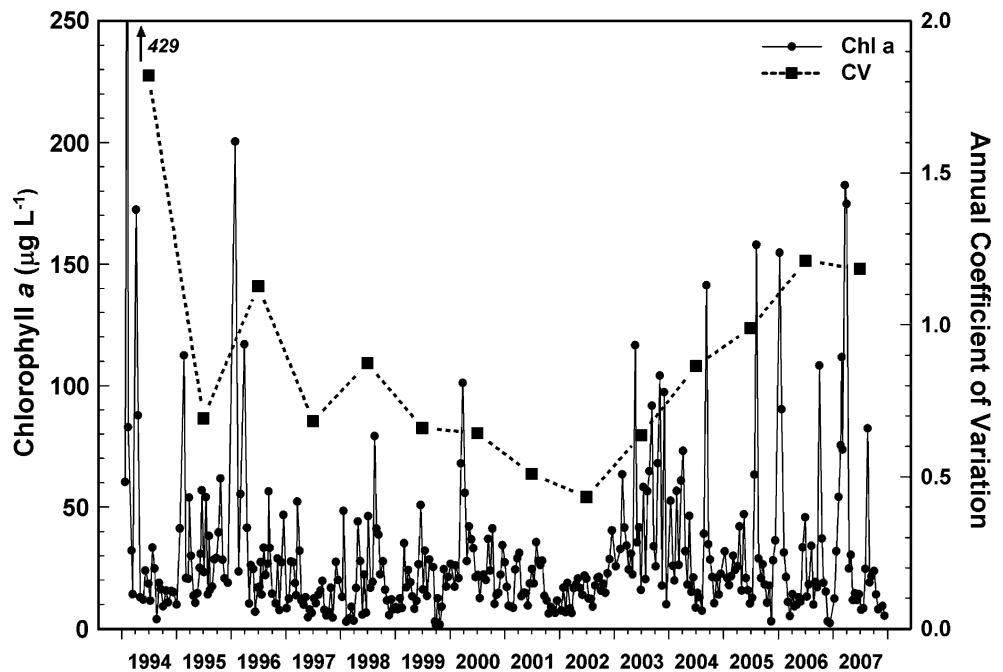
Hydrologic Variability as a Driver of Phytoplankton Community Dynamics

Hydrologic variability occurred over a wide range of temporal scales. Multi-annual, seasonal, and event-induced changes in river discharge, and the resulting changes in estuarine flushing and water residence times, affected total phytoplankton biomass and differentially affected specific phytoplankton groups as a function of their contrasting growth characteristics (Figs. 1 and 5). However, there were also indications in the longer-time series of factors other than hydrology exerting controls on the phytoplankton biomass (Figs. 1 and 5). Below, we focus on the impacts of climatic/hydrologic variability on phytoplankton community structure for the NRE.

Multi-year Patterns

Analysis of the 14-year ModMon database has revealed substantial multi-year changes in the NRE phytoplankton community. At a mid-estuarine location, Chl *a* concentration showed no significant long-term trend over the entire period, but large changes in variability did occur (Fig. 5). From 1994 to early 1996, several very large ($>80 \mu\text{g l}^{-1}$ chlorophyll *a*) phytoplankton blooms occurred in this region of the estuary (Fig. 5). However, this was followed by a >6 -year period (from mid-1996 to end of 2002) of few large blooms (e.g., only one bloom $>80 \mu\text{g l}^{-1}$ was recorded during this period; Fig. 5) and lower variability.

Fig. 5 Surface Chl *a* concentration ($\mu\text{g L}^{-1}$) at station 120, which is representative of patterns observed at stations 100–160 (see Fig. 2). *Circles* are the concentration values and *squares* indicate the coefficient of variation for each year. Note the increased variability and bloom potentials in phytoplankton from early 2003 onward



It is not clear what led to this prolonged period of relatively stable phytoplankton biomass. Phytoplankton growth and biomass in this region of the estuary is N limited throughout much of the year (Paerl et al. 1995, 2004, 2006c; Rudek et al. 1991), and thus blooms tend to follow freshwater discharge with accompanying “new” N loads. From 1996 to 2002, there were several significant freshwater pulses, and many smaller pulses, to this part of the estuary (Fig. 3). Nonetheless, large blooms did not develop, although the reason for this remains unclear. Through ongoing analysis of archived samples, we are currently exploring the possibility of changes in food web structure (i.e., top-down controls) that may have impacted bloom potentials in this region of the estuary. Beginning in early 2003 at the same site, the NRE again began to experience an increase in phytoplankton bloom frequency and magnitude (indicated by increasing Chl *a* variability) that continues to the present (Fig. 5).

Accompanying the increase in bloom occurrences were shifts in the taxonomic composition of the phytoplankton community, as determined by HPLC pigment analysis samples collected by the FerryMon program. Several chemotaxonomic pigments became more variable, accompanying the increased variability in Chl *a* and bloom frequencies. This was most obvious for peridinin and alloxanthin (representing dinoflagellates and cryptophytes, respectively; Fig. 6), all of which became more variable, and showed increased numbers of blooms. Multiple regression analyses of peridinin and alloxanthin on temperature, salinity, flow, and DIN loading showed that only salinity was significantly related to the two pigments. Both alloxanthin and peridinin were higher under lower salinity

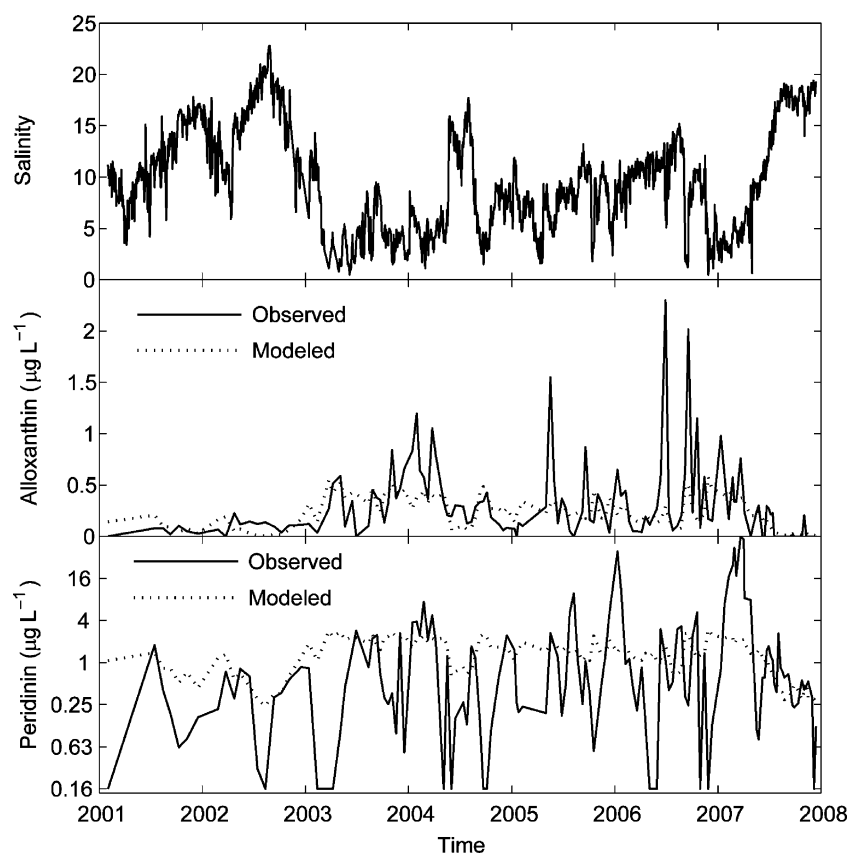
conditions. For alloxanthin, the negative relationship with salinity was quite strong ($R^2=0.37$, $p<1\times 10^{-14}$). For peridinin, the relationship with salinity was much weaker ($R^2=0.06$, $p=3.1\times 10^{-3}$). Despite the relationships with salinity, it is clear that salinity alone did not predict the high variability in alloxanthin and peridinin brought on by the large bloom events that began in early 2003 (Fig. 6).

Further analyses are warranted to determine the mechanisms leading to the prolonged multi-year changes in the frequency/magnitude of blooms in the NRE and other estuaries worldwide. While these shifts at particular locations within the estuary do not necessarily imply system-wide increases or decreases in productivity, the spatial extent is still large enough to be of ecological significance. In particular, shifts in bloom potentials may lead to regions of the estuary becoming more/less important in terms of trophic transfer, and may also refocus the sinking flux of phytoplankton-derived organic matter to regions of the estuary that vary in susceptibility to hypoxia formation from the breakdown of phytoplankton blooms (Paerl et al. 1998). Finally, from a water quality assessment perspective, shifts in bloom frequency between different regions of the NRE may change determinations of compliance or failure of the Chl *a*-based TMDL for regions of the estuary where bloom frequency has increased (North Carolina Department of Environment and Natural Resources 1998, 2001).

Seasonal Patterns

Zeaxanthin concentrations, which primarily represent cyanobacteria, exhibited seasonal fluctuations, with minimal

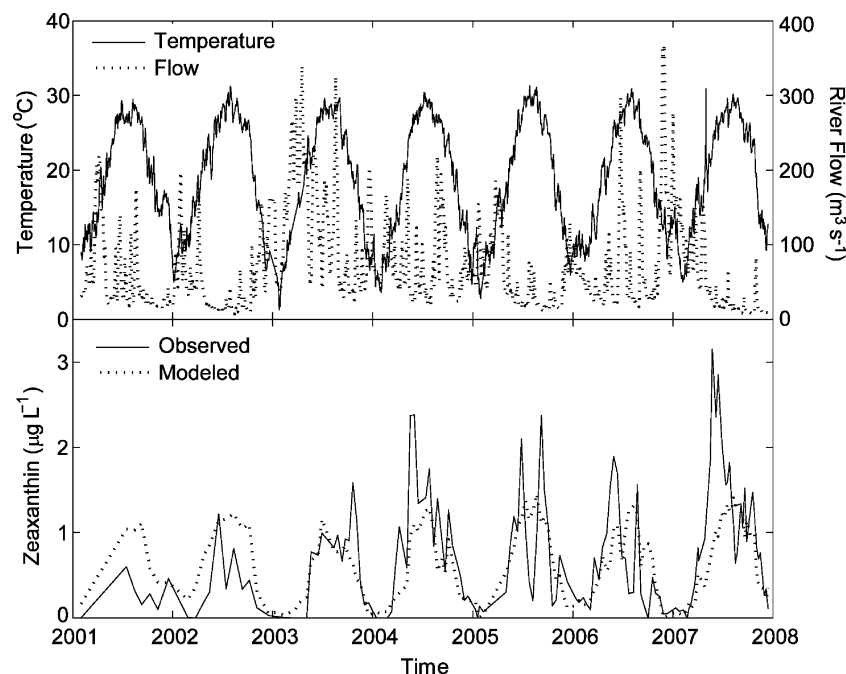
Fig. 6 **a** Daily mean salinity from FerryMon along the Neuse River ferry transect. Salinity data for 10 November 2002 through 22 June 2003 are from the USGS station 0209265810 located midway along the ferry transect. **b** Observed alloxanthin concentration from FerryMon and alloxanthin concentrations modeled by multiple regression of alloxanthin on salinity. Alloxanthin = $[0.78 - 0.036 \times \text{Salinity}]^2$. $N=141$. $R^2=0.37$. $F=81.6$, $p < 1 \times 10^{-14}$. **c** Observed peridinin concentration from FerryMon and peridinin concentrations modeled by multiple regression of peridinin on salinity. Note the log scale. Peridinin = $[1.75 - 0.063 \times \text{Salinity}]^2$. $N=141$. $R^2=0.062$. $F=9.1$, $p=3.1 \times 10^{-3}$. Pigment data from 18 May 2005 through 3 April 2007 were collected by ModMon at station 120



concentrations during colder winter months and maximal concentrations in warmer months (Fig. 7). A multiple regression analysis of zeaxanthin on temperature, river discharge, salinity, and DIN loading showed a strong positive relationship between zeaxanthin and temperature

($p=3.4 \times 10^{-13}$) and a weaker negative relationship with riverine discharge ($p=4.7 \times 10^{-3}$). Neither salinity nor DIN loading was retained as a significant factor by the stepwise regression. Overall, cyanobacteria have relatively slow growth rates and prefer warm waters for optimal growth

Fig. 7 **a** Daily mean river flow at Kinston, NC and daily mean temperature from FerryMon at the Neuse River Ferry transect. **b** Observed zeaxanthin concentration from FerryMon at the Neuse River Ferry transect and zeaxanthin concentration modeled by multiple regression of zeaxanthin on temperature and river flow. Zeaxanthin = $[0.16 + 0.035 \times \text{Temperature} - 0.0016 \times \text{Flow}]^2$. $N=141$. $R^2=0.51$. $F=73.0$, $p_{\text{Overall regression}} < 1 \times 10^{-15}$. T tests of significance of individual regression coefficients: $p_{\text{Temperature}}=3.4 \times 10^{-13}$, $p_{\text{Flow}}=4.7 \times 10^{-3}$. Data gaps in pigments, salinity, and temperature were filled as in Fig. 6



(Paerl and Huisman 2008; Reynolds 2006). The negative relationship between cyanobacterial biomass and river discharge is most likely due to the effect of decreasing residence time with increasing flow. Size-fractionated pigment data indicate that nearly all of the zeaxanthin is in the picoplankton ($<3 \mu\text{m}$) size range (Fig. 8) and microscopic examination of samples from the NRE have revealed many *Synechococcus*-like cells (Gaulke, A., personal observation). These observations confirm earlier studies showing picocyanobacteria to be an important and at times dominant fraction of phytoplankton in estuarine and coastal ecosystems spanning a range of trophic states (Marshall and Alden 1990; Murrell and Lores 2004; Philips et al. 1999). Because of their small size, picoplankton-dominated food webs tend to have much longer food chains and exhibit lower sinking fluxes than larger phytoplankton-dominated food webs (Hansen et al. 1994; Murrell and Lores 2004; Pedrós-Alió et al. 2000). Given predicted climatic changes, especially global warming, the strong

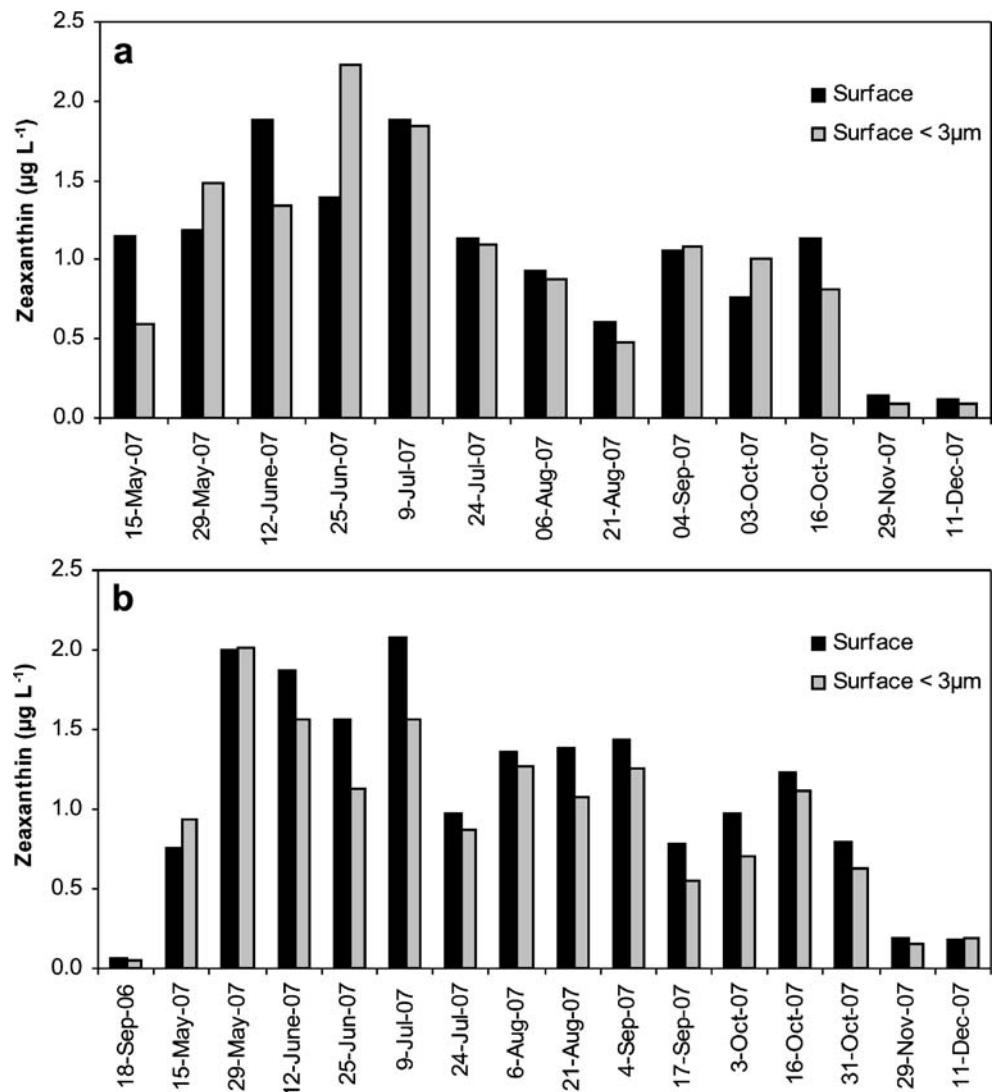
positive relationship between picocyanobacteria biomass and temperature increases will be important to follow from ecosystem dynamics and management perspectives (Paerl and Huisman 2008).

Event Scale Variability

Episodic events, such as abnormal periods of high or low freshwater discharge as well as storms, can represent important drivers of phytoplankton dynamics in estuarine ecosystems. The wet spring of 2003 is an example of how short-term hydrologic forcing can profoundly affect phytoplankton biomass and community composition in the NRE. Photopigment concentrations began to increase soon after large freshwater pulses in the spring of 2003 and continued with a rainy summer (see Figs. 1, 6, and 7).

With respect to taxonomic composition of the Chl *a* response, fast-growing cryptophytes, characterized by alloxanthin, showed an immediate, rapid response to

Fig. 8 Size-fractionated zeaxanthin concentration from surface waters of stations 180 (a) and 70 (b). The data illustrate that most of the zeaxanthin belongs in the picoplankton ($<3 \mu\text{m}$) size class



increased flow and nutrient enrichment associated with the commencement of the rainy period (Fig. 6). Then, as summer progressed and flow decreased, cyanobacteria, characterized by zeaxanthin, became a more dominant feature of the phytoplankton community (Fig. 7). These sequential differential taxonomic responses to the rainy spring–summer of 2003 demonstrate the roles flow, and hence water residence time, play in selecting for specific taxonomic responses. Fast-growing taxa like cryptomonads tend to respond to initial rapid increases in nutrient-laden flow, while slower-growing taxa that prefer warmer periods, typified by the cyanobacteria (Paerl and Huisman 2008), will tend to dominate during summer months.

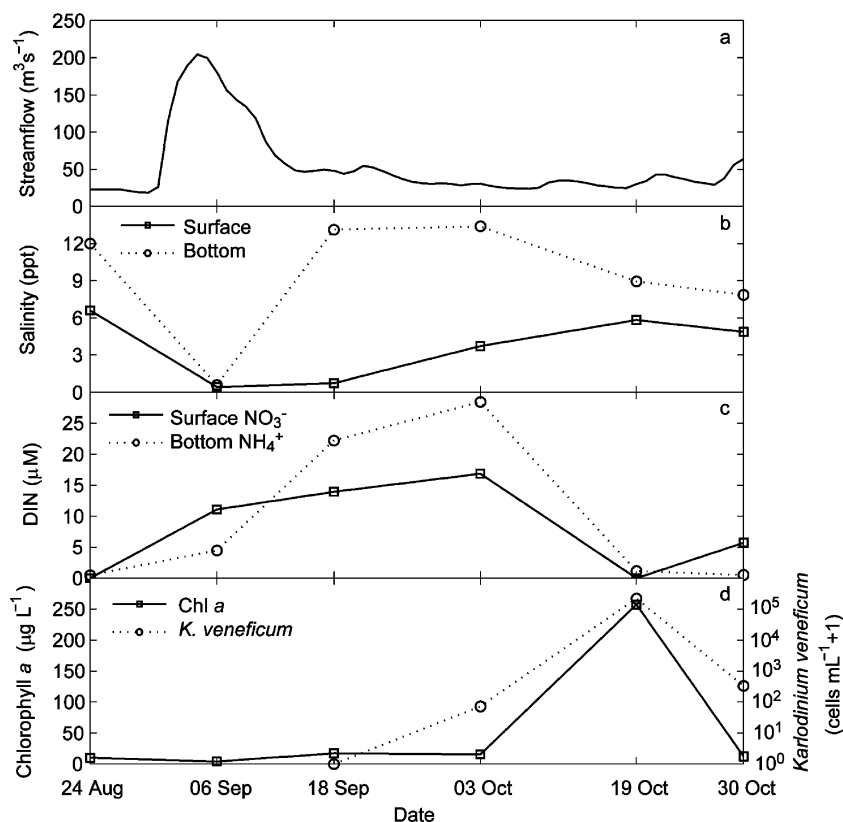
Dinoflagellates, characterized by peridinin, also showed an increase in biomass following the elevated discharge in spring of 2003 (Fig. 6). However, like the cyanobacteria, dinoflagellates have relatively slow growth rates (Reynolds 2006), which may have kept their bloom response more modest than fast-growing cryptomonads during this high flow period. Unlike the cyanobacteria, dinoflagellates prefer cooler, relatively low light conditions in conjunction with periods of elevated hydrologic discharge to the NRE (Paerl et al. 1995). Indeed, they showed more profound bloom responses in fall and winter months following periods of freshwater discharge as experienced in 2005 and 2006 (Fig. 6).

Alterations in estuarine phytoplankton biomass and community structure have also been documented in response to

the passage of other large, flood-inducing hurricanes, including Fran in 1996, Floyd, Dennis, and Irene 1999 (Paerl et al. 1998, 2001; Peierls et al. 2003), although it is not clear how representative the effects of those storms are given that storms span a continuum in terms of their characteristics (i.e., high rainfall vs. low rainfall; strong vs. light winds, etc.). Considerable effort has been put forth to understand the effects of different types of storms on phytoplankton dynamics in the NRE (Paerl et al. 2001, 2006a, b; Wetz and Paerl 2008). For instance, we found that relatively large storms such as Hurricane Isabel, which passed near the NRE as a category 2 hurricane in 2003, do not necessarily promote conditions favorable for phytoplankton growth. Isabel produced high winds (which would presumably mix nutrients from the sediments into the water column) and localized freshwater input, but nonetheless conditions became less favorable for phytoplankton growth after the storm. The potential stimulatory effects of Isabel's nutrient inputs were believed to be dampened in part by the fact that water column was already mixed and nutrient concentrations relatively high prior to the storm's passage (Paerl et al. 2006a, b; Wetz and Paerl 2008).

In contrast to Isabel which was a relative large and intense storm, but which did not elicit a phytoplankton response, several smaller storms have caused very large phytoplankton blooms. For instance, Tropical Storm Helene passed over the region in 2000 and led to a prolonged

Fig. 9 Environmental conditions leading to the development of a toxic *Karodinium veneficum* bloom. **a** Neuse River flow at Kinston, NC showing the runoff pulse from Tropical Storm Ernesto during the fall of 2006. **b** Time series of surface (0 m) and bottom (3 m) salinity at station 60 where the peak bloom biomass was observed on 19 October 2006. **c** Time series of the dominant sources of dissolved inorganic nitrogen to the bloom area; riverine NO_3^- input to the surface waters and accumulation of regenerated NH_4^+ in the bottom waters at station 60. **d** Time series of Chl *a* and *Karodinium veneficum* cell abundance at station 60



(several weeks) increase in phytoplankton biomass in the mesohaline section of the NRE (Wetz and Paerl 2008). A more recent example was the impact of Tropical Storm Ernesto, which struck the North Carolina coast during September 2006 (Hall et al. 2008). This storm generated over 30 cm of rainfall within the NRE watershed and produced a flood pulse that temporarily increased flow by a factor of six over the previously dry conditions that dominated during the summer of 2006 (Fig. 9). The freshet delivered a large infusion of inorganic nitrogen primarily as nitrate (NO_3^-). Additionally, the strong salinity-based stratification and prevailing weak winds following the freshet prevented vertical mixing (Fig. 9) and allowed anomalously high concentrations of regenerated ammonium (NH_4^+) to accumulate in the saline bottom waters (Fig. 9). The unusual high nutrient availability fueled phytoplankton growth (Fig. 9), and a short-lived frontal circulation pattern additionally concentrated vertically migrating cells. The result was an intense ($>200,000$ cells ml^{-1}) but highly localized bloom of the toxic dinoflagellate *Karlodinium veneficum* in the mid-estuarine region (Fig. 2). The collapse of the bloom coincided spatially and temporally with several fish kills for which there was no other obvious cause of mortality (Hall et al. 2008).

Conclusions

More than a decade of intensive monitoring (ModMon, FerryMon) indicates that hydrological changes accompanying climatic events occurring on multiple timescales play a fundamentally important role in determining phytoplankton biomass and community composition in the NRE. Throughout this period, the system has been impacted by anthropogenic nutrient enrichment and subsequent management efforts. Complicating the nutrient management efforts are climatic changes, which are clearly playing an increasingly important role in mediating delivery of nutrients and freshwater to the estuary, both of which have been shown to strongly modulate phytoplankton community biomass and composition in the estuary.

The most recent decade of climatically influenced phytoplankton community data has shown highly significant shifts in composition and biomass, rivaling what was observed during the more anthropogenically influenced period of the 1970s–early 1990s, where increases in nutrient loading were the key drivers of ecological change in this community. While anthropogenic stressors (nutrient over-enrichment and sedimentation) still play a fundamentally important role in shaping phytoplankton production and compositional dynamics, the impacts of these stressors have been modified by climatic changes. To clarify and ultimately predict the trophic and biogeochemical cycling

responses of estuaries to climate change, we must develop an understanding of the impacts of climate change on phytoplankton community structure and function.

Of additional concern is how potential shifts in phytoplankton community structure due to climatic changes alter habitat range. Using geographically diverse evidence, Paerl and Huisman (2008) proposed that cyanobacterial habitats have expanded northward in the Northern hemisphere due to global warming. In addition, it was shown that longer optimal growing seasons worldwide have enabled cyanobacterial species to be more competitive in a variety of habitats, including estuaries. This was attributed to the fact that, as a taxonomic group, cyanobacteria prefer higher temperatures for optimal growth than other taxa (i.e., diatoms, chlorophytes). Furthermore, changing hydrologic characteristics such as more extensive droughts would favor cyanobacterial bloom species, as they prefer long residence time conditions. Conversely, a more stormy time period, where frequent pulses of freshwater discharge dominate, would favor fast-growing taxa such as chlorophytes, cryptophytes, and diatoms. To some extent, this scenario may be unfolding in the NRE over the past decade.

Therefore, in managing (reducing) nutrient inputs to control eutrophication and minimize unwanted events, such as harmful algal blooms, parallel climatic changes need to be taken into consideration. Evidence presented here indicates that climatic changes and oscillations can at times overwhelm nutrient inputs in terms of controlling algal bloom thresholds, their duration, and spatial extent. This calls for water quality, eutrophication, and habitat models that incorporate hydrologic and other physical changes (i.e., water temperature, stratification, and circulation) in efforts to better predict phytoplankton community responses to nutrient inputs in estuarine and coastal ecosystems.

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