Impacts of inorganic nutrient enrichment on phytoplankton community structure and function in Pamlico Sound, NC, USA

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Abstract

Human activities in the watersheds of the tributaries of Pamlico Sound (PS) in North Carolina have resulted in increased riverine loading of nutrients. Pamlico Sound is a regionally important aquatic resource, and provides crucial foraging and nursery habitats for Southeast Atlantic fisheries. Changes in phytoplankton community composition that may result from increased frequency and quantity of inorganic nutrient inputs could have negative ecological effects on PS. In this study we conducted a series of nutrient bioassays to assess the relationship between increased inorganic nutrient concentration and phytoplankton community structure and function. Experiments were conducted on the native phytoplankton community of the southwest basin of PS. We utilized nutrient addition treatments and all-but-one nutrient treatments in bioassays. This allowed the comparison of the effect of adding one potentially growth-limiting nutrient (e.g. nitrogen) to adding all potentially limiting nutrients except one (e.g. all except nitrogen). Data from these bioassays indicated that the phytoplankton community in PS is primarily nitrogen (N) limited. Dissolved inorganic N concentrations in PS were relatively stable during this study. The biology of its estuarine tributaries, as has been shown for the Neuse River Estuary (NRE), acted as an effective filter for most of the nutrients transported from upstream. We found stoichiometric predictors of phytoplankton community nutrient limitations to be reliable in some instances, but inaccurate in others. Some taxa-specific responses to nutrient additions were observed, however there were no consistent patterns throughout the experiments. Results indicated that changes in the PS phytoplankton community could result from changes in nutrient regime, and changes may not be consistent across phytoplankton taxonomic groups. Unlike the NRE where pulses of riverine N have significant effects on phytoplankton community structure, the PS phytoplankton community did not appear to be subjected to these periodic N enrichments.

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Keywords: bioassay inorganic nutrient enrichment; phytoplankton community; Pamlico Sound, NC

1. Introduction

Pamlico Sound (PS) is the largest lagoonal estuary in the United States and it provides crucial foraging and nursery habitats for mid-Atlantic and southeastern US fisheries (Copeland and Gray, 1991). Nitrogen (N) has been identified as the nutrient most likely to limit primary production in PS tributaries, including the Neuse River (Paerl et al., 1995) and Pamlico River estuaries (Hobbie et al., 1972). Agricultural, urban and industrial expansion in its estuarine tributary watersheds has resulted in increased riverine loading of the limiting nutrient N (Stanley, 1988; Dodd et al., 1993). Increased N loading has been associated with eutrophication and declines in water quality (Vitousek et al., 1997), symptoms of which include harmful algal blooms, hypoxia (low $O_2$) and anoxia (no $O_2$) events, fish kills and trophic disruption (Rosenberg, 1985; Paerl, 1997; Paerl et al., 1998; Boesch et al., 2001). A mechanistic understanding
of eutrophication in the estuarine tributaries of Pamlico Sound through experimental manipulations has been explored at great depth (Paerl and Bowles, 1987; Rudek et al., 1991; Paerl et al., 1995; Piehler et al., 2002). However, little research has been conducted in PS to determine the relationship between the major drivers (inorganic nutrients) and end products (carbon in phytoplankton) of eutrophication.

There is some evidence that chlorophyll a concentrations near the mouth of the Neuse River have increased in the past 10 years (Paerl et al., in preparation). However, sufficient data do not exist to establish a cause and effect relationship between nutrient inputs and responses in phytoplankton production in PS. Recent research in PS following three hurricanes in 1999 demonstrated that in extreme flow conditions PS is subjected to elevated nutrient concentrations and ensuing elevated phytoplankton biomass (Paerl et al., 2000, 2001). Because hurricane intensity and frequency may increase in the near future (Goldenberg et al., 2001), the input of considerable nutrients to PS in freshwater runoff may require more serious consideration.

Changes in phytoplankton community composition that may result from increased frequency and quantity of inorganic nutrient inputs could have negative ecological effects in PS. Harmful and nuisance algal blooms, bottom water hypoxia/anoxia, alterations in the quality of phytoplankton and zooplankton as a food source for higher trophic levels, and declining fisheries are all potential consequences of major shifts in phytoplankton community composition. Some evidence of these symptoms was found following the hurricanes in 1999 (Paerl et al., 2000, 2001), however direct relationships between a changing nutrient input regime and phytoplankton community composition have yet to be established and tested with manipulative experiments in PS.

In this study, we conducted a series of nutrient manipulation bioassays to assess the relationship of increased inorganic nutrient concentration and phytoplankton community structure and function. Experiments were conducted on the native phytoplankton community of the southwest basin of PS. We utilized nutrient addition treatments (Paerl and Bowles, 1987) and all-but-one treatments (Twomey and Thompson, 2001) in tandem to compare the effect of adding a potentially growth-limiting nutrient to adding all potentially limiting nutrients except one. These data provided a more rigorous assessment of phytoplankton nutrient relationships than a single type of treatment alone, and perhaps also allowed for a more comprehensive determination of the nutrient(s) that limit phytoplankton productivity and biomass. Data from bioassay experiments and monitoring were used to test the following hypotheses. First, we proposed that phytoplankton primary productivity and biomass accumulation were directly related to availability of inorganic N in PS. Second, we hypothesized that varying nutrient concentrations would change phytoplankton community composition in PS. Finally, we used bioassay data to compare nutrient limitation inferred from ambient nutrient stoichiometry (e.g. DIN/DIP) to phytoplankton responses to nutrient manipulations.

2. Methods

2.1. Site description

Pamlico Sound (PS) is a 4350-km² coastal lagoon in the southeastern United States that receives surface water inputs from multiple sources. Riverine sources include the Neuse and Tar-Pamlico Rivers, and there is also input to PS from Albemarle Sound. Pamlico Sound has a relatively long residence time because it is bounded by the Outer Banks. Since late fall 1999, a water quality monitoring program has been in place in PS that has included monthly measurements of physical, chemical, and biological factors (Peierls et al., 2003).

2.2. Experimental design

Experiments were performed during October 2000, January 2001, April 2001 and July 2001 to assess representative nutrient and phytoplankton community regimes from each season. Water was collected using a non-destructive diaphragm pump in 20-l carboys from 0.5 m below the surface in the southwest basin of PS (Fig. 1) and transported in the dark to the UNC-CH Institute of Marine Sciences (IMS). The water samples were in transit for approximately 2 h. The all-but-one bioassays were based on a modification of the method described by Twomey and Thompson (2001). Treatments included additions of nitrogen, phosphorus, silica, vitamins and trace metals (Table 1) based on the recipe of Harrison et al. (1980). Concentrations were designed to be high enough to saturate initial growth rates, and thus were higher concentrations than the addition bioassays described below. Four treatments were prepared for the all-but-one bioassays: ALL-N, which included all nutrients with the exception of N; ALL-P, which included all of the nutrients with the exception of PO₄³⁻; ALL, which had the full nutrient compliment added; and a control with unamended PS water. To assess the relationship of phytoplankton community structure and growth with nutrient availability, the growth of the control was compared to growth of the ALL treatment. If the ALL treatment produced higher biomass, faster growth or increased productivity than the control, then it was assumed that one or more of the nutrients were limiting on that particular day. If the control and ALL treatment showed similar growth, then it was unlikely that the phytoplankton community was nutrient limited. When the potential for nutrient limitation was detected,
comparisons of the growth responses of the major treatments with those of the control and the ALL treatments identified the particular nutrient or combinations of nutrients most likely responsible for nutrient limitation. For example, to test for phosphorus limitation, all nutrients were added except for PO$_4^{3-}$. If the treatment growth and biomass were high, approaching the levels of the ALL treatment, then PO$_4^{3-}$ was replete in the sample and was therefore unlikely to limit phytoplankton growth. If the treatment growth and biomass accumulation were low, similar to the control, then it was likely that PO$_4^{3-}$ was limiting phytoplankton growth. Three additional treatments included the addition of 20 μM inorganic N as NO$_3$ (N), 5 μM PO$_4^{3-}$ (P) and N and P combined (NP). These concentrations were selected following historical data analysis of the nutrient concentrations in the NRE and were an approximation of the concentrations seen in the estuary following a riverine discharge event.

Four replicates of each treatment were incubated in 10-l Cubitainers (Hedwin Corp., Baltimore, MD) that are chemically inert and transmit 85% of incident photosynthetically active radiation (PAR) (Paerl, 1987). Cubitainers were placed outside the IMS in holding ponds filled with water from Bogue Sound for temperature and irradiance control and incubated for 84 h. Neutral density screening was used to prevent photoinhibition when ambient irradiance was above 800 μmol quanta m$^{-2}$ s$^{-1}$. Pinckney et al. (1999) found 48 h a sufficient incubation period to detect effects of nutrient addition treatments in NRE phytoplankton communities. We utilized an 84 h incubation to ensure sufficient time for the growth of slower growing phytoplankton that could be present in PS. Cubitainers were inverted twice daily to minimize settling of biomass. Our design did not account for differential top-down controls in treatments. However, because we had a relatively short incubation time we believe that differential grazing effects were unlikely.

### 2.3. Analytical

The chlorophyll $a$ concentration was measured using the non-acidification fluorometric method (Welschmeyer, 1994). Fifty milliliter sub-samples were filtered onto 25 mm Whatman GF/F glass microfiber filters (0.8 μm nominal pore size) and stored at $-20^\circ$C.

**Table 1**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Compound</th>
<th>Concentration (μM)</th>
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</thead>
<tbody>
<tr>
<td>Nitrogen</td>
<td>NaNO$_3$</td>
<td>137</td>
</tr>
<tr>
<td>Phosphate</td>
<td>Na$_3$HPO$_4$·7H$_2$O</td>
<td>21.8</td>
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<tr>
<td>Silicate</td>
<td>Na$_2$SiO$_3$·9H$_2$O</td>
<td>105.6</td>
</tr>
<tr>
<td>Iron</td>
<td>FeCl$_3$·6H$_2$O</td>
<td>6.56</td>
</tr>
<tr>
<td>Chelator (CH$_3$N(CH$_2$COOH)CH$_2$COONa)$_2$·2H$_2$O</td>
<td>6.56</td>
<td></td>
</tr>
<tr>
<td>Trace metals</td>
<td>MnSO$_4$·H$_2$O</td>
<td>2.42</td>
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<tr>
<td>CoSO$_4$·7H$_2$O</td>
<td>0.0569</td>
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</tr>
<tr>
<td>ZnSO$_4$·7H$_2$O</td>
<td>0.254</td>
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</tr>
<tr>
<td>Na$_2$MoO$_4$·2H$_2$O</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>NiCl$_2$·6H$_2$O</td>
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<td></td>
</tr>
<tr>
<td>NaSeO$_3$</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Chelator (CH$_3$N(CH$_2$COOH)CH$_2$COONa)$_2$·2H$_2$O</td>
<td>8.3</td>
<td></td>
</tr>
<tr>
<td>Vitamins</td>
<td>B1 (Thiamine HCl)</td>
<td>0.297</td>
</tr>
<tr>
<td></td>
<td>B12 (Cyanocobalamin)</td>
<td>0.0015</td>
</tr>
<tr>
<td></td>
<td>B6 (Biotin)</td>
<td>0.0041</td>
</tr>
</tbody>
</table>

Chelator appears twice because it was added both with the iron addition and with the trace metal addition.
Ten milliliters of acetone (90% acetone, 10% DI) was added to filters in polypropylene centrifuge tubes. Filters in tubes were then sonicated on ice for 30 s and samples were extracted for approximately 12 h at -20 °C.

Dinitrogen (N₂) fixation (nitrogenase activity) was estimated using the acetylene reduction assay (Stewart et al., 1967). N₂ fixation was measured because we know from previous work that N₂ fixers can be active in the Neuse River Estuary periodically in late summer, and when present can affect N cycling (Piehler et al., 2002). Fifty milliliter aliquots of sample water were added to 72-ml serum vials that were then capped with flanged rubber stoppers. Acetylene was generated by adding CaC₂ to deionized water (DI) and was injected into the headspace of the vial (~20% of the total volume). Serum vials were incubated in situ, in a rack at the water surface in the pond at IMS, for 4 h under one layer of neutral density screening (ambient PAR was reduced by about 50%). Following the incubation, 5-ml headspace sub-samples were transferred to evacuated 4-ml blood collection vials for storage. Sub-samples were transferred to 2-ml evacuated autosampler vials for gas chromatographic analysis of ethylene using a Shimadzu GC 9A gas chromatograph equipped with a flame ionization detector (FID). The GC was fitted with a 2-m stainless steel Poropak T column held at 80 °C with a relative value of the total chlorophyll a.

Phytoplankton community composition was determined using diagnostic photopigment analyses (Millie et al., 1993; Tester et al., 1995; Jeffrey et al., 1997). Samples (100 ml) were collected from Cubitainers at specified time intervals, filtered onto 25 mm Whatman GF/F filters, and frozen (~80 °C). Photopigments were extracted using a 90% aqueous acetone solvent and sonication. High performance liquid chromatography (HPLC) was used to quantify the relative biomass of algal groups (cyanobacteria, diatoms, chlorophytes, cryptophytes and dinoflagellates) in the phytoplankton community based on biomarker photopigment (chemosystematic chlorophylls and carotenoids) concentrations. An in-line photodiode array spectrophotometer (PDAS, Shimadzu SPD M10av) provided identification of individual photopigments based on characteristic absorption spectra (380–700 nm) (Rowan, 1989). Pigment concentrations from HPLC were analyzed using CHEMTAX to calculate the relative abundances of the major phytoplankton groups (Mackey et al., 1997). CHEMTAX is a matrix factorization program for calculating abundances of algal classes from concentrations of chemosystematic marker photopigments (chlorophylls and carotenoids) (Mackey et al., 1996, 1997; Wright et al., 1996; Pinckney et al., 1998). The resulting class composition matrix was expressed as a relative value of the total chlorophyll a.

Sub-samples of selected treatments were collected for microscopic counts to corroborate the phytoplankton taxonomic group data from HPLC. Lugol’s solution was used to preserve 50 ml samples in polypropylene bottles. Phytoplankton were counted under an inverted phase contrast microscope (Wild) using the Utermöhl technique (Utermöhl, 1958) with PhycoTech counting chambers.

Water samples for dissolved inorganic nutrient analyses were gently filtered through 25 mm diameter Whatman GF/F filters. Nitrate + nitrite (NO₃⁻ + NO₂⁻), ammonium (NH₄⁺), orthophosphate (PO₄³⁻) and dissolved silica (DSi) concentrations were determined with a Lachat Quick-chem 8000 auto-analyzer (Lachat, Milwaukee, WI) using standard protocols (Lachat Quik-chem methods 31-107-04-1-C, 31-107-06-1-A, and 31-115-01-3-C, respectively). The limits of detection were 0.26 μM for NO₃⁻ + NO₂⁻, 0.31 μM for NH₄⁺, 0.02 μM for PO₄³⁻ and 1.24 μM for DSi.

2.4. Ambient physical, chemical and biological conditions

Bioassays were run concurrently with a water-quality monitoring program in PS (see Peierls et al., 2003). Physical, chemical and biological data were collected from PS monthly. Vertical profiles of temperature, salinity and dissolved oxygen were obtained using a YSI 6600 sonde (YSI Inc., Ohio). Both surface and
bottom water samples were collected at each station for analysis of nutrient concentrations and chlorophyll a concentrations (procedures were the same as described above). Portions of these data are included to provide an environmental context for the bioassays described here.

2.5. Data analysis

Community responses were analyzed using a one-way analysis of variance (ANOVA). Data were natural log transformed when necessary to satisfy the normality assumption. Post-hoc comparisons of means were made using a Bonferroni multiple comparisons of means (Moore and McCabe, 1993).

3. Results

3.1. Background field data

During the period of this study, dissolved inorganic nutrient concentrations and chlorophyll a concentrations were measured monthly at the site from which samples were obtained for these bioassays. Data are presented for surface (0–0.1 m) and bottom waters (0.5 m above the bottom) from monthly monitoring, and for surface water (0.5 m) only for samples taken at the time of the experiments (Fig. 2). Chlorophyll a values ranged from 3 to 15 μg l⁻¹ for the period of this study. The chlorophyll a concentrations at the time of the experiments were generally in the low end of this range and were similar to the monthly monitoring data during those times (Fig. 2A). Nitrate concentrations were also quite low (<1 μM) and showed little difference between surface and bottom values (Fig. 2B). Nitrate concentrations were somewhat higher in the late fall through early spring than the rest of the year. Phosphate concentrations were always below 1 μM, and were highest in September 2000 and June 2001 (Fig. 2C). Ammonium concentrations were generally low in both bottom and surface waters throughout the period of the experiments. Concentrations of ammonium ranged from 0.5 to 1.5 μM throughout the study (Fig. 2D), with the exception of July 2001 when the ammonium concentration was nearly 8 μM in the bottom water. At this time point, the water column was highly stratified and the bottom water was hypoxic (dissolved oxygen ~1.5 mg/l) indicating that the high NH₄⁺ concentration was likely due to a build up of NH₄⁺ released from the sediments. Silica concentrations ranged from 5 to 70 μM during the study (Fig. 2E).

3.2. Bioassays

3.2.1. Fall 2000 bioassay

The October 2000 bioassay occurred during a period of relatively low overall dissolved inorganic nutrient concentrations and a very low DIN/DIP (Fig. 2). Primary productivity in the ALL and the NP addition treatments was significantly greater than the control, indicating potential nutrient limitation (P < 0.05, Fig. 3A). Primary productivity rates were stimulated by N to levels significantly greater than the control by treatments that included N (Fig. 3A). Rates of primary productivity in the ALL-P treatment were greater than the control but less than the ALL treatment. Primary productivity in the NP treatment was significantly higher than the N addition alone. There was, however, no evidence of a difference in primary productivity to the P addition compared with the control. The pattern of treatment response was identical in the chlorophyll a measurements for the experiment (Fig. 4A). The phytoplankton taxonomic group biomass response to the treatments relative to the control is detailed in Table 2. The ALL treatment increased the relative percentages of chlorophytes and diatoms compared to the control (Fig. 5, Table 2). The N treatment caused increases in the range of 12–28% in the proportion of diatoms in the total phytoplankton community relative to the control (Fig. 5). The increase in the proportion of diatoms appeared to be at the expense of cyanobacteria, which decreased between 7 and 28% relative to the control in treatments with added N (Fig. 5). However, the decline in cyanobacterial contribution to overall phytoplankton biomass was not statistically significant (Table 2). Dinoflagellates were a very small proportion of the overall population, but decreased significantly in several of the treatments (Table 2).

3.2.2. Winter 2000/01 bioassay

DIN/DIP was higher in the January 2001 experiment than in October 2000, but dissolved inorganic nutrient concentrations were still low (Fig. 2). Increases in primary productivity relative to the control only occurred in treatments that included N. Once again the ALL-P treatment was significantly greater than the control, but the NP treatment was not higher than the N treatment in this experiment (Fig. 3B). Chlorophyll a responses to treatments were similar to those seen in primary productivity (Fig. 4B). In the NP treatment, the proportion of cyanobacteria declined relative to the control (between 2 and 13%), whereas the proportion of diatoms increased relative to the control (between 2 and 17%, Fig. 5). Both the reduction in cyanobacterial contribution to the phytoplankton community and the increase in the contribution of diatoms with the NP treatment were statistically significant (Table 2).

3.2.3. Spring 2001 bioassay

The April 2001 bioassay was conducted during another period of low inorganic nutrient concentrations, but the DIN/DIP with a lower bound of 46 was much...
higher than in any of the other experiments (Fig. 2). The response of primary productivity was an increase relative to the control in treatments that included N (Fig. 3C). Primary productivity in the ALL-P was significantly lower than in the ALL treatment, while the N and NP treatments were not significantly different (Fig. 3C). The response of chlorophyll \(a\) to the treatments was again similar to that observed with primary productivity, except chlorophyll \(a\) in the NP treatment was higher than in the N treatment (Fig. 4C). The proportion of chlorophytes increased in the ALL, N and NP treatments compared to the proportions in the control (Fig. 5, Table 2). There was little other evidence of phytoplankton community shifts in response to bioassay treatments, except for the N and NP treatment, which caused a decrease in the proportion of cyanobacteria relative to the control and the NP treatment increasing the proportion of diatoms (Fig. 5, Table 2).

3.2.4. Summer 2001 bioassay

In July 2001 inorganic nitrogen was present at concentrations similar to those measured in January 2001, but the DIN/DIP molar ratio was considerably lower (3.7) (Fig. 2). Responses of primary productivity and chlorophyll \(a\) to nutrient treatments were similar in pattern to those in the other experiments, but with fewer statistically significant differences. There was no significant stimulation above the control by any treatment other than the ALL treatment (Figs. 3D and 4D). Diatoms were stimulated in the ALL treatment and increased their percent contribution to overall phytoplankton biomass in the ALL treatment (Fig. 5, Table 2). Many treatments decreased the contribution of specific taxonomic groups to overall phytoplankton biomass (Table 2). Chlorophytes and dinoflagellates decreased in the ALL treatments (Table 2), apparently being replaced by diatoms.

Fig. 2. Ambient dissolved inorganic nutrient concentrations and chlorophyll \(a\) concentrations throughout the study period. Nutrient concentrations are \(\mu\)M and chlorophyll \(a\) concentrations are \(\mu\)g l\(^{-1}\). circles are bottom water, triangles are surface water and stars are concentrations at the time of the four bioassays. Samples that were below the detection limit are presented as the detection limit concentration. The limits of detection were 0.26 \(\mu\)M for NO\(_3\) + NO\(_2\), 0.31 \(\mu\)M for NH\(_4\), 0.02 \(\mu\)M for PO\(_4^{3-}\) and 1.24 \(\mu\)M for dissolved silica. Data presented are chlorophyll \(a\) (A), nitrate (B), phosphate (C), ammonium (D), and dissolved silica (E).
Microscopic phytoplankton surveys confirmed the general patterns of phytoplankton taxonomic group abundance identified by HPLC in the four experiments. The acetylene reduction assay was conducted during each of the bioassays and never detected nitrogenase activity. Pico-coccoid types dominated the cyanobacterial community, and there was no evidence of N₂ fixing cyanobacteria identified by microscopy.

4. Discussion

Identifying the factors that limit phytoplankton primary productivity is an early goal in assessing potential impacts of changes in nutrient regimes on a body of water. In situ nutrient bioassays have been used extensively to try to provide this information (D’Elia et al., 1986; Paerl and Bowles, 1987; Sanders et al., 1987). Results from nutrient addition bioassays show the response of the native phytoplankton to identifiable changes in nutrient conditions, but generalizations from the experiments must be drawn with significant consideration (Hecky and Kilham, 1988).

Bioassays are often conducted on a relatively small scale, and it has been argued that the responses observed in them can have little to do with the overall ecology of the system in which they are conducted (Carpenter, 1996). There is also the issue of whether stimulation of primary productivity or biomass accumulation in response to addition of a nutrient is sufficient evidence that the nutrient limits primary productivity or biomass accumulation. In order to limit these concerns, we attempted to interpret our data within the context of knowledge of the overall system, and determined the response of the phytoplankton community to two different types of nutrient manipulations. Because we measured the response of the phytoplankton community to additions of nutrients likely to limit productivity and biomass and to the addition of all of the potentially growth-limiting nutrients except for one, we believe that when the responses were consistent, the evidence of the relationship between nutrients and phytoplankton was stronger.

Primary productivity provided an instantaneous assessment of the effects of the treatments on phytoplankton community function, while chlorophyll a concentrations were a cumulative measure of treatment...
effects on community size. Photopigment data offered the most detail about changes in community structure resulting from the bioassay treatments. There were some general patterns among the responses to treatments in the experiments. Primary productivity, chlorophyll \(a\) concentration and phytoplankton taxonomic group distribution responded similarly to the bioassay treatments. Treatments that included N almost always stimulated productivity and chlorophyll \(a\), however P additions alone never stimulated either productivity or chlorophyll \(a\). These results indicated that DIN concentration (i.e. availability) exerted the strongest control on phytoplankton productivity and biomass accumulation. Additionally, when DIN was added productivity and biomass generally increased and when all growth-limiting nutrients except N were added (ALL-N treatment) there was generally no change in productivity and biomass. There were several occasions when the bioassay responses to ALL treatments were higher than the response to NP. There are several explanations for the larger response to the ALL treatment. Among them are the fact that the concentrations of N and P were higher in the ALL treatment than in the NP treatment and the fact that the ALL treatment also included dissolved silica, vitamins and trace metals. In our study, DSi concentrations always exceeded 5 \(\mu M\) and DSi/DIN

<table>
<thead>
<tr>
<th>Table 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments shown caused significant increases in the taxonomic group listed relative to the control (Bonferroni, (P &lt; 0.05))</td>
</tr>
<tr>
<td>October-00</td>
</tr>
<tr>
<td>Chlorophytes</td>
</tr>
<tr>
<td>Cryptophytes</td>
</tr>
<tr>
<td>Cyanobacteria</td>
</tr>
<tr>
<td>Diatoms</td>
</tr>
<tr>
<td>Dinoflagellates</td>
</tr>
</tbody>
</table>

Parenthetic signs (+ or −) indicate whether the treatment increased or decreased the relative contribution of the taxonomic group to the overall phytoplankton. Data are from HPLC photopigment analysis processed with the CHEMTAX matrix factorization program. The treatments were ALL (A), ALL-N (A−N), ALL-P (A−P), nitrogen (N), phosphorus (P) and nitrogen & phosphorus (N+P).

Fig. 5. Percent composition of the phytoplankton community by taxonomic group using HPLC photopigment data processed with the CHEMTAX matrix factorization program. Data shown are from the fall (A), winter (B), spring (C) and summer (D) experiments, respectively. Bars are the mean of four replicates.
ratios were always greater than 4. Diatom growth in marine waters is likely to be limited by dissolved silica (DSi) when DSi/DIN ratios are less than 1 (Redfield et al., 1963; Brzezinski, 1985) and DSi concentrations when DSi/DIN ratios are less than 2 μM (Dortch and Whitledge, 1992). While we generally argue that stoichiometry may provide erroneous determination of the nutrient that limits phytoplankton productivity and growth, DSi and DSi/DIN are much greater than values that would be indicative of potential Si limitation. This indicates that silica was not likely to have limited diatom growth in the southwestern basin of PS during this study. In addition, a single bioassay experiment using water collected from the Neuse River Estuary (the largest tributary of the southwest basin of the PS) found no effect of DSi additions (Hall, unpublished data).

Nitrogen limitation of phytoplankton growth is common in coastal systems (Nixon, 1986) and this appeared to also be the case in PS. In fact, an early study in PS stated phytoplankton primary productivity was primarily N limited, followed by N and P dual limitation (Woods, 1967), though the methods used to make these determinations were not clear. Dugdale (1967) and Ryther and Dunstan (1971) were among the first to elaborate on coastal N limitation. Because the NRE phytoplankton community is primarily N limited (Paerl and Bowles, 1987; Rudek et al., 1991; Boyer et al., 1994; Pinckney et al., 1999) the determination of phytoplankton N limitation in PS nutrient bioassays is not surprising. Nitrogen limitation of phytoplankton biomass accumulation has also been reported in the higher salinity portions of Chesapeake Bay, which is similar to PS in morphology and scale (Fisher et al., 1999).

The possibility of secondary limitation by P of primary productivity and biomass accumulation by phytoplankton in PS was raised by the responses in some of the bioassays. There were a few instances in which responses to treatments that included N and P were higher than N alone. Additionally, the ALL-P treatment also had consistently lower responses than the ALL treatment. It is likely that the nutrient manipulations drove the phytoplankton community to be secondarily limited by P during the incubation. What these data mean for the functioning of the ecosystem is less clear. Because the PS lies downstream of the Neuse River Estuary it is less likely to see pulses of DIN. If, however, a large pulse did occur (such as was seen during the hurricanes of 1999; Paerl et al., 2001), it is possible that P could become secondarily limiting to phytoplankton productivity and biomass accumulation. Overall however, data from this study indicate that N is the primary, and often sole, limiting nutrient for phytoplankton productivity and biomass accumulation in PS.

Because the NRE is more prone to large pulses of nutrients from the watershed during the relatively wet spring runoff period and large summer-fall tropical storm events, the seasonal relationship between phytoplankton community structure and function and nutrient levels in the NRE is likely different from that in the PS. In the spring bioassay, the ambient nutrient conditions in PS closely resembled those seen at other times of the year and did not appear to be affected by the elevated riverine spring load. Apparently, there was enough biological uptake and removal of N through the river and estuary to buffer the sound from large pulses of N the vast majority of the time. In the NRE, N laden spring runoff has been shown to affect phytoplankton nutrient limitation and community composition (Pinckney et al., 1999). The absence of large seasonal pulses of DIN during this study appeared to further strengthen N limitation of phytoplankton in PS.

Predicting phytoplankton dynamics and devising management strategies in estuarine and coastal waters often involves using stoichiometric relationships of inorganic nutrient concentrations. The N/P ratio has long been used as a predictor of phytoplankton nutrient limitation in aquatic ecosystems (Redfield, 1958). In certain systems, N/P ratios have been found to be good predictors of the level of dominance of types of phytoplankton such as cyanobacteria (Smith, 1990). In these bioassays, the pre-experiment ambient DIN/DIP ratios were most often low, and predicted the N limitation of the phytoplankton community that was observed in the bioassays. The bioassay responses in the April 2001 experiment indicated N limitation, but stoichiometry would have predicted P limitation since the DIN/DIP ratio of the water collected for the experiment (46) was much higher than the Redfield ratio (16) (Redfield, 1958). Luxury uptake of PO₄³⁻ prior to water collection for the experiment could have provided necessary intracellular P for several rounds of cell division, decoupling P sufficiency in the 4-day experiment from ambient DIP concentrations (Malone, 1980). Alternatively, the phytoplankton may have been utilizing an organic source of P rather than using α-PO₄³⁻ as their main P source. In marine systems, the concentration of dissolved organic forms of P often greatly exceeds the concentrations of DIP (Bjorkman and Karl, 1994; Ammerman et al., 1994; Michaels et al., 1996). There is a growing awareness that most if not all phytoplankton have the ability to produce alkaline phosphatase enzymes (Scanlan and Wilson, 1999), which cleave phospho-ester bonds of organic P molecules, freeing DIP for transport through the phytoplankton cell membrane (Bjorkman and Karl, 1994). Alkaline phosphatase production is stimulated in response to low DIP concentrations (Scanlan and Wilson, 1999). It is very possible that the very low DIP concentrations in this experiment stimulated the production of alkaline phosphatase allowing the phytoplankton to utilize DOP thus alleviating P limitation.
Because ambient nutrient concentrations are measures of the residual nutrients after biological activity, they may not reflect phytoplankton nutrient limitation in the system. Analyses of historic nutrient conditions in the NRE have provided detailed accounting of changes in N/P ratios and offered them as potential predictors of phytoplankton nutrient relationships (Qian et al., 2000). Our data indicate these relationships may not be predictable in PS, and support the need for a combination of approaches to determine nutrient limitation of phytoplankton in coastal waters (Granéli et al., 1990). Factors that confound determining a direct and generalizable relationship between phytoplankton productivity and biomass accumulation and water column nutrient conditions include exchange of nutrients between the sediments and the water column, denitrification, N₂ fixation, variable residence time and stored pools of nutrients in phytoplankton. All of these factors can vary enormously, both within single estuaries and between different estuaries. Geider and LaRoche (2002) suggested that variations in the N/P ratio at which phytoplankton become limited by either N or P are likely to occur in marine waters.

These results confirm that when supplied with limiting nutrients, some phytoplankton taxa have the ability to increase their relative contribution to total community biomass at the expense of others. There was some evidence of seasonal variability in phytoplankton taxa response to variability in nutrient supply. The greatest change resulting from experimental additions was observed in the relative contribution of diatoms and cyanobacteria to the total phytoplankton community biomass. Several studies have suggested that diatoms grow faster and have a higher affinity for nutrients than other phytoplankton taxa under nutrient replete conditions (Parsons et al., 1978; Sanders et al., 1987; Harding, 1994). This view is consistent with our results during summer, autumn and winter, which showed that when N was limiting, the relative concentration of diatoms increased in treatments with added N. The relatively low proportion of diatoms in spring compared with the rest of the year was probably caused by competitive exclusion by cyanobacteria. Cyanobacteria often compete well in warmer water temperatures and lower N concentrations (Andersson et al., 1994). The high initial biomass of cyanobacteria compared with the other phytoplankton during spring was expected given that their biomass is greatest in the major tributary, the NRE, during spring each year (Pinckney et al., 1998). N₂ fixation was not observed in any of the bioassays, indicating the cyanobacteria in this experiment were not gaining a competitive advantage by obtaining atmospheric N. Dinoflagellates showed little response to the various bioassay treatments. It was most likely that dinoflagellates were competitively excluded by other taxa during nutrient replete conditions due to their slower growth rates and higher nutrient affinities, which have been observed in diverse studies (Chan, 1980; Levasseur et al., 1984; Tang, 1996). Alternatively, these results may be a consequence of the difficulty associated with growing dinoflagellates out of their native habitat (Guillard and Keller, 1984).

Nutrient pollution resulting from human activities is largely responsible for the alteration of trophic status of diverse aquatic ecosystems (Vollenweider, 1982; Smol et al., 1991; Nixon, 1995; Boesche et al., 2001). Solutions to control excessive phytoplankton carbon loading that is driven by excessive nutrient supplies are being investigated and implemented in regions throughout the world (Vollenweider, 1982; Elmgren and Larsson, 2001; Mitsch et al., 2001). In the NRE deterioration of water quality has prompted a mandated nutrient management strategy intended to halt the decline and provide eventual improvement in water quality. Management plans for the NRE include a nitrogen loading cap and an overall 30% reduction in N loading. Our results suggest that if nutrient re-mediation schemes alter the N and P dynamics of PS, changes to the relative proportion of taxa in the phytoplankton community might result. The ecological consequences of a taxonomic shift could be beneficial if palatable organisms proliferate, thereby ensuring trophic transfer of nutrients through the food chain into higher order, commercially valuable organisms. Alternatively, if harmful organisms proliferate, reduced trophic transfer, large aggregations of nuisance organisms, oxygen depletion and loss of biodiversity could result. Cyanobacteria are favored when the concentration of N is low, while diatoms are favored when N is available at higher concentrations. However, historical research has shown that when N and P are at concentrations high enough to saturate phytoplankton growth rates, nuisance blooms are likely to occur (Paerl, 1988; Richardson, 1997). Thus, the challenge for natural resource managers is to achieve a balance, where N and P are supplied at rates and concentrations which stimulate production of useful algal species, while preventing excessive C loading or production of ecologically harmful species.

The PS phytoplankton community has received attention recently because of the interest in determining its response to large climatic perturbations (e.g. tropical storms) that have recently occurred (Paerl et al., 2000, 2001). Hurricanes can cause enormous hydrologic extremes that transport upstream freshwater to areas in the sound that would not normally receive this type of discharge. These investigators found a generally bio-stimulatory effect of hurricane related discharge on PS phytoplankton, likely attributable to riverine DIN transport to PS. Following the hurricane related studies, questions persisted about the relationship of nutrients and phytoplankton during more normal hydrologic conditions. Data from bioassays in this study indicate...
that the phytoplankton community in PS is primarily N limited. Secondary P limitation was occasionally possible in the bioassays, however it most often occurred after a period of incubation with N levels much higher than those normally seen in PS. Nitrogen concentrations in PS were relatively stable during this study; the biology of the NRE apparently acted as an effective filter for riverine nitrogen transported from upstream (Kennedy, 1984). We found stoichiometric predictors of phytoplankton community nutrient limitations to be reliable in some instances, but inaccurate in others. Some taxaspecific responses to nutrient additions were observed, however predictable patterns were not evident throughout the experiments. These results indicate that changes in the PS nutrient regime could affect the phytoplankton community, and these changes may not be consistent throughout phytoplankton taxonomic groups.

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