Use of long-term data and multivariate ordination techniques to identify environmental factors governing estuarine phytoplankton species dynamics

by

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Abstract

A continuous, 13-yr record of environmental data and phytoplankton species and assemblage structure in the mesohaline Neuse River Estuary (biweekly, April–October; monthly, November–March) was used to evaluate phytoplankton assemblage responses to changing environmental conditions. Ordination techniques including non-metric multidimensional scaling (NMDS), indicator species analysis, and BIO-ENV software were used to investigate potential environmental predictors of phytoplankton assemblage patterns under chronic eutrophication. Phytoplankton assemblages were strongly related to temperature and total nitrogen : total phosphorus ratios, with expected seasonal changes in species composition. Inter-annual changes in river discharge influenced whether phytoplankton assemblages were dominated by diatoms and phototrophic flagellates, or by mixotrophic and heterotrophic dinoflagellates. Increasing ammonium concentrations also have been an important influence on phytoplankton assemblages. Raphidophytes (including the potentially toxic species *Heterosigma akashiwo*), haptophytes, chlorophytes, and the bloom-forming dinoflagellate *Heterocapsa rotundata* increased in more recent years (2000-2006), concomitant with increasing ammonium concentrations. Abundance of the potentially toxic dinoflagellates *Prorocentrum minimum*, and the grouping *Pfiesteria* spp., “pfiesteria-like” dinoflagellates, and *Karlodinium veneficum* remained stationary over time and rarely exceeded $10^3$ cells mL$^{-1}$. Abundance of *P. minimum* was positively related to dissolved organic nitrogen and suspended solids concentrations, whereas the highest abundance of the grouping *Pfiesteria* spp., “pfiesteria-like” dinoflagellates and *K. veneficum* occurred during summer and fall, related to high total phosphorus concentrations, temperature, and salinity. Overall, this study provides new species-level insights to advance understanding about anthropogenic influences on phytoplankton assemblages. The data suggest an increasingly
important role of ammonium in controlling phytoplankton assemblage structure, including increased abundance of some harmful species, in eutrophic estuaries.

**Introduction**

Cultural eutrophication, the nutrient over-enrichment of surface waters by human activity, is a major environmental problem in estuaries worldwide (National Research Council 2000). Stimulation of noxious and toxic algae (harmful algal blooms, or HABs) is among the most pernicious effects of chronic eutrophication. Environmental damage caused by HABs includes mass mortalities of wild and farmed fish and shellfish, human illness and death from toxin-contaminated seafood or from toxin exposure through inhalation or water contact, and illness and death of various invertebrates, seabirds and marine mammals (Burkholder 1998). Of particular concern in eutrophic estuaries is the potential relationship between the frequency of HABs and increased anthropogenic nutrient inputs to coastal waters (Glibert et al. 2005).

Previous studies have established that phytoplankton assemblages in affected systems respond to excessive nutrient inputs with increased biomass and decreased diversity, and that nutrient enrichment generally promotes a shift in dominance from diatoms to filamentous green algae, flagellates and cyanobacteria, some of which can be toxic to fish and wildlife (Burkholder 2000). Other research has shown that variations in nutrient loadings and supply ratios can regulate algal growth rates and community composition (Rudek et al. 1991; Burkholder 2000). Despite many investigative efforts to understand the factors regulating phytoplankton assemblage composition and bloom formation, the environmental conditions contributing to the success of individual species and the forces governing phytoplankton dynamics, diversity and stability remain poorly understood, especially in estuaries (Cloern and Dufford 2005). In addition, few studies have examined the long-term dynamics of phytoplankton assemblages
(Rojo and Alvarez-Cobelas 2000) to identify the ecological variables that regulate assemblage structure and selection for potentially harmful bloom-forming species under chronic eutrophication.

The Neuse Estuary Monitoring and Research Program of North Carolina State University’s Center for Applied Aquatic Ecology (CAAE) represents one of few sustained observational programs in shallow lagoonal estuaries that has acquired long-term (14 yr ongoing) environmental and phytoplankton data sufficient to assess long-term trends in nutrient concentrations and loadings, and phytoplankton response. While numerous phytoplankton studies have been conducted in the Neuse River Estuary (NRE), the earlier research has primarily assessed the collective productivity of the phytoplankton assemblage or of large taxonomic groups in relation to nutrients and consumers (Boyer et al. 1993; Valdes-Weaver et al. 2006; Paerl et al. 2007). Most of these studies used high-performance liquid chromatography (HPLC) to quantify concentrations of phytoplankton diagnostic photopigments, used to estimate the relative abundance of major phytoplankton groups in the estuary. The argument for this approach is that higher levels of biological organization are often more predictable indicators of nutrient enrichment because of the statistical variability inherent in phytoplankton species datasets (Cottingham and Carpenter 1998; Valdes-Weaver et al. 2006). A major shortcoming, however, is that the photopigment method does not enable identification of the ecological variables that may be selecting for less common species, or for potentially harmful bloom-forming species over time during the eutrophication process.

Phytoplankton assemblages have been well studied in tidal embayments (Cloern and Dufford 2005), relative to what is known about their dynamics in shallow, turbid, non-tidal estuaries. This study of a shallow, turbid, non-tidal lagoonal estuary under chronic eutrophication
focused where possible beyond phytoplankton functional groups to the species level, which has been recommended as an important next step in research to advance understanding about long-term relationships among phytoplankton assemblage structure, individual nuisance or harmful species, and environmental factors (Reynolds et al. 2000; Cloern and Dufford 2005; Marshall et al. 2005). Compositional variation in the phytoplankton data was addressed using ordination, which has been described as a group of methods for data reduction leading to hypothesis generation (Kent and Coker 1992). Ordination reveals the strongest patterns in species composition by representing complex ecological relations in low-dimensional space (McCune and Grace 2002). As a result, the ordination analyses used in this study provided interpretations of species-environment relationships and enabled consideration of questions such as: Did the species structure of phytoplankton assemblages and harmful species in this representative turbid, eutrophic lagoonal estuary change over the 13-yr study (1994-2006) and, if so, how were these biological changes related to climate? What environmental factors best explain dominant species composition and overall phytoplankton assemblage structure over time? By addressing these important questions, this study addresses an identified urgent need (Cloern 2001) to gain a better understanding of the complex mechanisms that influence the response of phytoplankton assemblages to chronic cultural eutrophication.

Study area

The Neuse River and Estuary flow ~320 km through the Piedmont and Coastal Plain of North Carolina to Pamlico Sound (Fig. 1). The watershed drains 16,000 km² of productive and rapidly developing urban, industrial, and agricultural areas. The watershed contains ~1.35 x 10⁶ people (about 16% of the state’s population), with 54% of the basin population located in ~10% of the land area within the upper watershed (Burkholder et al. 2006).
The NRE is a sub-estuary and major tributary of Pamlico Sound, a bar-built estuary characterized by shallow depth and reduced tidal action (Luettich et al. 2002; Reed et al. 2004), especially in the upper reaches of the NRE where this study was conducted. Pamlico Sound is geographically confined by the Outer Banks with only a few narrow inlets, resulting in reduced water exchange with the Atlantic Ocean and attenuating the coastal ocean astronomical tide. Mean water depth is shallow (~4.4 m) and characterized as wind-mixed with negligible tidal effect (Leuttich et al. 2002; Reed et al. 2008). The NRE is poorly flushed with a relatively long residence time in major tributaries (mean over an annual cycle, 50-100 days depending upon river discharge and coastal ocean water levels; Christian et al. 1991). It exhibits a high degree of salinity stratification due to freshwater input from upriver and high-salinity water input via estuarine circulation from downriver. A previous study addressing physical-chemical structure (Reed et al. 2004) showed that approximately 92% of water-column profiles obtained over an eight-year period indicated vertical density stratification due to salinity differences and was seasonally dependent. The study area is also impacted by bottom-water hypoxia, especially in the summer when water temperatures are at a maximum (Burkholder et al. 2006). This low-oxygen water can be upwelled due to changes in wind direction, leading to fish kills (Glasgow et al. 2001; Reynolds-Fleming and Luettich 2004) in addition to documented Pfiesteria fish kills. Due to the shallow nature of the NRE, the predominant wind field, especially in the form of high wind events, is the most significant water column mixing forcing factor (Giffin and Corbett 2003; Reynolds-Fleming and Luettich 2004; Reed et al. 2008). Wind events have also been implicated as an important factor in resuspension of sediments and significant nutrient flux to the water column (Giffin and Corbett 2003).
The waters of the Neuse River basin have been classified by the state as “nutrient-sensitive” because they sustain bottom-water hypoxia, massive fish kills, and noxious and toxic algal blooms from anthropogenic nutrient over-enrichment (Burkholder et al. 2006). The relative isolation of the poorly flushed NRE from ocean inputs (Christian et al. 1991), and its “hurricane-prone” character (Burkholder et al. 2004; Mallin and Corbett 2006) are other characteristics that make the Neuse system especially sensitive to adverse effects from anthropogenic nutrient loading. Major storms have caused flooding of confined animal feeding operations and municipal wastewater treatment plants that have led to the release of large amounts of raw animal and human wastes for weeks after the storms (Burkholder et al. 2004; Mallin and Corbett 2006). Recent efforts to characterize climatic and anthropogenic influences on nitrogen (N) and phosphorus (P) concentrations and loadings to the NRE have revealed that the system has sustained a 500-fold increase in ammonium (NH₄⁺N) concentrations over the past decade (Burkholder et al. 2006). Blooms of potentially toxic cyanobacteria in the lower freshwater river (e.g., *Microcystis aeruginosa*) give way to estuarine flagellate blooms (Mallin 1994; Pinckney et al. 1997). Laboratory studies and studies of algal blooms in other systems have demonstrated that the abundance of some of these harmful species (e.g., *Prorocentrum minimum*) is strongly linked to nutrient over-enrichment, including increasing ammonium concentrations (Fan et al. 2003; Glibert et al. 2005).

**Methods**

*Environmental data.* Because our intent was to focus on changes in the abundance of bloom-forming and harmful species in response to environmental conditions, the data on environmental conditions used in this analysis were selected from the complete 13-yr dataset based on seasonal chlorophyll *a* maxima for each year. Data on salinity, temperature, dissolved oxygen
(DO), suspended solids (SS), total Kjeldahl nitrogen (TKN), NH$_4^+$N, nitrate+nitrite (hereafter referred to as nitrate, NO$_3^-$N), total phosphorus (TP), soluble reactive phosphate (SRP), silicate, and phytoplankton assemblages (Burkholder et al. 2006) were collected during biweekly (April – October) or monthly sampling (November - March) from 1994 through 2006 at 22 historical and current sampling stations located throughout the NRE (Fig. 1). The chlorophyll and phytoplankton assemblage data corresponding to seasonal maxima were taken using integrated water-column samples from the upper or lower water column or, in shallow areas, a total integrated water-column sample. Temperature, salinity, DO, and light data were used from depth 0.5 m for chlorophyll maxima from the upper water column; from depth 1.5 m for total integrated water-column samples; and from depth 2.5 m for chlorophyll maxima that occurred in the lower water column. Nutrient and SS data were used from the upper or lower water column or from total integrated water-column samples to match the corresponding samples for chlorophyll maxima.

**Phytoplankton samples and data preparation.** Samples for analysis of phytoplankton assemblages were selected, as mentioned, based on seasonal chlorophyll $a$ maxima for each year (10 samples with the highest chlorophyll $a$ in a season within a given year x 4 seasons x 13 yr = ~520 samples analyzed). Phytoplankton samples were preserved with acidic Lugol’s solution (Vollenweider 1974) in the field and archived at 4°C in darkness until analysis. Precipitation data for New Bern were obtained from the North Carolina State Climate Office. Neuse River discharge rates were measured by the United States Geological Survey (USGS) at Kinston, North Carolina, ~70 km upstream from the oligohaline edge of the estuary (Mills Branch) under average flow conditions (Burkholder et al. 2004). Taxa were quantified following Lund et al. (1958). Although chemosystematic classification has become popular for estimating the relative abundance of phytoplankton taxonomic groups (Paerl et al. 2007; Valdes-Weaver et al. 2006), it is frequently
misapplied, as noted by Lewitus et al. (2005); moreover, phytoplankton marker pigments cannot be used to distinguish between individual nuisance species or phytoplankton functional groups. For example, *Scenedesmus* and *Euglena* both have the marker pigment chlorophyll $b$, and cannot be differentiated using a chemosystematic approach, but these two genera (as well as species within each genus) may have very different nutritional strategies (Wetzel 2001).

This analysis required information, where possible, beyond phytoplankton dynamics at the class level. Identifications were taken to the lowest taxonomic level possible using light microscopy (Olympus IX70 inverted microscope, phase contrast, 600X). Many identifications were taken to species or genus level, but for some flagellates, individual genera were difficult to consistently differentiate from one another in acidic Lugol’s-preserved samples and were mostly identified at the class level (e.g. cryptophytes – Cryptophyceae; haptophytes – Haptophyceae). Raphidophytes were identified to species in light microscopy (supported by molecular probe analyses; Coyne et al. 2005) only for blooms, as they tend to preserve poorly (although better at 4°C in darkness; O’Halloran et al. 2005). Finally, along with *Pfiesteria piscicida* Steidinger and Burkholder and *Pfiesteria shumwayae* Glasgow and Burkholder (Marshall et al. 2006), cells identified as “pfiesteria-like” included cryptoperidiniopsoids and other physically similar taxa (Seaborn et al. 2006) under light microscopy. Although the obligate phototrophic and mixotrophic species, *Karlodinium veneficum* (Ballantine) J. Larsen is not functionally similar to the heterotrophic *Pfiesteria* spp. and cryptoperidiniopsoids, and is readily distinguished from *Pfiesteria* spp. under light microscopy in fresh and recently preserved material (Burkholder et al. 2001), it was difficult to consistently and accurately distinguish *K. veneficum* from *Pfiesteria* spp. and “pfiesteria-like” dinoflagellates in older acidic Lugol’s-preserved samples. Therefore,
*Pfiesteria* spp., “pfiesteria-like” species, and *K. veneficum* were grouped together for this analysis.

Taxa abundances, including filamentous and colonial forms, was quantified by enumerating single cells. Species data were compiled into a matrix of mean cell number by season and year, resulting in a final matrix of ~90 columns of species cell counts by 52 sample rows (13 yr x 4 seasons). The averaging reduced the visual clutter that resulted from treating the points individually and enhanced our ability to display the relevant findings of the ordination. Seasonal categories were as follows: December – March = winter; April and May = spring; June – September = summer; October and November = fall. These categories were determined by comparing monthly means for the water quality variables and choosing the grouping that best discriminated into seasons. In general, nutrient concentrations for each month within the designated seasonal categories were significantly different from those of months in other seasons, but not from one another. To reduce the “noise” (variability) in the phytoplankton dataset and enhance detection of assemblage patterns in relation to environmental factors, rare taxa (defined as present in < 5% of the samples) were removed prior to analysis (McCune and Grace 2002), and average abundances of the remaining ~70 species were log-transformed after 1 was added as a constant.

Every phytoplankton sample used in the analysis had a corresponding suite of physical and chemical measurements that could be related to ordinations of samples based on phytoplankton composition data. Quantitative environmental variables were also averaged by season and year to correspond to the phytoplankton species matrix, and included Neuse River discharge rates (m$^3$ s$^{-1}$), temperature, salinity, photosynthetically active radiation (PAR), dissolved oxygen (DO), suspended solids (SS), total nitrogen (TN), total Kjeldahl nitrogen
(TKN), NH$_4^+$N, NO$_3^-$N, total phosphorus (TP), soluble reactive phosphate (SRP), silicate, and TN:TP ratios. Season was also included in the non-metric multidimensional scaling (NMDS) analyses (described below) as a categorical variable.

**Statistical analysis.** Ordination techniques, which order samples along axes expressing the main trends or gradients in the data, were used to investigate potential environmental predictors of phytoplankton assemblage patterns. NMDS, a geometric mapping technique for data that expresses the ranked distances (or difference) between the objects of a set, was chosen because it is currently considered the most effective ordination method for ecological data (McCune and Grace 2002). NMDS is well suited to data that are non-normal or are on arbitrary or discontinuous scales (McCune and Grace 2002). Like most species abundance data, the distribution of phytoplankton abundance in this study was skewed, with many samples containing a fairly small number of each species. As a result, NMDS was chosen among other ordination techniques to establish seasonal and year-to-year differences in phytoplankton assemblage structure, and to interpret those differences in terms of environmental conditions. Some authors suggest that the best way to accurately describe the relationship between assemblage structure and environmental variables is to apply two principally different ordination methods to the same data (Ter Braak 1986, Økland 1996). Thus, canonical correspondence analysis (CCA) also was used to explore relationships between phytoplankton assemblage structure and abiotic variables. The information supplied by CCA was complementary and supportive of NMDS results, and did not offer insights beyond those supplied by NMDS. Therefore, only the results of NMDS are presented here.

All six NMDS ordinations (Table 1) were carried out using PC-ORD version 4.0 software in the “slow and thorough” autopilot mode using a Sørenson distance matrix. Although NMDS
was also run on samples before averaging, the key results are consistent with those found after averaging. As a result, in all of the NMDS ordination diagrams presented, each point on the diagram represents average abundance of all phytoplankton species in 10 samples from a given season and year. The relative distance between the averaged samples reflects relative similarity or dissimilarity in species composition. A large distance between two averaged samples represents a large difference in phytoplankton species composition. After averaged samples were arranged in space according to their similarity (or dissimilarity) in species composition, vectors representing environmental variables or phytoplankton species were superimposed on the ordination diagram to indicate the direction of maximum increase. The length of the vector represents the magnitude of the correlation between the environmental variable (e.g., nutrient concentration) or species abundance and sample phytoplankton composition. In each diagram, only the environmental or species vectors with a greater r-square value than the indicated cutoff are shown. R-square ($r^2$) cutoff values were set at different levels in each diagram to reduce clutter for easier interpretation of the results. For example, in the ordination of winter and summer samples, a large number of species were more abundant in either winter or summer samples. As a result, the $r^2$ cutoff value for species vectors was set higher so that only the most highly correlated species are shown. When there were not as many highly correlated variables, $r^2$ cutoff values were lowered. Although these $r^2$ values may appear low, ordination methods differ from inference testing in that they are primarily descriptive, summarize complex relationships, and are used to identify other variables most closely associated with compositional variation. Heterogeneous datasets such as the one used here have much underlying complexity, defy simple summarization, and often result in a low percentage of variance represented by ordination axes (McCune and Grace 2002). Because the primary purpose of ordination is filtering a signal from
noise (Gauch 1982), ordinations should not be interpreted on the basis of correlation coefficients or $r^2$ values alone.

In addition to using NMDS to describe relationships between phytoplankton assemblage structure and environmental variables, indicator species analysis (Dufrêne and Legendre 1997) was performed on the phytoplankton dataset (~100 taxa; log-transformed abundance) by sample matrix (~520 samples). The full dataset was used for indicator species analysis because seasonal averages would have obscured species preferences based on abundance and presence or absence in samples. Phytoplankton indicator taxa (abundant and consistently “faithful” to a particular group; significant index value $\alpha \leq 0.05$) were determined by running indicator species analysis separately by season (groups ≡ winter, spring, summer, fall), TN:TP ratios (groups ≡ < 8:1, ≥ 8:1 and ≤ 16:1, >16:1), NH$_4$N concentrations (groups ≡ <50 μg NH$_4$N L$^{-1}$ and >50 μg NH$_4$N L$^{-1}$), nitrate concentrations (groups ≡ <100 μg NO$_3$N L$^{-1}$ and >100 μg NO$_3$N L$^{-1}$), TKN concentrations (groups ≡ <1500 μg TKN L$^{-1}$ and >1500 μg TKN L$^{-1}$), SRP concentrations (groups ≡ < 50 μg SRP L$^{-1}$ and > 50 μg SRP L$^{-1}$), TP concentrations (groups ≡ <150 μg P L$^{-1}$ and >150 μg P L$^{-1}$), and SS concentrations (groups ≡ < 20 mg L$^{-1}$ and > 20 mg L$^{-1}$). PC-ORD’s Multi-response Permutation Procedures (MRPP) with a Sørensen distance matrix were used to determine the compositional significance ($\alpha < 0.05$) of each of the groupings used for indicator species analysis.

Finally, relationships between phytoplankton species assemblage patterns and the recorded set of environmental factors were explored using the BIO-ENV procedure (Clarke and Ainsworth 1993) in R software version 2.4.0 (R Development Core Team 2006). This method uses the weighted Spearman rank correlation ($\rho_s$) to correlate the biotic pattern (represented in the phytoplankton sample similarity index) with the resemblances (Bray-Curtis distances).
between samples computed for each environmental parameter separately \((k = 1)\) and for all possible combinations \((k = 2, 3, \ldots, n)\). It was then possible to identify the environmental parameter or suite of parameters that correlates best with – and, by implication, may most strongly influence – phytoplankton species assemblages in the NRE. Environmental variables used in the BIO-ENV analysis were Neuse River discharge rates, temperature, salinity, DO, SS, TN, TKN, \(\text{NH}_4^+\)N, \(\text{NO}_3^-\)N, TP, SRP, silicate, and TN:TP ratios \((n = 13)\). Temperature was expected to have a highly significant effect on phytoplankton species composition that would obscure the effect of the other environmental variables. Therefore, the BIO-ENV procedure was run on the full dataset (seasonal averages; 13 yr x 4 seasons) and within each of the four seasons defined above.

**Results**

During this study, the NRE was affected by 9 tropical cyclones with high flooding and a sustained 3-yr drought (2000-2002). Precipitation was high in 1996, which included Hurricane Fran (level 3; early September), and in 1999 which included Hurricanes Dennis (late August and early September), Floyd (September), and Irene (October). The path of Hurricane Fran followed the length of the Neuse watershed, and Hurricane Floyd caused record flooding in some areas of the Neuse Basin (Burkholder et al. 2004). In marked contrast, the sustained drought during 2000-2002 was evaluated as the worst sustained by North Carolina in 100 yr, and much of the state was declared a federal drought disaster area for agriculture (Southeast Regional Climate Center 2007; Burkholder et al. 2006). The sustained drought was followed by above-average precipitation in 2003. That year the path of Hurricane Isabel tracked the length of the watershed (September; State Climate Office of North Carolina 2003). Precipitation in 2004-2006 was close
to the long-term average (on basis of 100-yr annual mean for North Carolina; Southeast Regional Climate Center 2007).

Neuse River discharge rates were positively associated with precipitation patterns (Fig. 2A). Depending on seasonal precipitation and river discharge rates, seasonal mean surface salinities varied from 0.5 to 15.1 (Fig. 2B), and seasonal mean temperatures varied from 6.7°C to 29.3°C (Fig. 2C). Seasonal mean SS concentrations, which varied from 5.2 to 44.7 mg L⁻¹ (Fig. 2D), and photosynthetically active radiation (PAR) indicate substantial turbidity in this estuary, especially in the spring and fall (Fig. 2E). DO concentrations also varied seasonally with higher concentrations generally occurring in the winter (winter means 9.8 mg L⁻¹ to 13.1 mg L⁻¹) and lower concentrations in summer (summer means 5.6 mg L⁻¹ to 8.4 mg L⁻¹) (Fig. 2F). Lowest DO concentrations occurred in fall of 2001 and 2002 during the sustained drought.

The nutrient trends indicated by these samples are similar to those reported in Burkholder et al. (2006), indicating that the subset of samples selected for this phytoplankton analysis was representative of changing environmental conditions in the NRE. TN and TP concentrations (grand means ± 1 SE for samples in this analysis, 1088 ± 23 μg TN L⁻¹, range 353-4130 μg TN L⁻¹; 133 ± 5 μg TP L⁻¹, range 34-557 μg TP L⁻¹) reflected eutrophic conditions (Burkholder 2000; Wetzel 2001; Fig. 2 G, H). TN:TP ratios (molar basis) were lowest in summer (grand mean ± 1 SE, 6.6 ± 0.2) and highest in winter (13.1 ± 0.4) and spring (11.2 ± 0.7; Fig. 2I). NH₄⁺N concentrations (38 μg L⁻¹, range 0-670 μg L⁻¹) increased from 1994 to 2006, and generally were higher in summer and fall (Fig. 2J). The highest NH₄⁺N concentrations occurred in summer and fall of 2002 at the end of the sustained drought.

A total of 86 taxa were identified in the mesohaline NRE (Table 2, Fig. 3). These include 13 cyanobacteria, 26 diatoms, 27 dinoflagellates, 2 raphidophytes, various ochrophytes (2
chrysophytes, 1 silicoflagellate), 3 euglenoids, 13 green algae, and 1 prasinophyte).

Cryptophytes and haptophytes were included as well but, as mentioned, individual taxa often could not be distinguished in older acidic Lugol’s-preserved samples.

Cyanobacteria, especially Oscillatoria spp. and Anabaena spp., were dominant in summer, and cyanobacteria (Oscillatoria spp.) and small chlamydomonad-like flagellates were co-dominant in fall (Figs. 4A,B and 5). In winter, either dinoflagellates or diatoms were the dominant functional group (Fig. 4C). During years when dinoflagellates were dominant (Fig. 4C), Prorocentrum minimum, Heterocapsa rotundata, and Heterocapsa triquetra were most abundant (Fig. 6). In years when diatoms were dominant (Fig. 4C), Leptocylindrus minimus, Chaetoceros, and Cyclotella were most abundant (Fig. 6). The dominant phytoplankton group during spring varied from year to year (Fig. 4D). The dinoflagellate P. minimum, chlamydomonads, Oscillatoria spp., and Thalassiosira spp. were often among the two most abundant taxa (Fig. 6). Cryptomonads, which previously have been reported as abundant in the upper and middle NRE (Mallin 1994, Pinckney et al. 1998), were among the top three most abundant taxa, but never the most abundant, in some years during summer (1994, 1999, 2001, 2002, 2003, 2006), fall (1997, 1999, 2005), and winter seasons (1997, 1999, 2000, 2004) (Figs. 5 and 6). Their maximum abundance, however, ranged from $10^2$ to $10^3$ cells mL$^{-1}$, and did not exceed $9 \times 10^3$ cells mL$^{-1}$ (attained in one summer). During seasons when they were most abundant, they contributed fewer than 10% of the total cells among the top three most abundant taxa except during winter 1997 (14.1%); summer, fall and winter 1999 (11.8 - 32.2%); and summer 2002 (27.2%).

NMDS of phytoplankton species abundance considering all samples indicated, as expected, that species composition varied seasonally (Fig. 7). The relative distance between
points reflects relative similarity (points close together) or dissimilarity (points further apart) in species composition. Averaged samples from the same season, indicated by color in the first ordination diagram and shape in following diagrams, are close to one another and more distant from averaged samples from other seasons (Fig. 7). TN:TP ratios and TN, TKN, and SS concentrations were highest during winter and spring (Fig. 7A) when phytoplankton assemblages were dominated by diatoms and dinoflagellates as indicated by their vectors lying between the winter and spring groupings (Fig. 7B). TN:TP ratios were low and SRP concentrations were high in summer (Fig. 7A) when cyanobacteria were dominant (Fig. 7B). Certain dinoflagellates such as *Peridinium* spp. and *Protoperidinium pellucidum* were also most abundant in summer (Fig. 7B) as indicated by the large vectors pointing in the direction of the summer samples. Fall samples, with intermediate TN:TP ratios and SRP concentrations, were dominated by small flagellates such as chlamydomonads, or by cyanobacteria (Fig. 7).

NMDS of summer and fall samples collectively indicated that cyanobacteria and certain dinoflagellates (e.g., *Gymnodinium* spp.) were more abundant in summer in association with higher temperatures and TP and SRP concentrations, and that small flagellates such as chlamydomonads were more abundant during fall in association with higher TN:TP ratios (Fig. 8). A river discharge-salinity gradient was also discerned in this ordination, with the vector for river discharge pointing in the opposite direction as the vector for salinity (Fig. 8A). High river discharge rates, low salinities, and high NO$_3$-$N$ concentrations, which occurred during years with hurricanes (fall 1996, 1999, 2003; Fig. 8A), coincided with higher abundance of the diatoms *Leptocylindrus minimus* and *Thalassiosira* spp., as indicated by environmental and species vectors all pointing in the same direction (Fig. 8B). Although there were no hurricanes in the fall of 2000, species composition continued to have higher abundance of diatoms post-hurricane year.
1999. Low river discharge rates and high salinity (Fig. 8A) coincided with higher cell numbers of the dinoflagellate *Oxyrrhis marina* (Fig. 8B).

The fluctuation between diatom dominance and dinoflagellate dominance was again apparent when NMDS was run on winter and spring samples only. This ordination indicated that phytoplankton composition not only differed between winter and spring samples, but also between samples with high NO$_3$N concentrations (coinciding with high discharge rates) and those with lower NO$_3$N concentrations and high salinities (Fig. 9). As in fall, river discharge rate was related to species dominance, but in winter and spring samples, different diatom and dinoflagellate species were abundant. The dinoflagellates *Prorocentrum minimum* and *Heterocapsa triqueta* were dominant in both winter and spring when NO$_3$N concentrations were lower and salinity and silicate were elevated (Fig. 9). *Heterocapsa rotundata*, cryptomonads, and the diatoms *Leptocylindrus minimus*, *Skeletonema costatum*, and *Rhizosolenia* spp. were more abundant in winter when NO$_3$N concentrations and river discharge were high (Fig. 9). Chlorophytes (prasinophytes, chlamydomonads) and the diatoms *Thalassiosira* spp. were more abundant during spring (Fig. 9B), in association with higher temperatures and high TN and TKN concentrations (Fig. 9A).

Removal of the spring and fall samples from the NMDS analysis emphasized differences between the phytoplankton assemblages of winter and summer. *Heterocapsa triqueta*, *H. rotundata*, *Rhizosolenia* spp., *Cerataulina pelagica*, *Leptocylindrus minimus*, *Chaetoceros* spp., and *Skeletonema costatum* were more abundant in winter samples, which had higher NO$_3$N and DO concentrations and higher TN:TP ratios (Fig. 10). Summers were characterized by higher SRP and TP concentrations (Fig. 10A), and higher abundance of cyanobacteria, euglenoids, and certain dinoflagellates (Fig. 10B). *Heterosigma akashiwo* and other small flagellates
(chlamydomonads, haptophytes) increased in more recent samples (~2003-2006) coinciding with increasing NH$_4^+$N concentrations (Fig. 10). Although *H. rotundata* was one of the three most abundant taxa in the mesohaline NRE (based on seasonal averages) in 9 of the 13 winter seasons analyzed, this species reached exceptionally high densities (1.4 x 10$^5$ cells mL$^{-1}$) in more recent individual samples (2000-2001, 2004-2006; Figs. 6 and 10B).

Ordination of averaged winter samples by NMDS showed that dinoflagellates *Prorocentrum minimum* and *Heterocapsa triquetra* were dominant during winter conditions of high salinity and high TKN and SS concentrations (Fig. 11). These samples also had higher silicate concentrations. In contrast, a diatom assemblage with abundant *Leptocylindrus minimus*, *Chaetoceros* spp. and *Rhizosolenia* spp. dominated winter samples with low salinity, lower silicate, TKN and SS concentrations, and higher NO$_3^-$N concentrations (Fig. 11). The dinoflagellate *Heterocapsa rotundata* and cryptomonads were also more abundant in winter samples with higher NO$_3^-$N concentrations (Fig. 11); the potentially harmful dinoflagellate *A. sanguinea* was more abundant in winter samples with high salinity and lower NO$_3^-$N concentrations (Fig. 11). High river discharge rates, low salinities, and high inorganic N concentrations also coincided with higher abundance of cryptophytes (mostly reflecting the extremely high-water-year 1999; Figs. 9 and 11).

During summer seasons with high salinity, the *Pfiesteria*—“pfiesteria-like” dinoflagellates -*Karlodinium veneficum* grouping was abundant (Fig. 12). As in winter, mixotrophic and heterotrophic dinoflagellates were also most abundant in samples with high TKN concentrations. *Heterosigma akashiwo* was one of the two most abundant species in the estuary in summer 2000, and among the five most abundant species in summer 2002-2004 and fall 2005 (Fig. 5). Increased abundance of this species in summer coincided with higher NO$_3^-$N concentrations (Fig.
12). NH$_4$N concentrations, which did not meet the $r^2$-cutoff value and thus were not indicated on the ordination diagram, were also high in summer and fall samples when *H. akashiwo* was dominant.

Indicator species analyses supported NMDS results (Tables 3-5). The best indicators of high NH$_4$N concentrations, or taxa that were consistently abundant in samples with NH$_4$N > 50 μg NH$_4$N L$^{-1}$, were cryptomonads, *Cyclotella* spp., *Heterosigma akashiwo*, *Heterocapsa rotundata*, and *Leptocylindrus minimus* (Table 5). *Pseudo-nitzschia* spp., present in fewer than 5% of the samples, also emerged as an indicator of high NH$_4$N concentrations because the samples in which *Pseudo-nitzschia* spp. were present had > 50 μg NH$_4$N L$^{-1}$. *Prorocentrum minimum* commonly formed extensive and persistent blooms (≥ 1 x 10$^4$ cells mL$^{-1}$) during winter and spring seasons (1994-1996, 1999, 2002, 2005-2006; Fig. 6), and was an indicator species for both high TKN and high SS concentrations (Table 5). Various other mixotrophic and heterotrophic dinoflagellates also were indicators of high TKN and SS concentrations (Table 5). *Anabaena* spp. and *Oscillatoria* spp. were indicators of high SRP and TP concentrations and low TN:TP ratios (Tables 4 and 5). Euglenoids, the silicoflagellate *Dictyocha fibula*, and several dinoflagellate taxa (including toxin-producing species) were also indicators of higher P concentrations and lower TN:TP ratios, which occurred primarily during summers (Tables 3-5). The BIO-ENV comparison between phytoplankton and environmental data for the full dataset (all seasons) confirmed that, of all single environmental parameters ($k = 1$), temperature showed the highest affinity to phytoplankton distribution ($\rho_s = 0.37$). Phytoplankton assemblages were best correlated with temperature and also with TN:TP ratios ($\rho_s = 0.40$, Table 6), which ranged from 4:1 to 40:1 in winter and from 1:1 to 14:1 in summer. Various combinations of other parameters yielded lower $\rho_s$ values. When the BIO-ENV procedure was run on individual
seasons to reduce the effect of temperature, other combinations of environmental parameters correlated best with phytoplankton species assemblages in the NRE. For example, NH$_4$+N concentrations emerged as important in regulating phytoplankton assemblages in all four seasons and river discharge was important in regulating species composition in spring and fall (Table 6).

**Discussion**

*Changes in phytoplankton assemblages in relation to environmental gradients.* The seasonal dynamics of the phytoplankton assemblages at the functional group or class level were partly consistent with descriptions from previous studies of the NRE, but new insights also were gained about major factors that influenced certain potentially harmful species in shallow, non-tidal, turbid estuaries such as the Neuse. Cyanobacteria were dominant during summer months, and diatoms and dinoflagellates predominated in late winter-early spring (Mallin 1994; Pinckney et al. 1998). BIO-ENV analyses indicated that temperature (likely as a controlling influence on algal growth rates) and TN:TP ratios were important in regulating phytoplankton species assemblages on an annual basis. The N$_2$-fixing ability of many cyanobacterial taxa found in the NRE would afford them a competitive advantage in the summer when TN:TP ratios are lower. Although TN:TP ratios can be important in regulating phytoplankton assemblages, BIO-ENV analyses suggested that within the summer season, the amount and form of N is important in influencing species composition. NMDS analysis of summer samples also suggested that increasing inorganic N concentrations coincided with increased densities of small flagellates, including the raphidophyte *Heterosigma akashiwo*. Higher TN and TKN concentrations during summers were associated with increased abundance of dinoflagellates *Akashiwo sanguinea* and *Peridinium* spp.
In contrast, high salinities and P concentrations during summer were associated with an increase in other dinoflagellates including the grouping *Pfiesteria*—“pfiesteria-like” species— *Karldinium veneficum*, which were indicators of high TP and SRP concentrations and low TN:TP ratios. These observations about potentially toxic *Pfiesteria* spp. (Burkholder et al. 2005; Moeller et al. 2007) in the NRE are supported by laboratory experiments showing that cell production of *Pfiesteria piscicida* and *P. shumwayae* is stimulated by an increase in phosphate availability, and by phosphate or N (especially urea and NH$_4^+$) enrichment (Burkholder and Glasgow 1997; Glibert et al. 2006). The potentially toxic mixotroph *K. veneficum* also thrives in eutrophic estuarine waters based on field and experimental laboratory studies (Li et al. 2000). Overall, the grouping *Pfiesteria*—“pfiesteria-like” dinoflagellates—*K. veneficum* did not increase in abundance over the 13-yr period, and rarely exceeded 1000 cells mL$^{-1}$. *Pfiesteria* spp., in particular, have been described as sensitive to washout from tropical storms, which have increased in the NRE since the mid-1990s (Burkholder et al. 2004).

Although taxa (mainly cyanobacteria) that were dominant in summer continued to be present during the fall, their contribution to total phytoplankton abundance generally diminished with decreasing temperatures and increasing TN:TP ratios. The fall season coincided with increased dominance of small flagellates such as chlamydomonads, cryptomonads and, in more recent samples (2000-2006), haptophytes. The contribution of these small flagellates and some diatom taxa, such as *Leptocylindrus minimus* and *Thalassiosira*, increased in the fall season when river discharge rates and nitrate concentrations were high, especially in association with Hurricane Fran in 1996 and three consecutive tropical cyclones in 1999 (Dennis, Floyd, and Irene). Although seasonal mean abundance of dinoflagellates decreased, certain species such as *Oxyrrhis marina* were significant indicators of the fall season and were more abundant during
drier fall seasons (1997, 1998, 2006) with reduced river discharge rates and higher salinities. Cryptomonads were also among the most abundant species during fall and winter of 1999 following the four tropical cyclones of September - October. The importance of Neuse River discharge rates in regulating fall phytoplankton assemblages was indicated in the BIO-ENV analysis. Similar patterns for fall phytoplankton assemblages have been reported in other estuaries such as Chesapeake Bay (Marshall et al. 2005; Buchanan et al. 2005). More generally over an annual cycle, Bayesian modeling studies have indicated that water residence time or flushing rate, not tracked in this study, is also important in regulating the Neuse estuarine phytoplankton assemblage (Arhonditsis et al. 2007).

In accord with previous work (Mallin 1994; Valdes-Weaver et al. 2006), Neuse River discharge rates and hydrologic inputs of nutrients were also important in determining the dominant species of winter-spring phytoplankton assemblages in the mesohaline estuary. Mean dinoflagellate abundance decreased and diatom abundance increased during winter-spring seasons that immediately followed hurricane years (1997, 2000), and during winter-spring seasons with elevated rainfall and river discharge rates (1998, 2003). The positive association between diatom abundance and elevated river discharge in this study was also supported by the increase in diatom species under conditions of low salinity, high DO, and high inorganic N concentrations. The most frequently occurring taxa during diatom-dominated years were *Leptocylindrus minimus*, *Skeletonema costatum*, *Rhizosolenia*, *Cerataulina pelagica*, *Chaetoceros* spp., and *Thalassiosira* spp. The fact that many of these diatom taxa were also indicators of high nitrate concentrations is consistent with previous studies which have shown that diatom blooms frequently are associated with high nitrate supply (Takahashi and Fukazawa 1982, Lomas and Glibert 1999).
Increased vertical mixing during some periods following elevated rainfall and river flow (Burkholder et al. 2004) may also have selected for diatom species, although it should be noted that vertical mixing in the NRE is highly wind-dependent (Reynolds-Fleming and Leuttich 2004). Increased mixing is known to inhibit some dinoflagellate species (Thomas and Gibson 1990; Havskum et al. 2005), giving diatoms a potential competitive advantage during these periods of increased vertical mixing. In addition, diatoms, with heavy siliceous frustules, tend to sink out of the water column and require sufficient vertical mixing from high river flow to remain suspended. Dissolved silicate that was restored to surface waters from the sediments during these periods of vertical mixing was likely rapidly taken up by diatom populations, which may partly explain why high diatom cell numbers in this study were associated with lower water-column silicate concentrations in winter. Si:N and Si:P ratios in the NRE during these winter-spring periods of diatom dominance and lowered silicate concentrations were ~2 and ~20 respectively, which is approaching the range (1:1 for Si:N) where the transition from diatom dominance to flagellates generally takes place (Sommer 1994). Data were not available on water-column regenerative processes and sediment-water fluxes of dissolved silicate in the NRE, but the lowest dissolved silicate concentrations in the mesohaline Chesapeake Bay have been shown to occur during later stages of the spring biomass maximum (Conley and Malone 1992).

In contrast, dinoflagellates dominated during the winter-spring seasons with lower rainfall and river flow (1994, 1996, 1999, 2002, 2004-2006) and were positively associated with higher salinity. The stable water column in drier years (shown by Reed et al. 2004) and the reduced flushing associated with drier conditions may have facilitated dinoflagellate movement in the water column by reducing interference and physical damage from water column mixing.
In deeper systems, shifts from diatoms to dinoflagellate-dominated assemblages have occurred during reduced vertical mixing (Parsons et al. 1978), and dinoflagellate blooms often have occurred during upwelling relaxation periods (Blasco 1977). Unlike the multi-species diatom blooms that occurred in the NRE, winter-spring dinoflagellate blooms were dominated either by *Prorocentrum minimum* and *Heterocapsa triquetra* or by *Heterocapsa rotundata*. Winter-early spring blooms of the mixotrophic dinoflagellates *P. minimum* and *H. triquetra* have been described as a common feature in the mesohaline NRE since the early 1990s (Mallin et al. 1991; Mallin 1994; Springer et al. 2005). This study showed that high densities of *P. minimum* were associated with high TKN and SS concentrations, and that densities of *H. rotundata* increased with increasing ammonium concentrations during winter.

Other modeling efforts such as Bayesian approaches have indicated that hydrologic forcing, mainly from river flow fluctuations, strongly regulates plankton dynamics in the upper NRE, with nutrient concentrations becoming more important in the mesohaline, more slowly flushed segments (Arhonditsis et al. 2007). As in other estuarine systems (Cloern and Dufford 2005), grazing pressure from herbivorous zooplankton is known to interact with nutrient supplies and other environmental conditions in controlling phytoplankton assemblage composition and abundance (Mallin and Paerl 1994). It has been hypothesized that grazing controls may become more important in the lower NRE under longer water residence times (Arhonditsis et al. 2007), but additional research is needed to assess the role of zooplankton in structuring the phytoplankton assemblages.

*Bloom-forming and potentially toxic species.* Whereas abundance of the *Pfiesteria* spp.-“pfiesteria-like” species-*Karlodinium veneficum* grouping was positively related to high phosphorus and low TN:TP ratios (above), other dinoflagellates were linked to high N. Previous
studies have shown that the potentially toxic species *Prorocentrum minimum* exhibits higher growth rates on urea and ammonium than on nitrate (Fan et al. 2003), and large increases in urea were found to precede blooms of *P. minimum* in several tributaries of Chesapeake Bay (Glibert et al. 2001). This may help to explain why *P. minimum* densities in this study were positively correlated with TKN concentrations, which represents the sum of organic N compounds, including urea, and ammonium. Other mixotrophic (*Heterocapsa triquetra*) and heterotrophic dinoflagellates (*Oblea rotunda, Peridinium* spp.) also were positively related to TKN concentrations. Although bacteria and/or free dissolved enzymes degrade dissolved organic N to ammonium or urea in natural waters (Berman et al. 1999), these dinoflagellates could also be utilizing organically bound N for growth through direct uptake (Fan et al. 2003, Glibert et al. 2005; Twomey et al. 2005), or through use of cell-surface enzymes that can provide amino acids for assimilation or degrade amino acids to ammonium (Palenik and Morel 1990; Stoecker and Gustafson 2003).

In addition to being an indicator for high TKN concentrations, *P. minimum* was also an indicator for high SS. This species can grow under low light conditions (Grzebyk and Berland 1996) and can supplement its nutrient demands through mixotrophic grazing of cryptophytes (Stoecker et al. 1997). It appears to be an excellent competitor during periods of high turbidity in the NRE. The positive association between *P. minimum* and other mixotrophic and heterotrophic dinoflagellates with both high TKN and SS suggests that the combination of low light and rich organic substrates may select for phytoplankton species capable of meeting their nutrient requirements by utilizing dissolved organic substances (resorption) or organic particles (phagotrophy).

In contrast, *Heterocapsa rotundata* was positively related to inorganic N, especially ammonium, and high cell concentrations of *H. rotundata* in the NRE were more closely related
to high densities of diatoms than to other dinoflagellates. *H. rotundata* was abundant in years when diatoms dominated the winter-spring assemblage (1997, 1998, 2000, 2001, 2003). The contribution of this species to the overall phytoplankton assemblage was especially high in winter of 2004, when densities reached $1.4 \times 10^5$ cells mL$^{-1}$ in association with ammonium concentrations in excess of $200 \mu g \text{NH}_4^+ \text{N L}^{-1}$. Dense blooms of *H. rotundata* have been reported in other estuaries (Hilmer and Bate 1991; Marshall et al 2005), but the nutritional ecology of this species is poorly known. Although mixotrophic capability has been documented, *H. rotundata* exhibited very low ingestion rates (Jeong et al. 2005) and may rely primarily on photosynthesis for growth. Considering that the abundance of *H. rotundata* and diatoms in this study were positively related to one another and to inorganic N, *H. rotundata* may be more similar to co-occurring diatoms than to other dinoflagellates in its N nutrition.

For all seasons, the contribution of small flagellates (notably raphidophytes, haptophytes, and chlorophytes) to overall algal abundance increased over the study period, concomitant with increasing ammonium concentrations. In addition, the potentially toxic raphidophyte *Heterosigma akashiwo* and the bloom-forming dinoflagellate *Heterocapsa rotundata* increased in more recent samples (2000-2006). These two species were indicators of concentrations in excess of $50 \mu g \text{NH}_4^+ \text{N L}^{-1}$. Cryptophytes also were indicators of high NH$_4$+N, and are common in eutrophic estuaries where they serve as a food source for grazers including mixotrophic harmful algal species (Mallin 1994; Burkholder et al. 2008). Phytoplankton generally exhibit an uptake preference for ammonium (Dugdale and Goering 1967). Although ammonium, nitrate and urea all can be N sources for *H. akashiwo*, ammonium was shown to support more rapid exponential growth rates than nitrate or urea (Herndon and Cochlan 2006). In addition, ammonium is acquired more readily by *H. akashiwo* than other N substrates at both saturating
and sub-saturating N concentrations (Herndon and Cochlan 2006). Temperature and salinity also
were related to high densities of *H. akashiwo*. As in other regions (Smayda 1998), blooms of *H.
akashiwo* appeared mostly in summer samples, and highest densities (> $2 \times 10^4$ cells mL$^{-1}$)
ocurred at $> 20^\circ$C and low to moderate salinities (5-12). Blooms of *H. akashiwo* in other
regions have been associated with mortality of cultured and wild fish (Smayda 1998).

*Pseudo-nitzschia* spp. also emerged as indicators of high inorganic N concentrations in
this study. Although this genus was rare (< 800 cells mL$^{-1}$, and found in fewer than 5% of the
samples analyzed in this study), the ability of several *Pseudo-nitzschia* species to produce the
neurotoxin domoic acid (Bates et al. 1998), and their appearance in more recent samples (after
2001) with NH$_4^+$N $> 50$ μg L$^{-1}$ is noteworthy. Growth of other *Pseudo-nitzschia* species (e.g.,
*Pseudo-nitzschia multiseries* (Hasle) Hasle) has been inhibited in laboratory studies at
ammonium concentrations $\sim 360$ μg NH$_4^+$N L$^{-1}$ (Hillebrand and Sommer 1996), but potentially
toxic *Pseudo-nitzschia* blooms have been reported in coastal environments that are rich in
ammonium and organic N (Bates et al. 1998). Aside from the association with high ammonium
concentrations in this study, *Pseudo-nitzschia* spp. were present in the NRE too rarely to identify
patterns in their abundance in relation to other environmental and seasonal gradients. Field
observations of *Pseudo-nitzschia* spp. in other coastal areas indicate that there has been an
increase in their abundance in association with increased nutrient loading (Parsons et al. 2002).
Continued monitoring for *Pseudo-nitzschia* spp. in the NRE and the use of additional
identification techniques (molecular analysis and scanning electron microscopy), culturing
studies, and toxin assays to distinguish between potentially toxic and non-toxic representatives of
this genus are recommended, especially considering the $\sim 500$-fold increase in ammonium
concentrations over the past decade (Burkholder et al. 2006).
This long-term study considered species-specific data where possible and used ordination techniques to assess relationships among phytoplankton assemblage structure, individual nuisance or harmful species, and environmental variables over a long-term (13-yr) period. The data suggest that the ecology of estuarine phytoplankton assemblages in shallow, turbid lagoonal, wind-mixed estuaries such as the Neuse contrasts with paradigms about phytoplankton ecology that have been developed from observations in tidal estuaries and embayment. In deeper, tidal systems, sedimentation and turbulence have been hypothesized to be among the most important factors controlling phytoplankton assemblages. As Cloern and Dufford (2005, p.18) wrote, “Margalef (1978) postulated that the ‘combination of sedimentation with turbulence’ shapes communities as the pelagic varies between extremes of a fertile-turbulent state (promoting diatom growth) and exhausted-stratified state (promoting growth of flagellates and dinoflagellates that migrate vertically to exploit nutrient gradients; Smayda and Reynolds 2001).” Blooms result when phytoplankton are released from strong light limitation by runoff-induced salinity stratification, or increased depth of the euphotic zone with decreased suspended solids during neap tides.

In contrast, in this shallow lagoonal estuary, temperature and TN:TP ratios were the most important factors influencing phytoplankton assemblages. During colder seasons, dinoflagellates dominate at TN:TP > 1, whereas other dinoflagellate species, other flagellates, cyanobacteria and euglenoids dominate in warmer seasons at TN:TP > 8. Conditions that support blooms vary depending upon the season: In winter and early spring, the major factors appear to be the amount and form of nitrogen, and water discharge. Under high nitrate and high discharge, winter-early spring blooms in the NRE consisted of the small photosynthetic dinoflagellate *Heterocapsa rotundata*, co-dominant with diatoms such as *Leptocylindrus minimus* and...
Conditions of high DON, low water discharge, and low light from high suspended solids favored blooms of larger mixotrophic dinoflagellates. During summer, the amount and form of N, as well as P availability, were major factors influencing blooms. High DON favored dinoflagellates such as *Akashiwo sanguinea* and *Protoperidinium pelucidum*; high ammonium favored *Heterosigma akashiwo*, green flagellates, and euglenoids; and high P favored other mixotrophic dinoflagellates including toxin producers such as *Pfiesteria* spp. and *Karlodinium veneficum*. In contrast, during the “hurricane season” of late summer and fall, water discharge was very important in controlling phytoplankton assemblages, as well as N form. Diatoms dominated when discharge was high, whereas mixotrophic and heterotrophic dinoflagellates dominated in seasons of low discharge, high salinity and high DON. BIO-ENV results also revealed that within each season, increasing ammonium concentrations in the NRE were an important influence on species composition and assemblage structure.

Overall, the ordination techniques used in this study provide insights about the abundance and distribution of phytoplankton taxa, including some potentially toxic and bloom-forming species, along physical-chemical and seasonal gradients in an estuary under chronic anthropogenic nutrient enrichment. These analyses also suggest that increasing ammonium concentrations have been important, especially within recent years, in governing phytoplankton assemblage structure. In future work, the use of additional identification techniques (molecular analysis and scanning electron microscopy) and the inclusion of data on micronutrients (e.g., iron and manganese) and phytoplankton consumers will continue to strengthen understanding about the interactive processes that regulate phytoplankton assemblage structure in estuaries.

Few studies have characterized the dynamics of natural phytoplankton communities during the eutrophication process with data sufficient to assess long-term trends in relationships
between nutrient enrichment and blooms of certain HAB species. Identifying the environmental factors that govern phytoplankton community change through eutrophication using long-term data series will strengthen the ability of scientists, public health officials, and resource managers to predict the likelihood of a phytoplankton bloom and the species most likely to cause that bloom given a specific combination of environmental factors.
References


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Table 1. Descriptions of the six datasets used for NMDS ordinations, and the questions targeted by each of the six ordinations. The various data groupings were chosen to better extract and compare phytoplankton species variation *between* seasons and years and develop hypotheses about long-term relationships among phytoplankton assemblage structure, individual nuisance or harmful species, and environmental factors. The 13 quantitative environmental variables were Neuse River discharge rates, temperature, salinity, DO, SS, TN, TKN, NH$_4^+$N, NO$_3^-$N, TP, SRP, silicate, and TN:TP ratios and the categorical variable was season. Rare species present in < 5% of the samples were removed and phytoplankton cell numbers were log-transformed prior to the analysis.

<table>
<thead>
<tr>
<th>Dataset</th>
<th>Species Matrix Dimensions</th>
<th>Environmental Matrix Dimensions</th>
<th>Targeted Questions</th>
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<tbody>
<tr>
<td>Full dataset, seasonal averages</td>
<td>70 species x 52 samples</td>
<td>14 parameters x 52 samples</td>
<td>How does phytoplankton composition vary seasonally?</td>
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<tr>
<td>Summer and fall samples, seasonal averages</td>
<td>68 species x 26 samples</td>
<td>14 parameters x 26 samples</td>
<td>How does summer and fall phytoplankton composition relate to year-to-year changes in environmental conditions?</td>
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<tr>
<td>Winter and spring samples, seasonal averages</td>
<td>70 species x 26 samples</td>
<td>14 parameters x 26 samples</td>
<td>How does winter and spring phytoplankton composition relate to year-to-year changes in environmental conditions?</td>
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<tr>
<td>Summer and winter samples, seasonal averages</td>
<td>67 species x 26 samples</td>
<td>14 parameters x 26 samples</td>
<td>How does summer and winter phytoplankton composition relate to year-to-year changes in environmental conditions?</td>
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<tr>
<td>All winter samples</td>
<td>43 species x 128 samples</td>
<td>13 parameters x 128 samples</td>
<td>How are environmental gradients related to abundances of winter bloom-forming species?</td>
</tr>
<tr>
<td>All summer samples</td>
<td>46 species x 126 samples</td>
<td>13 parameters x 126 samples</td>
<td>How are environmental gradients related to abundances of summer phytoplankton taxa?</td>
</tr>
</tbody>
</table>
Table 2. Phytoplankton taxa identified in acidic Lugol’s preserved samples from the mesohaline NRE (1994-2006). Asterisks (*) indicates species that are potentially toxic.

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**Phylum Chlorophyta**
Class Chlorophyceae
  Order Volvocales
    “Chlamydomonad” species
    *Golenkinia* spp.
    *Gonium* spp.
    *Pandorina* spp.
  Order Sphaeropleales
    *Ankistrodesmus* spp.
    *Crucigenia* spp.
    *Kirchneriella* spp.
    *Oocystis* spp.
    *Pediastrum duplex* Meyen
    *Scenedesmus* spp.
    *Selenastrum* spp.
  Order Zygnematales
    *Closterium* spp.
    *Staurastrum* spp.

Class Prasinophyceae
  Order Pyramimonadales
    *Pyramimonas* spp.

**Phylum Cryptophyta**
Class Cryptophyceae
  Order Cryptonodales
    *Cryptomonas* spp.
    *Chroomonas* spp.
    *Teleaulax amphioxeia* Conrad (Hill)

**Phylum Cyanobacteria (Phylum Cyanophyta)**
Class Chroococcales
  Order Chroococcales
    *Aphanocapsa* spp.
    *Chroococcus* spp.
    *Merismopedia* sp.
    *Merismopedia tenuissima* Lemmermann
    *Microcystis* spp.
    *Snowella* spp.
Table 2 (cont’d.)

<table>
<thead>
<tr>
<th>Class</th>
<th>Order</th>
<th>Species</th>
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<td>Class Dinophyceae</td>
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<td></td>
<td>Polykrikos kofoidii Chatton</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Polykrikos schwartzii Bütchli</td>
</tr>
<tr>
<td></td>
<td>Order Peridiniales</td>
<td>Ceratium furca (Ehrenberg) Claparède and Lachman</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cystodinium sp.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heterocapsa rotundata (Lohmann) Hansen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heterocapsa triqueta (Ehrenberg) Stein</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oblea rotunda (Lebour) Balech</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oxytoxum spp.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peridinium aciculiferum Lemmermann</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peridinium spp.</td>
</tr>
<tr>
<td></td>
<td><em>Pfiesteria piscicida</em> Steidinger et Burkholder</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Pfiesteria shumwayae</em> Glasgow et Burkholder</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Protoperidinium bipes (Paulsen) Balech</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Protoperidinium pellucidum Bergh</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Scrippsiella trochoidea (Stein) Loeblich III</td>
</tr>
</tbody>
</table>
Table 2 (cont’d.)

Order Prorocentrales
   *Prorocentrum micans* Ehrenberg
   *Prorocentrum minimum* (Pavillard) Schiller

**Phylum Euglenophyta**

Class Euglenophyceae
   Order Euglenales
      *Euglena* spp.
      *Eutreptia* spp.
      *Trachelomonas* spp.

**Phylum Haptophyta**

Class Haptophyceae
   Order Pavlovales
      *Pavlova* sp.
   Order Prymnesiales
      *Chrysochromulina* spp.

**Phylum Ochrophyta**

Class Bacillariophyceae
   Order Aulacoseirales
      *Aulacoseira* spp.
   Order Achnanthales
      *Achnanthes* spp.
      *Achnanthidium* spp.
      *Cocconeis* spp.
   Order Bacillariales
      *Cylindrotheca closterium* (Ehrenberg) Reimann et Lewin
      *Nitzschia* spp.
      *Pseudo-nitzschia* spp.
   Order Chaetocerales
      *Chaetoceros* spp.
   Order Coscinodiscales
      *Actinoptychus* spp.
      *Coscinodiscus* spp.
   Order Cymbellales
      *Cymbella* spp.
<table>
<thead>
<tr>
<th>Order</th>
<th>Genus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Order Fragilariales</td>
<td>Asterionella spp.</td>
</tr>
<tr>
<td></td>
<td><em>Asterionellopsis glacialis</em> (Castracane) Round</td>
</tr>
<tr>
<td></td>
<td><em>Synedra</em> spp.</td>
</tr>
<tr>
<td>Order Hemiaulales</td>
<td><em>Cerataulina pelagica</em> (Cleve) Hendey</td>
</tr>
<tr>
<td>Order Leptocylindrales</td>
<td><em>Leptocylindrus minimus</em> Gran</td>
</tr>
<tr>
<td>Order Naviculales</td>
<td><em>Gyrosigma</em> spp.</td>
</tr>
<tr>
<td></td>
<td><em>Navicula</em> spp.</td>
</tr>
<tr>
<td></td>
<td><em>Pinnularia</em> spp.</td>
</tr>
<tr>
<td>Order Rhizosoleniales</td>
<td><em>Dactyliosolen fragilissimus</em> Bergon (Hasle)</td>
</tr>
<tr>
<td></td>
<td><em>Rhizosolenia</em> spp.</td>
</tr>
<tr>
<td>Order Thalassionematales</td>
<td><em>Thalassionema nitzschioides</em> (Grunow) Mereschkowsky</td>
</tr>
<tr>
<td>Order Thalassiosirales</td>
<td><em>Amphora</em> spp.</td>
</tr>
<tr>
<td>Order Triceratiales</td>
<td><em>Cyclotella</em> spp.</td>
</tr>
<tr>
<td></td>
<td><em>Skeletonema costatum</em> (Greville) P.T. Cleve</td>
</tr>
<tr>
<td></td>
<td><em>Thalassiosira</em> spp.</td>
</tr>
<tr>
<td>Class Dictyophyceae</td>
<td></td>
</tr>
<tr>
<td>Order Dictyochales</td>
<td><em>Dictyocha fibula</em> Ehrenberg</td>
</tr>
<tr>
<td>Order Pedinellales</td>
<td><em>Apedinella radians</em> (Lohmann) Campbell</td>
</tr>
<tr>
<td>Class Raphidophyceae</td>
<td><em>Chattonella</em> spp.</td>
</tr>
<tr>
<td></td>
<td><em>Heterosigma akashiwo</em> (Hada) Hada ex Hada et Chihara*</td>
</tr>
</tbody>
</table>
Table 2 (cont’d.)

<table>
<thead>
<tr>
<th>Class Synurophyceae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Order Synurales</td>
</tr>
<tr>
<td><em>Mallomonas</em> spp.</td>
</tr>
<tr>
<td><em>Synura uvella</em> Ehrenberg</td>
</tr>
</tbody>
</table>
Table 3. Results of indicator species analysis for season. A significance level of $\alpha = 0.05$ was used to determine the indicator species for each season.

<table>
<thead>
<tr>
<th>SEASON</th>
<th>$n$</th>
<th>INDICATOR SPECIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fall</td>
<td>126</td>
<td><em>Akashiwo sanguinea</em>, <em>Amphidinium</em> spp., <em>Oxhyrris marina</em>, <em>Polykrikos</em> spp., haptophyte species</td>
</tr>
</tbody>
</table>
**Table 4.** Results of indicator species analysis for TN:TP ratios (molar basis). A significance level of $\alpha = 0.05$ was used to determine the indicator species for each TN:TP range.

<table>
<thead>
<tr>
<th>TN:TP</th>
<th>n</th>
<th>INDICATOR SPECIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 8:1</td>
<td>308</td>
<td><em>Anabaena</em> spp., <em>Dictyocha fibula</em>, euglenoids, <em>Oscillatoria</em> spp., <em>Pfiesteria</em> spp.-“pfiesteria-like” species-<em>Karlodinium veneficum</em>, <em>Protoperidinium</em> spp., <em>Scrippsiella trochoidea</em></td>
</tr>
<tr>
<td>≥ 8:1 and ≤ 16:1</td>
<td>161</td>
<td><em>Akashiwo sanguinea</em>, <em>Chaetoceros</em> spp., chlamydomonads</td>
</tr>
</tbody>
</table>
Table 5. Phytoplankton indicator taxa (abundant and consistently “faithful” to a particular group; significant index value $\alpha \leq 0.05$) determined by running indicator species analysis separately for two concentration ranges of each nutrient.

<table>
<thead>
<tr>
<th>NUTRIENT RANGE</th>
<th>n</th>
<th>INDICATOR SPECIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{NO}_3^-$ $&lt; 100$ $\mu g$ $L^{-1}$</td>
<td>371</td>
<td><em>Anabaena</em> spp., chlamydomonads, euglenoids, <em>Oscillatoria</em> spp., <em>Prorocentrum minimum</em>, <em>Protoperidinium pellucidum</em>, <em>Scrippsiella trochoidea</em></td>
</tr>
<tr>
<td>$\text{NO}_3^-$ $&gt; 100$ $\mu g$ $L^{-1}$</td>
<td>128</td>
<td><em>Cerataulina pelagica</em>, <em>Heterocapsa rotundata</em>, <em>Leptocylindrus minimus</em>, <em>Pseudo-nitzschia</em> spp., <em>Thalassiosira</em> spp.</td>
</tr>
<tr>
<td>$\text{NH}_4^+$ $&lt; 50$ $\mu g$ $L^{-1}$</td>
<td>402</td>
<td><em>Amphidinium</em> spp., <em>Dictyocha fibula</em>, <em>Prorocentrum minimum</em></td>
</tr>
<tr>
<td>$\text{NH}_4^+$ $&gt; 50$ $\mu g$ $L^{-1}$</td>
<td>97</td>
<td>Cryptomonads, <em>Cyclotella</em> spp., <em>Heterosigma akashiwo</em>, <em>Heterocapsa rotundata</em>, <em>Leptocylindrus minimus</em>, <em>Pseudo-nitzschia</em> spp.</td>
</tr>
<tr>
<td>TKN $&lt; 1500$ $\mu g$ $L^{-1}$</td>
<td>450</td>
<td>No significant indicators</td>
</tr>
<tr>
<td>TKN $&gt; 1500$ $\mu g$ $L^{-1}$</td>
<td>49</td>
<td><em>Amphidinium</em> spp., <em>Heterocapsa triquetra</em>, <em>Oblea rotundata</em>, <em>Peridinium</em> spp., <em>Prorocentrum minimum</em></td>
</tr>
<tr>
<td>SS $&lt; 20$ $\mu g$ $L^{-1}$</td>
<td>439</td>
<td>No significant indicators</td>
</tr>
<tr>
<td>SS $&gt; 20$ $\mu g$ $L^{-1}$</td>
<td>60</td>
<td><em>Dictyocha fibula</em>, <em>Heterocapsa triquetra</em>, <em>Oblea rotundata</em>, <em>Peridinium</em> spp., <em>Prorocentrum minimum</em></td>
</tr>
<tr>
<td>TP $&lt; 150$ $\mu g$ $L^{-1}$</td>
<td>361</td>
<td><em>C. pelagica</em>, <em>Chaetoceros</em> spp., <em>H. rotundata</em>, <em>H. triquetra</em>, <em>Leptocylindrus minimus</em>, <em>Rhizosolenia</em> spp., <em>Skeletonema costatum</em></td>
</tr>
<tr>
<td>TP $&gt; 150$ $\mu g$ $L^{-1}$</td>
<td>138</td>
<td><em>Akashiwo sanguinea</em>, <em>Anabaena</em> spp., <em>Gymnodinium</em> spp., <em>Oscillatoria</em> spp., <em>Oxytoxum</em> spp., <em>Pfiesteria</em> spp./“pfiesteria-like” species/<em>Karlodinium veneficum</em>, <em>Protoperidinium pellucidum</em></td>
</tr>
<tr>
<td>SRP $&lt; 50$ $\mu g$ $L^{-1}$</td>
<td>391</td>
<td><em>Chaetoceros</em> spp., <em>Heterocapsa rotundata</em>, <em>Heterocapsa triquetra</em>, <em>Leptocylindrus minimus</em>, prasinophytes, <em>Prorocentrum minimum</em>, <em>Rhizosolenia</em> spp., <em>Skeletonema costatum</em></td>
</tr>
<tr>
<td>SRP concentrations $&gt; 50$ $\mu g$ $L^{-1}$</td>
<td>108</td>
<td><em>Anabaena</em> spp., <em>Chroococcus</em> spp., <em>Dictyocha fibula</em>, euglenoids, <em>Oscillatoria</em> spp., <em>Oxytoxum</em> spp., <em>Pfiesteria</em> spp./“pfiesteria-like” species/<em>Karlodinium veneficum</em>, <em>Protoperidinium pellucidum</em>, <em>Scrippsiella trochoidea</em></td>
</tr>
</tbody>
</table>
Table 6. Results from the BIO-ENV analyses, showing the best overall combination of environmental parameters ($k =$ the number of parameters with the largest rank correlation [$\rho_s$] between biotic and environmental matrices) for each individual season and for the whole dataset.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>$k$</th>
<th>BEST VARIABLE COMBINATION ($\rho_s$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summer</td>
<td>4</td>
<td>Temperature, TKN, TN, NO$_3^-$, NO$_3^{+}$N, NH$_4^{+}$N ($0.36$)</td>
</tr>
<tr>
<td>Fall</td>
<td>3</td>
<td>TN:TP, river discharge, NH$_4^{+}$N, SRP ($0.27$)</td>
</tr>
<tr>
<td>Winter</td>
<td>5</td>
<td>Salinity, TN:TP, NH$_4^{+}$N, silicate ($0.49$)</td>
</tr>
<tr>
<td>Spring</td>
<td>4</td>
<td>Temperature, river discharge, NH$_4^{+}$N ($0.37$)</td>
</tr>
<tr>
<td>Full dataset</td>
<td>2</td>
<td>Temperature, TN:TP ($0.37$)</td>
</tr>
</tbody>
</table>
Figure Legends

**Figure 1.** Current and historic sampling stations for salinity, temperature, dissolved oxygen, suspended solids, nutrient concentrations, phytoplankton biomass as chlorophyll *a*, and phytoplankton assemblages in the Neuse River Estuary, NC. Samples were collected during biweekly (April – October) to monthly (November – March) sampling from 1994 through 2006.

**Figure 2.** Seasonal mean (*n* = 10 samples/season/year, corresponding with samples chosen for phytoplankton analysis) (A) Neuse River discharge rates measured by U.S. Geological Survey at Kinston (about 70 km upstream from the upper edge of the study area at Mills Branch) in m³ s⁻¹, (B) Salinity, (C) Temperature, (D) Suspended solids, (E) Photosynthetically active radiation (PAR), (F) Dissolved oxygen at depth 1.5 m, (G) TN, (H) TP, (I) TN:TP, and (J) NH₄⁺N from 1994 to 2006.

**Figure 3.** Examples of phytoplankton found in samples from the mesohaline NRE (scale bars = 20 µm): A) *Prorocentrum minimum*, B) *Odontella mobiliensis*, C) *Eutreptia* sp., D) *Aulacoseira* sp., E) *Heterocapsa rotundata*, F) *Gyrosigma* sp., G) *Heterocapsa rotundata*, H) *Scrippsiella trochoidea*, I) *Pseudo-nitzschia* sp., J) *Chaetoceros* sp., K) *Oxyphysis oxytoxoides*, and L) *Coscinodiscus* sp.

**Figure 4.** Relative abundance (calculated from mean cell number, *n* = 10) of dinoflagellates, diatoms, cyanobacteria, and other flagellates in the NRE during A) Summer, B) Fall, C) Winter, and D) Spring from 1994-2006.

**Figure 5.** The three most abundant taxa (based on mean cell number, *n* = 10) in the mesohaline NRE for (A) summer and (B) fall of each year. Arrows indicate species that were among the two most abundant species for only one year.
**Figure 6.** The three most abundant taxa (based on mean cell number, \( n = 10 \)) in the mesohaline NRE for (A) winter and (B) spring of each year. Arrows indicate species that were among the two most abundant species for only one year.

**Figure 7.** Ordination of all samples by phytoplankton data, showing seasonal differences in environmental conditions and phytoplankton species composition. Each point on the diagram represents mean abundance of all phytoplankton species in 10 samples from a given season and year (the same points are shown in both panels). The relative distance between samples reflects relative similarity in species composition. (A) Vectors indicate strength and direction of environmental gradients (vector \( r^2 \) cutoff value = 0.10). (B) Vectors represent strength of species gradients (vector \( r^2 \) cutoff value = 0.25). Abbreviated taxa are *Heterocapsa rotundata, Heterocapsa triquetra, Prorocentrum minimum*, and *Protoperidinium pellucidum*.

**Figure 8.** Ordination of summer and fall samples by phytoplankton data, showing differences in environmental conditions and phytoplankton species composition between summer and fall seasons (years and points shown in the first panel are the same in the second panel). (A) Vectors indicate strength of environmental gradients (vector \( r^2 \) cutoff value = 0.20; TEMP \( \equiv \) temperature). (B) Vectors represent strength of species gradients (vector \( r^2 \) cutoff value = 0.40). Abbreviated taxa are *Leptocylindrus minimus, Merismopedia tenuissima, Oxyrrhis marina, Protoperidinium pellucidum*, and *Scrippsiella trochoidea*.

**Figure 9.** Ordination by phytoplankton data, showing differences in environmental conditions and phytoplankton species composition between winter and spring seasons (years and points shown in the first panel are the same in the second panel). (A) Vectors indicate strength of environmental gradients (vector \( r^2 \) cutoff value = 0.20). (B) Vectors represent strength of species
gradients (vector $r^2$ cutoff value = 0.40). Abbreviated taxa are *Heterocapsa rotundata, Heterocapsa triquetra, Leptocylindrus minimum, Prorocentrum minimum, Rhizosolenia* spp., and *Skeletonema costatum*.

**Figure 10.** Ordination by phytoplankton data, showing differences in environmental conditions and phytoplankton species composition between winter and summer seasons (years and points shown in the first panel are the same in the second panel). (A) Vectors indicate strength of environmental gradients (vector $r^2$ cutoff value = 0.10). (B) Vectors represent strength of species gradients (vector $r^2$ cutoff value = 0.50). Abbreviated taxa are *Cerataulina pelagica, Dictyocha fibula, Heterocapsa rotundata, Heterocapsa triquetra, Heterosigma akashiwo, Leptocylindrus minimus, Polykrikos hartmanii,* and *Skeletonema costatum*.

**Figure 11.** Ordination of 128 winter samples by phytoplankton taxa showing yearly differences in environmental conditions and phytoplankton species composition (the same points are shown in both panels). (A) Vectors indicate the strength and direction of environmental gradients (vector $r^2$ cutoff value = 0.10). (B) Vectors represent the strength of species gradients (vector $r^2$ cutoff value = 0.25). Abbreviated taxa are *Akashiwo sanguinea, Leptocylindrus minimus, Heterocapsa rotundata, Heterocapsa triquetra, Prorocentrum minimum,* and *Rhizosolenia* spp.

**Figure 12.** Ordination of 126 summer samples by phytoplankton taxa showing yearly differences in environmental conditions and phytoplankton species composition (the same points are shown in both panels). (A) Vectors indicate the strength and direction of environmental gradients (vector $r^2$ cutoff value = 0.10). (B) Vectors represent the strength of species gradients (vector $r^2$ cutoff value = 0.25). Abbreviated taxa are *Akashiwo sanguinea, Heterosigma akashiwo,* and *Merismopedia tenuissima.*
Figure 1
Figure 2
Figure 3
Figure 4
Figure 6
Figure 7
Figure 8
Figure 9
Figure 10
Figure 11