PICOPHYTOPLANKTON – AN IMPORTANT, UNEXPLORED COMPONENT OF THE NEUSE RIVER ESTUARY PHYTOPLANKTON COMMUNITY

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ABSTRACT

ALICIA GAULKE: Picophytoplankton – An important, unexplored component of the Neuse River Estuary phytoplankton community
(Under the direction of Hans Paerl)

Picophytoplankton (PP) is a dominant component of open ocean phytoplankton communities. Although this fraction may outcompete larger species in low nutrient, low light environments, its relative contribution is thought to decrease in eutrophic waters, focusing most PP research on oligotrophic systems. Recent studies suggest that PP may also be an important component of eutrophic estuarine phytoplankton assemblages. In the Neuse River Estuary (NRE) PP achieve significant biomass during certain seasons and conditions. In this study, PP biomass and primary productivity were determined to assess temporal and spatial variation of PP in the NRE. PP averaged 35.3-40.6% and 41.8-56.9% of the total biomass and primary productivity, respectively. Temperature, salinity, and nutrient concentrations were important in controlling PP dynamics; however, additional factors including grazing and estuarine residence time are likely influential. Little is known regarding the fate of PP carbon in estuaries, thus PP should not be overlooked when evaluating estuarine ecosystems.

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α	Light limited rate of photosynthesis
C	Carbon
Chl *a*	Chlorophyll *a*
DIN	Dissolved inorganic nitrogen
DIP	Dissolved inorganic phosphorus
DON	Dissolved organic nitrogen
h	Hour
HPLC	High performance liquid chromatography
in	Inch
km	Kilometer
L	Liter
m	Meter
mg	Milligram
ml	Milliliter
NH_4^+	Ammonium
NO_x	Nitrate + nitrite
NRE	Neuse River Estuary
P_m^B	Chlorophyll-specific light saturated rate of photosynthesis
PO_4^{3-}	Phosphorus (dissolved, inorganic)
PP	Picophytoplankton
s	Second

µCi	Microcurie
µg	Microgram
µm	Micrometer
µM	Micromolar
µmol	Micromole

CHAPTER 1

INTRODUCTION

Picophytoplankton (PP), the smallest size fraction of phytoplankton, is a ubiquitous and diverse component of marine and freshwater ecosystems (Waterbury et al. 1979, Johnson and Sieburth 1979, Chisholm et al. 1988, Stockner et al. 2000). While a definitive size structure has yet to be universally accepted, PP has been defined as the proportion of phytoplankton smaller than 3µm in length (Stockner and Antia 1986). Cyanobacterial genera such as *Synechococcus* and *Prochlorococcus* are known to comprise a large proportion of the picoplankton community, although recent studies have demonstrated that eukaryotic PP may also contribute significantly as well (Worden et al. 2004).

The small size of PP imparts many adaptive advantages that have likely contributed to their widespread abundance and distribution. Small cells have a greater surface area to volume ratio than larger cells, allowing for more resource (light and nutrients) acquisition area relative to internal cell structure. Small cells also have a thinner diffusive boundary layer surrounding their surface, allowing for more efficient nutrient uptake, and which is particularly advantageous in low nutrient environments (Raven 1986). Photon absorption rates are also higher for smaller cells, and hence PP is able to more efficiently utilize photons for photosynthesis and growth (Raven 1986). These adaptive advantages contribute to the overwhelming dominance of PP in low nutrient, low light environments.

Picophytoplankton can be responsible for a dominant proportion of the total phytoplankton biomass (Landry et al. 1996, Marañón et al. 2001) and primary production (Platt et al. 1983, Bell and Kalff 2001) in oligotrophic open ocean systems. Their relative contribution is, however, thought to decrease in more eutrophic waters where the higher nutrient uptake rates of larger phytoplankton species may lead them to outcompete smaller cells when nutrients are plentiful (Riegman et al. 1993). Thus, most research on the smaller phytoplankton size fraction has focused on open-ocean systems, and the potential importance of PP in eutrophic waters has not until recently been realized.

Estuaries are naturally rich in nutrients and are among the most productive systems on the planet. This high productivity, a result of their naturally eutrophic state, makes estuaries important natural and human resources. The phytoplankton community is inherently linked to the trophic status of an estuary, thus the types of phytoplankton inhabiting these systems must be well understood in order to accurately study and model the biogeochemical processes within the estuary.

Iriarte and Purdie (1994) studied photosynthetic picoplankton (< 1µm and < 3µm) in a southern England estuary and concluded that the contribution of PP decreases with increasing system biomass. According to their research, while PP in open ocean environments contribute more than 50% to total phytoplankton primary production, coastal system contribution should vary around 20% while PP contribution in estuaries should be less than 10%. Badylak and colleagues (2007) observed that cyanobacterial picoplankton were numerically dominant in Tampa Bay Estuary but were not dominant in terms of overall phytoplankton biovolume. Additionally, Ning et al. (2000) reported cyanobacterial

picoplankton was on average 15% of the total phytoplankton biomass in San Francisco Bay, and that their relative contribution decreased with increasing total phytoplankton biomass.

These studies and many others support the widely held assumption that the importance and relative contribution of PP decreases with increasing total system biomass. However, there exists a growing body of research demonstrating that this typical model of phytoplankton distribution in eutrophic versus oligotrophic waters may not be indicative of all estuarine systems. These studies have also shown PP are important, and often overlooked, components of food webs and biogeochemical cycles in estuaries—systems which are often nutrient over-enriched and eutrophic (Marshall and Nesius 1996, Phlips et al. 1999, Marshall 2002). Additionally, they demonstrate that PP can attain high biomass and dominate the total phytoplankton biomass in estuaries during certain seasons and conditions (Ray et al. 1989, Buchanan et al. 2005, Badylak and Phlips 2004, Murrell and Lores 2004, Phlips et al. 1999).

In Pensacola Bay, phytoplankton < 5µm averaged over 70% of the total phytoplankton community, with this trend being most significant during summer months (Murrell and Lores 2004). Warm summer temperatures, along with periods of high residence times, also contributed to *Synechococcus* blooms in Florida Bay (Phlips et al. 1999). Picoplanktonic cyanobacteria have also been shown to comprise a significant proportion of the phytoplankton biomass in the York River, a tributary of Chesapeake Bay (Ray et al. 1989). These studies suggest that high summer temperatures, periods of low river flow, and increased residence times are conditions favorable to high picoplankton abundance, particularly cyanobacterial species.

Despite their importance, PP have largely been overlooked in nutrient-productivity studies of estuaries, many of which are threatened by adverse effects of nutrient over-enrichment and eutrophication (hypoxia, fish kills, habitat loss) (Nixon 1995). Since estuarine phytoplankton communities are closely tied to eutrophication potentials (Malone et al. 1988, Cloern 2001, Paerl et al. 2003), numerically-dominant PP must be included in assessments of this process in these vitally important ecosystems.

In North Carolina's Neuse River Estuary (NRE), high nitrogen loads have promoted algal blooms, hypoxia, and fish kills (NC DENR 2001, Paerl et al. 1998; 2007). Despite recent efforts to reduce nitrogen loading, nutrient-enhanced eutrophication remains a pressing issue in the NRE (NC DENR 1998; 2001; Paerl et al. 2007). The roles and responses of the total phytoplankton community in relation to eutrophication have been extensively studied (Pinckney et al. 1997, Paerl et al. 1998, 2006). In the NRE, cyanobacteria, likely falling into the PP size fraction, are a dominant component of the phytoplankton community during the summertime (Pinckney et al. 1998, Paerl et al. 2003, Valdes-Weaver et al. 2006). Recent research suggests that in the NRE most of the carotenoid zeaxanthin, a diagnostic cyanobacterial indicator pigment, largely falls into the $< 3\mu m$ size fraction (Paerl et al. 2009, in press). However, size-fractionated analysis of the total phytoplankton biomass and primary productivity has not been a component of most of these earlier studies in the NRE. Periods of significant phytoplankton production are also concurrent with bottom water hypoxia events observed during summer months, a prime causative agent of fish kills in the estuary (Paerl et al. 1998). The potential contribution of PP to primary productivity warrants further investigation and is necessary to understand the causes and mechanisms underlying eutrophication and its harmful effects.

As mentioned earlier, eutrophication potentials in estuaries are closely tied to the phytoplankton community structure within these systems. In addition to overall species composition, community biomass and rates of primary productivity are often the most telling indicators of change within the environment (Paerl et al. 2007). Investigating the most influential environmental factors affecting seasonal and spatial patterns in phytoplankton biomass and productivity is necessary to predict the effects caused by variation in these factors. Time series analysis and complex modeling programs have been used to elucidate the obvious and not so obvious physical, chemical, and biological factors at work in the NRE (Rudek et al. 1991, Paerl et al. 2006, Arhonditsis et al. 2007). However, relatively little is known about the factors most influential to PP in this system. In the current study, an attempt was made to investigate a select few of the factors known to influence the total phytoplankton community, and to compare those influences between size fractions.

Studies of phytoplankton dynamics in nearly all temperate systems often show trends of highest system biomass during warm summer months, although exceptions do exist. Picoplankton is a ubiquitous component of the phytoplankton community, and while they are found from pole to pole, PP like cyanobacteria tend to favor warmer waters (Stockner et al. 2000, Paerl and Huisman 2008, 2009). Studies of PP in estuaries are few, and many are limited to warm summer months. Thus, the difference in responses between the PP and larger size fractions in estuarine systems is not fully known.

Dissolved inorganic nitrogen (DIN) is an important and often limiting form of nitrogen in estuarine systems. DIN, in the forms of ammonium (NH_4^+) and nitrate + nitrite (NO_x), have been shown to increase biomass and primary production in the NRE (Mallin et al. 1991, Boyer et al. 1994). Dissolved organic nitrogen (DON) is another significant

component of total nitrogen and may be an important source of nitrogen for some phytoplankton species in the NRE (Twomey et al. 2005). However, most research concerning the influence of nitrogen on phytoplankton biomass and productivity has focused on the most biologically-available inorganic component. Thus DIN (NO_x and NH_4^+) will be the only nitrogen species discussed in the current study. Dissolved inorganic phosphate (PO_4^{3-}) is not typically limiting in the NRE; however, it is a vital macronutrient required for photosynthesis and growth and was included as a potential influence on size-fractionated biomass and productivity.

There is strong evidence that PP accounts for a highly significant fraction of estuarine primary production, and studies suggest that they may at times be a significant component of the total phytoplankton community in the Neuse River Estuary (Pinckney et al. 1997, Paerl et al. 2003). The goal of this study was to analyze the spatial and temporal variation of PP in this eutrophic estuary. Recent research in the NRE and other eutrophic estuaries suggests that Iriarte and Purdie's (1994) estimate of low overall PP abundance at high total biomass does not hold true for all systems. I additionally compared abiotic parameters to size-fractionated biomass and primary productivity to estimate under what conditions (nutrient concentrations, salinity, and temperature) PP abundance varies, and to what degree these factors are important.

CHAPTER 2

MATERIALS AND METHODS

2.1 Study site

North Carolina's Neuse River Estuary is the largest sub-estuary of the Albemarle-Pamlico Sound System, which is the second largest estuary in the United States. The NRE is a shallow (2.2m average depth) oligohaline to mesohaline, microtidal estuary (Luettich et al. 2000). Relatively long residence times, on the order of weeks to many months, ensure that nutrients entering the system are effectively utilized and retained (Paerl et al. 1998; 2006). In the last few decades, the NRE's 16,000 km^2 watershed has undergone rapid increases in urban, row crop, and industrial-scale swine and poultry farming development, which has contributed to an increase in point and non-point sources of nitrogen (N) and phosphorus (P) (Stow et al. 2001). These events have contributed to symptoms of human-inducted eutrophication in the estuary, including increased algal biomass, algal blooms, bottom water hypoxia and anoxia, and fish kills (Stow et al. 2001, NC DENR 2001, Paerl et al. 1998; 2007). Phytoplankton blooms in the NRE have been closely linked with periods of enhanced runoff and nutrient loading, particularly N loading, although salinity and temperature regimes are also important factors controlling phytoplankton dynamics (Paerl et al. 1995, Cloern 2001, Peierls et al. 2003).

2.2 Sampling

Since 1994, the Neuse River Estuary Modeling and Monitoring Project (ModMon: http://www.unc.edu/ims/neuse/modmon/index.htm) has generated a continuous dataset aimed at assessing water quality trends on a long-term scale within the estuarine system (Luettich et al 2000). Sampling is conducted on a biweekly basis at 11 fixed sites along the central axis of NRE. For this study, five of these stations—representative of the range of NRE salinity and hydrology regimes—were selected for size-fractionated chlorophyll *a* (Chl *a*), primary productivity, and picoplankton cell enumeration (Fig. 1). The upper estuarine stations, 30 and 50, are river-dominated and as such most strongly affected by allochthonous nutrient loading. The mesohaline mid-estuarine stations 70 and 120 mark an area in which the estuary widens and residence time increases. In this part of the estuary, primary production is controlled by both allochthonous "new" nutrient inputs and regenerated nutrients (Christian et al. 1991; Paerl et al. 1995). The meso-polyhaline station 180 is located at the mouth of the estuary and is strongly influenced by regenerated production and exchange with Pamlico Sound, into which the NRE flows (Christian et al. 1991; Peierls et al. 2003). Sampling was conducted from June 2007 through September 2008 on a biweekly basis, except for the months of November 2007 to February 2008, when the estuary was sampled on a monthly basis. No samples were collected for station 180 on September 17, 2007 and October 31, 2007 due to rough weather conditions.

Physical (temperature, irradiance), and chemical (salinity, nutrients, pH) conditions at 0.5m intervals from near-surface to 0.5m above the sediments are also recorded at each station as part of the ModMon project. For the purpose of this study, only the data collected from near-surface waters are reported here, as PP analysis was conducted on only near-

surface samples. At each of the five previously mentioned stations, near-surface water samples were collected by hand or pump and stored in translucent polyethylene bottles in the dark for transport to the laboratory. All water samples were collected between 9:00 AM and 2:30 PM and transported back to the laboratory where subsamples were filtered immediately for determinations of Chl *a* concentrations and the following day for rates of primary productivity. It has been shown that PP is heavily grazed by microzooplankton (Lewitus et al. 1998; Juhl and Murrell 2005); therefore, overnight storage may lead to an underestimation of productivity parameters. Due to time constraints, it was not possible to determine rates of primary productivity on the same day samples were collected. However, total Chl *a* concentrations measured immediately after sampling on July 9, 2008 and after overnight storage on the morning of July 10, 2008 indicate that 6.1 to 25.0% of biomass may be lost overnight (Table 1). This may also have lead to underestimated rates of primary productivity.

2.3 Chlorophyll *a* size-fractionation and analysis

Phytoplankton biomass was estimated using fluorometrically-determined Chl *a* measurements. 50ml whole water subsamples from each station were filtered in duplicate under a gentle vacuum (< 5 in. Hg) through Millipore glass fiber filters (nominal pore size of 0.7µm) and Whatman GF/D glass fiber filters (nominal pore size of 2.7µm). Beginning on November 27, 2007, 50ml whole water subsamples were also filtered in duplicate through 3.0µm porosity polycarbonate Poretics membrane filters. Due to a discontinuation of this filter type, on the July 9, 2008 sampling date, I switched from 3.0µm Poretics membrane

filters to 3.0µm Millipore Isopore™ membrane filters. Preliminary tests revealed no significant difference in biomass retention between filter types (Table 2).

Two 50ml subsamples of filtrate from both GF/D and 3.0µm filtered samples were also collected on Millipore glass fiber filters. Chl *a* measurements on these pre-filtered samples represent chlorophyll concentrations from the picoplanktonic size fraction. Chl *a* values obtained from GF/D and 3.0µm filters were subtracted from Chl *a* values obtained from Millipore filters (total Chl *a*) to calculate picoplankton Chl *a* concentrations. Glass fiber and membrane filters were homogenized (by grinding), extracted overnight in 90% acetone, and analyzed using a Turner Designs TD-700 fluorometer.

During sample collection and analysis, it was noted that measurements of Chl *a* concentrations retained on GF/D filters and 3.0µm membrane filters differed noticeably. Based on a review of the literature, it was determined that membrane filters provide a more accurate estimate of biomass than GF/D filters due to problematic clogging effects when using glass fiber filters. Therefore, conversion factors were determined using the strongly linear relationship between GF/D and membrane filter Chl *a* concentrations from November 29, 2007 through September 29, 2008 and applied to GF/D only Chl *a* concentrations from June 12, 2007 through October 31, 2007. Correction factors were calculated individually for each station, and are shown in Table 3.

2.4 Primary productivity size-fractionation and analysis

Primary productivity measurements were conducted at approximately the same time each morning the day after sample collection, according to Paerl (2002). Water samples were stored overnight in ambient light and temperature conditions in outdoor ponds filled

and continually flushed with Bogue Sound water. Six light and one dark 125 ml Pyrex reagent bottles were filled with water from each NRE station and 0.3 ml ^{14}C-$NaHCO_3$ (58 $\mu Ci\ \mu mol^{-1}$ specific activity). The bottles were incubated for approximately 4 hours under a gradient of light conditions (100%, 45%, 28%, 15%, 8%, and 4% maximum irradiance) in an indoor environmental chamber, with the temperature controlled to match the average NRE surface water temperature during the sampling date. The light supply consisted of 6 Sylvania 48 in., 34-Watt fluorescent bulbs suspended above the samples, and 0, 1, 2, 3, 4, or 6 black screens placed over the bottles were used to obtain the gradient of six light conditions described above. Irradiance flux readings were made for each light condition, using a Biospherical Instruments QSL-100 irradiance meter.

At the end of the incubation period, 50 or 75 ml subsamples were filtered from each bottle onto glass fiber Millipore filters, GF/D, and (beginning November 29, 2007) Poretics or Millipore Isopore^{TM} 3.0µm membrane filters. A subtraction method used to determine picoplankton Chl *a* concentrations was also used to determine rates of primary productivity for the picoplankton. Additionally, correction factors to account for differences between GF/D and 3.0µm membrane filters were calculated and applied for productivity rates obtained from June 12, 2007 through October 31, 2007 (Table 3).

Filters were fumed for at least 2 hours with concentrated HCl to remove abiotically precipitated ^{14}C-$NaHCO_3$, placed in vials filled with 5ml CytoScint scintillation cocktail, and counted on a Beckman Coulter LS 6500 Multi-Purpose Scintillation Counter. The dissolved inorganic carbon content of the water samples was determined using a Shimadzu Total Organic Carbon Analyzer (TOC-5000A).

Rates of carbon fixation and Chl *a*-based biomass obtained for each size fraction and irradiance measurements for each light incubation level were used to calculate photosynthetic rate parameters using the computation and visualization program, MATLAB. This program utilized the hyperbolic tangent function of Jassby and Platt (1976) to fit photosynthesis-irradiance curves for the > 3 and < 3µm size fractions. These curves were used to calculate the chlorophyll-specific light saturated rates of photosynthesis (P_m^B) and the slope (α) of each curve—the light limited rate of photosynthesis. These rate parameters were used to compare differences in photosynthetic efficiencies of the two size fractions; however, due to inconsistencies in the data likely arising from light incubation chamber, the values were not used to compare overall trends in primary productivity over space and time. Rather, non-chlorophyll corrected rates of primary productivity (mg C m^{-3} h^{-1}) obtained at 45% total irradiance (maximum irradiance reduced by one screen) were included in all time series and linear regression analysis. The average irradiance flux for this light level was found to be 381.8 µmol m^{-2} s^{-1}, a rate well above those used in light-limitation experiments (Huisman 1999) and similar to those at which phytoplankton have been found to be light saturated (Ray et al. 1989, Boyer et al. 1993). Based on results presented in this study, this light level was not likely to induce photoinhibition.

2.5 Cell counts

Beginning September 4, 2007, 10ml whole water samples from each station were preserved in duplicate with gluteraldehyde (1% w/v final concentration), and refrigerated in the dark for slide preparation. Five ml of each fixed sample was filtered under gentle vacuum (< 5 in. Hg) onto 0.2µm black stained Nuclepore membrane filters. Each filter was

mounted on a glass slide, covered with a drop of immersion oil and glass coverslip, and stored in the dark at -20˚C. Each slide was then photographed using a Nikon Eclipse E800 epifluorescence microscope fitted with an Olympus DP71 digital camera. A blue (497) and green (571) exciter filter, dichromatic mirrors (375-410 and 513-552), and green (531) and red (627) barrier filters were used. These filter sets are similar to those recommended by MacIsaac and Stockner (1993) for enumerating autotrophic picoplankton. Thirty fields of view were photographed under 400X or 1000X magnification, and depending on cell concentrations a subset of those fields photographed were randomly selected and counted for PP enumeration. At high cell concentrations (< 50 cells per field of view), no fewer than 10 fields of view were counted, while at low cell concentrations (> 30 cells per field of view), up to 25 fields of view were counted. Using this method, at least 500 cells were counted from each sample, except for during periods of very low cell concentrations where counting all fields of view photographed would not have reached the 500 cell goal. These events were limited to late winter and early spring months.

Putland and Rivkin (1999) recommend that if picoplankton samples cannot be enumerated immediately, that they be filtered and frozen for storage rather than refrigerated in suspension. However, due to an unavoidable delay in the delivery of necessary filtration supplies, samples were occasionally refrigerated for several weeks before subsamples were filtered, frozen, and photographed. Qualitative analysis of samples photographed after refrigerated versus frozen storage indicates that fading of picoplankton fluorescence was reduced when samples were refrigerated rather than frozen, contrary to the results of Putland and Rivkin (1999). Additionally, fading of cells after storage seemed to be exacerbated in

cooler months (winter and early spring), when total Chl *a* concentrations and cell abundance were low.

2.6 Factors influencing phytoplankton biomass and productivity

A YSI multiparameter sonde equipped with a conductivity/temperature probe (Model 6560) was used to collect temperature and salinity measurements throughout the study period as part of the NRE ModMon program. NO_x, NH_4^+, and PO_4^{3-} concentrations were determined using a Lachat/Zellweger Analytics Quick Chem 8000 flow injection autoanalyzer (Milwaukee, WI, USA). Water samples were vacuum filtered using pre-combusted Whatman glass microfibre filters (GF/F). The filtrate was stored in high-density polyethylene bottles and frozen at -20˚C until analysis. Two replicates were run from the same bottle. If any nutrient were below the detection limit they were taken to be zero for the particular sampling date.

The statistical program, R (The R Foundation for Statistical Computing, version 2.4.1), was used for all statistical tests conducted on the data. Multiple linear regression models were fitted to response variables, natural-log transformed size-fractionated Chl *a* and primary productivity measurements pooled across stations and the following potentially explanatory variables: temperature, salinity, NO_x, NH_4^+, and PO_4^{3-}. Nutrient concentrations were natural-log transformed for model fitting while temperature and salinity measurements were not. Because values of zero cannot be log transformed, a correction of 0.001 was added to all values within a data set containing zeros. This small addition solved the log transformation problem and did not significantly change the nature or interpretation of the data.

Despite expectations that temperature and salinity would be positively correlated, these two variables were not significantly related, even though similar seasonal patterns existed for both. This is possibly due to the drought conditions from the summer of 2007, which caused salinity to remain high through the winter while temperature decreased. Temperature was correlated with nutrient concentrations, as was salinity; therefore neither temperature and nutrients nor salinity and nutrients were included together as explanatory variables in the multiple linear regression models. Thus, physical factors (temperature and salinity) and chemical factors (nutrients) were always grouped together in order to determine the influence and interactions of the selected explanatory variables.

2.7 Diagnostic photopigments

Phytoplankton photopigments (chlorophylls and carotenoids) were identified, separated, and quantified by high performance liquid chromatography (HPLC) coupled to an in-line photodiode array spectrophotometer (Jeffrey et al. 1997). Functional groups within the phytoplankton community can be identified based on photopigments specific to certain taxonomic groups. Diagnostic photopigments analysis has been conducted as a regular component of the ModMon sampling program since 1994, and a complete summary of the methods used can be found in Pinckney et al. (1998). Since 2006, size-fractionated diagnostic pigment analysis has been conducted at stations 70 and 180. In the current study, zeaxanthin concentrations were compared to fluorometrically-derived Chl *a* concentrations to determine the proportions of each photopigment falling into the PP size fraction.

CHAPTER 3

RESULTS

3.1 Abiotic environmental parameters

At any one time, the average surface temperature of the Neuse River Estuary varied little across stations (Fig. 2). The minimum temperature for all stations occurred on January 15, 2008 and ranged from 9.7˚C at station 180 to 10.5˚C at station 30. Maximum temperatures occurred on various sampling dates during mid-summer months of 2008 and ranged from 29.5˚C at station 180 to 31.3˚C at station 30. From June through September in both years and at all stations, surface water temperatures exceeded 25˚C. At these temperatures cyanobacteria exhibit maximum growth rates, approaching those of eukaryotic phytoplankton species such as diatoms and chlorophytes (Paerl and Huisman 2008).

Surface salinity was consistently lowest at station 30 and always increased with distance downstream. Maximum salinities at all stations were almost always observed during summer months (station 30 salinity maximum occurred in November of 2007), and minimum salinities occurred during the spring or early summer. Salinity trends differed between summers as a result of slightly lower mean monthly river flow from spring through fall, 2007, compared to 2008 (data not shown, US Geological Survey station 02091814). Persistent drought conditions of 2007 resulted in lower discharge, and extended periods of high summer salinity into the fall and winter. However by the end of the 2008 summer, rainfall increased and salinity sharply declined at most stations (Fig. 3).

Variation over time of nitrogen species for each station is shown in Figure 4. Concentrations of NO_x, representing allochthonous or "new" sources of DIN to the estuarine system, were highest during the spring, concurrent with periods of increased river flow. The highest concentrations of NO_x were observed at station 30, and they were rapidly attenuated downstream. At stations 120 and 180, NO_x levels were below detection throughout the entire timeline of this study. Additionally, NO_x levels were frequently below detection during mid-summer through early winter of 2007, and throughout the summer of 2008 in upper and mid-estuarine stations. While NH_4^+ is often a preferred form of DIN taken up by phytoplankton, NO_x is also rapidly utilized by Neuse River Estuary phytoplankton (Boyer et al. 1994, Twomey et al. 2005) and is an important factor influencing rates of primary productivity.

Similar to NO_x, NH_4^+ concentrations (recycled N) were lowest during summer months and highest in the spring (Fig. 5). These results were consistent with those reported by Boyer et al. (1994), although Paerl and colleagues (1995) observed no consistent seasonal pattern of NH_4^+ concentrations. Internal recycling of NH_4^+ released from sediments may contribute to higher NH_4^+ concentrations during summer months, although such was not observed during the time period of the current study. NH_4^+ is not a large component of DIN from runoff, so the attenuation effect of NH_4^+ with increasing salinity was less pronounced than for NO_x. Averages of summer NH_4^+ concentrations in 2007 were slightly lower than in 2008, ranging from 0.39 to 0.60 µM and 0.82 to 1.81 µM respectively.

Dissolved inorganic phosphorus (as PO_4^{-3}) concentrations also varied seasonally, but were highest during the summer, concurrent with periods of high temperature (Fig. 6). PO_4^{-3}

concentrations remained consistently low throughout the winter and spring at both stations. High summer concentrations were similar between years, although stations 50 and 120 were slightly higher in 2007.

3.2 Chlorophyll *a*

Size-fractionated Chl *a* concentrations were highly variable over space and time. At all stations, Chl *a* concentrations varied by season but were generally lowest in the winter and highest during summer months. Average seasonal Chl *a* concentrations in the > 3µm size fraction were similar in the spring through fall, but lower in the winter, however the < 3µm fraction showed similarly low Chl *a* values in the spring and winter months, with highest averages concentration in the summer and fall (Fig. 7). Additionally, peaks and maximum Chl *a* concentrations occurred almost exclusively during summer months for stations 30 and 50, while distinct spring peaks were observed at stations 70, 120, and 180, although summer peaks were observed as well (Figs. 8-12). Concentrations averaged over the entire study period showed a decrease in total Chl *a* from upper to lower estuarine regions, as did average concentrations for the > 3µm size fraction. Averages corresponding to the < 3µm size fraction showed a similar trend, although the station 120 average was second highest to station 30, followed by stations 50, 70, and 180 (Table 4).

Picoplankton relative contribution to total Chl *a* was also highly variable over space and time, although seasonal trends were more difficult to discern than for directly measured Chl *a* concentrations. Nearly all stations saw peaks in biomass in the < 3µm size fraction contribution to phytoplankton biomass during summer or fall months (74.5% in July 2007, 71.3% in October 2007, 74.5% in July 2007, 68.7% in August 2008 for stations 30, 50, 120,

and 180, respectively). The only exception was station 70, where the highest relative contribution (61.3%) was observed on January 15, 2008; however, most other peaks at station 70 occurred during summer months. This was somewhat unexpected, since summer and fall months were also periods of high total biomass—conditions under which the percent contribution of PP is expected to decrease. However, nearly all peaks in biomass for the > 3µm size fraction co-occurred with a minimum relative contribution of the smaller size fraction. Similar to station 70, station 120 also had high relative contribution of the < 3µm size fraction from the late fall through early winter, although relative contribution decreased to < 10% for both stations by the February sampling date. The February decline in PP relative abundance was accompanied by an increase in total system biomass. Increases in total system biomass during the spring of 2008 corresponded at all stations to a decrease in relative picoplankton contribution. This indicates that the spring bloom was likely dominated by larger phytoplankton species.

Surprisingly, there was little variation between stations in the average relative contribution of the < 3µm size fraction, while total Chl *a* showed more pronounced variation (Table 4). The average relative contribution at station 30 (40.6%), with the highest average total Chl *a* (31.7 µg L^{-1}), was nearly identical to the average relative contribution at station 180 (40.2%) which had the lowest total Chl *a* (9.2 µg L^{-1}). This observation is in direct contrast to the paradigm of decreasing picoplankton relative abundance with increasing total system biomass (Iriarte and Purdie 1994, Stockner et al. 2000). Figure 13 shows a slightly inverse relationship between PP relative abundance and total phytoplankton biomass, although considerable scatter at high and low biomass suggests that the above-mentioned paradigm does not always hold true in the NRE.

3.3 Primary productivity

Size-fractionated primary productivity measurements also showed considerable variation over space and time. For both size fractions, fluctuations in Chl *a* biomass were nearly always positively correlated with fluctuations in rates of carbon fixation, however this positive relationship was stronger for the > 3µm than for the < 3µm size fraction ($r^2 = 0.49$ and $r^2 = 0.28$, respectively). This may potentially indicate a bias due to differential chlorophyll extraction efficiencies between phytoplankton species, particularly poorer extraction obtained for cyanobacteria (Stauffer et al. 1979). However, our extraction method included filter grinding, which has been shown to improve chlorophyll extraction from cyanobacteria (Wasmund et al. 2006).

Like Chl *a*, rates of primary productivity for both size fractions demonstrated a seasonal trend, with slightly higher rates during the summer compared to winter months. For the > 3µm size fraction, seasonally averaged rates of productivity were highest during the fall and spring, with lower rates in the summer and winter (Fig. 14). Summer productivity, however, always exceeded winter productivity. Rates of productivity were highest in the summer and fall for the > 3µm fraction and similarly low during winter and spring months. However, maximum rates and other peaks in both the large and small fractions occurred at various times year-round. Productivity rates averaged by station over the duration of the study decreased from station 30 to station 180 in the > 3µm size fraction, although average rates in the mid-estuarine region (stations 50-120) varied little compared to upper and lower regions. For the < 3µm size fraction, average rates of primary productivity remained relatively constant from station 30 to 120, and only decreased somewhat at station 180 (Table 4).

The PP size fraction contributed significantly to primary productivity at all stations and during all seasons, rarely falling below 25% contribution at any location or sampling date (Figs. 15-19). As with rates of carbon fixation, the relative contribution to total carbon fixation for the < 3µm size fraction was at a maximum during summer and fall months for all stations. Though slightly lower during winter months, relative contribution was always > 25%, and occasionally > 50% at stations 70, 120, and 180. These periods of high relative contribution corresponded to periods of low total system biomass and primary productivity. Likewise, periods of low relative contribution occurred at all stations during the spring and were often concurrent with periods of high total system biomass.

A comparison of the size fractions indicated that the < 3µm fraction of PP contributes more to total carbon fixation than to total Chl *a* (Fig. 20), (i.e., the majority of points fall above the $y = x$ line). This may be attributed to the adaptive advantages of PP incurred by their small size, specifically a higher efficiency of light and nutrient utilization (Raven 1986).

3.4 Photosynthetic parameters

The computation and visualization program, MATLAB, was implemented to calculate photosynthetic parameters using the hyperbolic tangent function of Jassby and Platt (1976). Due to possible deficiencies in the design of the light gradient incubation chamber, calculated values of P_m^B and α were occasionally highly variable and therefore somewhat suspect. The model could not calculate photosynthetic parameters for several sampling dates, while other calculations were unrealistically high (P_m^B > 2500 mg C (mg Chl *a*) $^{-1}$ hr^{-1}). Therefore, in comparing P_m^B and α across size fractions, the medians rather than the means of each station were analyzed. Figure 21 shows boxplots of P_m^B values calculated for

each station and size fraction, pooled over time. The > 3µm size fraction typically had a wider range of P_m^B than did the larger size fraction. Spatially, median values of the different size fractions were relatively similar. However the smaller size fraction typically had higher P_m^B values than did the larger size fraction, although this difference is only statistically significant at station 70. Similarly, α values for each station showed a wider range of variability in the < 3µm size fraction (Fig. 22). Values within the same size fraction were similar between stations, though the medians were consistently higher for the smaller fraction. Only at station 30 were the differences between median α values not statistically significant.

3.5 Picophytoplankton biomass – cell counts

All PP observed were coccoid or oval-shaped, although long filaments were observed at several stations. While the individual cells within filaments fell into the PP size fraction, the length of each chain far exceeded the PP size definition. In this study, such filaments were not counted as a component of the PP community. Picophytoplankton appeared mainly as single cells or pairs of cells, though colonial PP were also observed in grape-like bunches and short chains. Most cells fluoresced red, although orange-fluorescing cells were also observed. Depending on their photosynthetic pigments, cyanobacterial picoplankton such as *Synechococcus* spp. may fluoresce red or orange (MacIsaac and Stockner 1993). For the purpose of this study, both red and orange cells were counted as a component of the picoplankton size fraction, and no effort was made to differentiate the abundances of the two color classes. The intensity of cell fluorescence fluctuated over space and time. Cells were generally dimmer during winter and early spring months and also in lower regions of the

estuary. Nevertheless, brightly fluorescing cells were often observed at all sampling stations, especially during summer months.

Picophytoplankton cell concentrations varied from 3.3×10^6 to 4.3×10^9 L^{-1} across all stations (Fig. 23). A seasonal trend was apparent with the highest cell concentrations observed in the summer on June 9, 2008 for stations 30 though 120 and on August 5, 2008 for station 180. Minimum cell concentrations were observed in the spring (April 14, 2008) for the upper estuarine stations 30 and 50, while minimum concentrations for mid and lower estuarine stations 70, 120, and 180 occurred in the winter on January 15, 2008. There was a strong positive correlation between PP cell abundance and temperature—a widely documented trend (Weisse 1993, Stockner et al. 2000, Iriarte and Purdie 1994, Phlips 1999, Murrell and Lores 2004).

3.6 Factors influencing variation in phytoplankton biomass and productivity

Table 5 provides a summary of all multiple linear regression models and results. The first model regressed size-fractionated Chl *a* and primary productivity (separately) against temperature and salinity. As previously mentioned, temperature was relatively constant throughout stations at any given time and showed uniform seasonal variation. Salinity increased from station 30 to 180 and was highest from summer through fall and lowest in the spring. Previous studies indicate that total phytoplankton biomass and productivity are positively correlated with temperature and negatively correlated with salinity. The model in the current study indicated that the > 3µm size fraction of Chl *a* was positively correlated with temperature and negatively correlated with salinity ($p < 0.01$), while the < 3µm fraction was positively correlated to temperature alone ($p < 0.001$). Likewise, the smaller size

fraction of primary productivity was strongly correlated only with temperature ($p < 0.001$), however temperature did not appear to influence the larger fraction of productivity but showed a negative correlation with salinity ($p < 0.01$).

The second multiple linear regression model regressed Chl *a* and primary productivity (separately) against NO_x and PO_4^{3-}. Based on previous studies, a positive correlation was expected between phytoplankton biomass and productivity and both nutrients (Mallin et al. 1991, Paerl et al 2006). The model indicated that the > 3µm size fraction of Chl *a* was not significantly influenced by NO_x or PO_4^{3-} alone ($p > 0.1$), although there was a slightly significant ($p < 0.05$) influence of their interactive effects. Neither was NO_x or PO_4^{3-} a significant influence on the > 3µm fraction of productivity, as predicted by the model. The < 3µm fraction of Chl *a* was positively related to PO_4^{3-} only ($p < 0.01$), while both nutrients strongly influenced the smaller fraction of productivity ($p < 0.001$). NO_x was negatively related and PO_4^{3-} was positively related to productivity.

The third multiple linear regression model regressed Chl *a* and primary productivity (separately) against NH_4^+ and PO_4^{3-}. Similar to the second model, a positive correlation was expected between phytoplankton Chl *a* and productivity and the nitrogen species, in this case NH_4^+. However, the > 3µm size fraction of Chl *a* was negatively correlated with NH_4^+ ($p < 0.01$) and strongly positively correlated with PO_4^{3-} ($p < 0.001$), although no interaction effects were predicted. The < 3µm fraction was also negatively correlated with NH_4^+ ($p < 0.01$) but not influenced by PO_4^{3-} alone, although the model indicated the interaction effects of the nutrients significantly influenced Chl *a* ($p < 0.01$). PO_4^{3-} was positively related to the

larger size fraction of primary productivity ($p < 0.001$) but not NH_4^+, while both nutrients strongly influenced the < 3µm size fraction of productivity ($p < 0.001$).

3.7 Diagnostic photopigments

For the past decade, photopigment analysis has provided important insights into abundance and distribution of the major taxonomic groups of phytoplankton in the NRE (Pinckney et al. 1998, Valdes-Weaver et al. 2006). Zeaxanthin is typically used as an indicator of cyanobacteria, and size-fractionated diagnostic photopigment analysis indicates that most of the zeaxanthin at stations 70 and 180 is limited to the < 3µm size fraction, most likely represented by cyanobacteria (Paerl et al. 2009, in press). In the current study,zeaxanthin concentrations were typically highest during warmer months, which was indicative of optimal growth conditions for cyanobacteria (Paerl and Huisman 2008). Additionally, Chl *a* and zeaxanthin concentrations in the < 3µm size fraction at stations 70 and 180 were strongly correlated (Spearman correlation test, $p < 0.01$ and $p < 0.001$, respectively), and pigment concentrations at each station varied concurrently over time (Figs. 24-25). These results provided further evidence that size-fractionated zeaxanthin was a good indicator of picoplankton biomass (c.f. Pinckney et al. 2001), and that this smaller size fraction was composed largely of cyanobacteria.

CHAPTER 4

DISCUSSION

4.1 Picophytoplankton biomass

Total Chl *a* concentrations and rates of primary productivity obtained in this study were similar to those previously reported in the NRE (Boyer et al. 1993). However, these results show slightly different Chl *a* and productivity distribution trends than what is typically expected for this system. In the NRE, the Chl *a* maximum is usually located within the mid-estuary (stations 70-120), as biomass accumulates where the system widens and residence times increase (Valdes-Weaver et al. 2006). In the present study, biomass and productivity were greatest in the upper estuary (station 30) and diminished downstream (Table 4), and the summer of 2007 was marked by a prolonged drought. Drier than normal conditions most likely shifted the Chl *a* maximum towards the upper estuary, with highest Chl *a* concentrations observed at station 30 from the summer through fall of 2007. Rainfall and river flow increased in the winter and spring of 2008 (data not shown), shifting the Chl *a* maximum further downstream. As a result, Chl *a* concentrations at mid-estuarine stations often exceeded those of station 30 from the winter of 2008 to the end of the study period (Figs. 8-12).

At all stations, the > 3µm size fraction of primary productivity and Chl *a*-specific biomass were more strongly correlated with total productivity and biomass than the < 3µm fraction. Additionally, the variations within the > 3µm fraction appeared to closely track

variations in both total Chl *a* biomass and primary productivity. Although the larger fraction typically contributed a larger proportion to total Chl *a* and productivity than the PP size fraction, dominance by the < 3µm fraction was observed at all stations during most seasons. In fact, PP contribution to productivity and biomass averaged 41.8-55.4% and 35.3-43.7%, respectively, over the course of the study period (Table 4), indicating PP may play an important role along the entire length of the estuary.

Barber and Hiscock (2006) demonstrated that growth conditions favoring the onset of oceanic diatom blooms will similarly affect the non-diatom community, including picoplankton. They cite results from the IronEx-1 and IronEx-2 ocean fertilization experiments to show that the background PP signal is "overprinted" rather than replaced by blooming diatoms. Both size fractions increase their biomass and growth rates in response to nutrient additions, and diatoms accumulate faster than their grazers can consume them while grazing of PP increases concurrently. Nutrient loading to the NRE may be controlling phytoplankton abundance and growth in a similar fashion. Chlorophyll *a* and primary productivity for both PP and larger phytoplankton increased with the onset of favorable growth conditions (including increased light and temperature), particularly in the spring and summer. Grazing experiments may be necessary to determine how grazers may differentially consume PP and larger species in the NRE.

Patterns of PP contribution to Chl *a*-specific biomass were similar to those observed in Florida estuaries (Murrell and Lores 2004, Phlips et al. 1999, Badylak and Phlips 2004). Chl *a* contribution by cells < 5µm averaged over 70%, and increased during summer months, as reported by Murrell and Lores (2004). In Florida Bay, periods of the highest *Synechococcus* biovolumes were concurrent with periods of highest Chl *a* concentrations

(Phlips et al. 1999). Temperate estuaries, however, typically have lower reported percent contributions by PP (Ray et al. 1989, Iriarte and Purdie 1994, Ning et al. 2000). Ning and colleagues (2000) reported that picocyanobacterial Chl *a* contribution fell to below 2% when Chl *a* exceeded 7 µg L^{-1} in the San Francisco Bay. Likewise, Iriatre and Purdie (1994) stated that PP contribution to biomass in estuarine environments is not likely to exceed 10%.

The NRE is a temperate estuary that clearly does not follow this paradigm. At all stations except 180, total Chl *a* exceeded 10 µg L^{-1} on more than half of the sampling dates. During these times, relative contribution of the < 3µm fraction ranged from 3.0 to 74.5%, averaging 38.5% (calculations of 0% relative contribution were recorded, but are assumed to be due to sampling error and were excluded in this range). Figure 13 shows that, regardless of total phytoplankton biomass, PP percent contribution in the NRE rarely falls below 10%, the maximum contribution predicted by Iriarte and Purdie (1994). These results also indicate that PP is a significant contributor to the total phytoplankton community during all seasons, not only during warm summer months.

There are several important differences to note between the NRE and the abovementioned San Francisco Bay, Southampton Water, and Florida estuaries. The NRE is a relatively shallow, micro-tidal estuary with a residence time on the order of weeks to months. The estuary flows into Pamlico Sound, where exchange with the Atlantic Ocean is limited to only three inlets (Giese et al. 1985). Both San Francisco Bay and Southampton Water are meso- to macro-tidal systems, with greater mean depths than the NRE (Ning et al. 2000, Cloern 1996, Iriarte and Purdie 1994). The long residence times and shallow depths of the NRE compare more closely to Florida estuaries, whose average depths do not exceed 3m. These systems are also influenced little by tidal fluctuations and are characterized by limited

exchange with surrounding Atlantic Ocean or Gulf of Mexico water (Phlips et al. 1999, Murrell and Lores 2004, Badylak and Phlips 2004).

Long residence time is a factor in the buildup of biomass which characterizes several Florida estuaries and the NRE, thus demonstrating the link between eutrophication and residence time (Christian et al. 1991). It is evident that residence time, along with depth and tidal influence, play a role in determining the ratio of PP to total phytoplankton biomass. Additionally, relatively long residence times and poorly flushed conditions contribute to a decrease in nutrient concentrations, a condition that would favor PP over larger cells. Deeper and more frequently flushed estuaries, with higher nutrient concentrations, like San Francisco Bay may hold to the paradigm of decreasing PP contribution with increasing total biomass. However, shallow estuaries with longer residence times like the NRE and some Florida estuaries clearly do not fit this into this model. Picoplankton is a dominant component of the NRE phytoplankton community during most seasons and conditions. Several factors in addition to the trophic state of a given system must be considered before assumptions regarding the role of PP are made.

4.2 Picophytoplankton primary productivity

Similarly to Chl *a*-specific biomass, size-fractionated primary productivity varied seasonally with summer highs and winter lows, but the relative contribution of PP to total productivity remained relatively high throughout all seasons (Figs. 15-19). Rates of carbon uptake obtained in this study agree with those reported in Boyer et al. (1993); however, the region of highest productivity occurs in the mid-estuary. Like the trend in Chl *a*, this is likely due to the prolonged drought experienced during the time period of the current study.

Rates of light saturated total photosynthesis (P_m^B) calculated by Boyer et al. (1994) are nearly identical to the size-fractionated rates obtained in this study, with the exception of unrealistically high P_m^B calculations previously mentioned. Despite possible errors in the calculations of P_m^B and α, the observation of higher rate parameters in the PP size fraction (at most stations) is also consistent with reports in the literature (Platt et al. 1983, Morán 2007). This is likely a consequence of the adaptive advantages incurred by small cell size. As mentioned earlier, small cells more efficiently take up and utilize photons and other resources, which contribute to higher Chl *a*-specific rates of photosynthesis compared to larger size fractions. Higher α values, like those calculated in this study, may also indicate PP in the NRE are better adapted to low light conditions since steeper slopes on photosynthesis-irradiance curves reach P_m^B at a lower irradiance.

Ray and colleagues (1989) reported significantly higher P_m^B values for the < 3μm size fraction than the larger fraction at high light levels, although the rates were not significantly different at low light levels. In the current study, neither P_m^B nor α are significantly different between size fractions at stations 30 and 120. However, differences at stations 50, 70, and 180 are more apparent. Additional research is needed to investigate the underlying causes specific to light adaptation and carbon uptake rates between size fractions along the length of the NRE. Figure 20 suggests that PP have an adaptive advantage over the larger size fraction in terms of Chl *a*-specific carbon uptake (*i.e.*, most points on the plot comparing PP contribution to total productivity versus PP contribution to total biomass fall above the $y = x$ line). The high turbidity of estuaries would favor PP which are better

adapted to low light conditions, and these cells would likely contribute more to total carbon uptake than total biomass.

4.3 Picophytoplankton abundance

Picophytoplankton abundance, determined by direct counts of autofluorescing cells, was higher than that reported in a Chesapeake Bay tributary (Ray et al. 1989); however, PP abundance in the lower Chesapeake Bay was on the same order of magnitude of that reported in this study (Affronti and Marshall 1994, Marshall and Nesius 1996). Counts of cells < 3µm were also slightly lower than those reported by Phlips et al. (1999) in Florida Bay, and as many as 2 orders of magnitude higher than cyanobacterial counts in a temperate English estuary (Iriarte and Purdie 1994). Seasonal patterns of high abundance in the summer and low abundance in the winter were observed in the current study and in all studies mentioned above. It is possible that estimates of PP abundance may be underestimated due to problematic fading of cells during sample storage (MacIsaac and Stockner 1993). However, throughout the course of the study most cells fluoresced brightly and were easily visible upon microscopic analysis.

4.4 Diagnostic photopigments

The use of diagnostic photopigments to rapidly and conveniently quantify algal groups within marine habitats has helped to broaden insight into phytoplankton community dynamics. In the current study, HPLC-derived pigment concentrations were compared to fluorometrically-derived Chl *a* concentrations, in order to estimate phytoplankton class abundance. Size-fractionated analysis of pigments indicates that most of the zeaxanthin

present in whole water samples is present in the < 3µm size fraction (Paerl et al. 2009, in press). In the current study, size-fractionated Chl *a* analysis shows PP to be a dominant component of total biomass across space and time, and that zeaxanthin concentrations are strongly correlated with < 3µm Chl *a* concentrations (Figs. 24-25). Zeaxanthin concentrations are also positively correlated with PP abundance (Fig. 26), which supports the assumption that size-fractionated pigment analysis is a useful indicator of cyanobacterial abundance. However, it is yet unclear whether zeaxanthin concentrations provide an accurate estimate of PP abundance. Picoeukaryotes are another ubiquitous component of the PP community (Li 1994, Worden et al. 2004, Not et al. 2005), and may be overlooked when zeaxanthin is used as the only indicator pigment. Therefore, a conglomeration of techniques, including diagnostic pigment analysis and microscopic analysis, may be necessary to accurately assess the complete PP size fraction.

4.5 Influential environmental factors

Temperature has been shown to be one of the most influential factors controlling picoplankton abundance (Waterbury et al. 1986, Stockner et al. 2000), resulting in higher biomass and productivity during warm summer months (Phlips et al. 1999, Murrell and Lores 2004, Affronti and Marshall 1994, Boyer et al. 1994). In the current study, the results of the first multiple regression model suggests that Chl *a*-specific biomass was strongly correlated with temperature. In this model, only the > 3µm size fraction of primary productivity was not well correlated with temperature. The highest rates of primary productivity in the larger fraction occurred during spring and fall months, which are not concurrent with warmest

water temperatures. It is highly likely, therefore, that additional factors control biomass and primary productivity in the larger size fraction.

Chlorophyll *a*-specific biomass and primary productivity were negatively correlated with salinity for the > 3µm size fraction, but not for the smaller fraction. Similarly Mallin et al. (1991) also observed a decrease in total productivity with increasing salinity in the NRE. Picoplankton biomass, averaged by station, decreased somewhat from the upper to lower regions of the estuary, but the degree of variation between the highest and lowest biomass station was not as great as for the larger size fraction (Table 4). This indicates that PP, relative to the larger size fraction, is a more stable component of the system and not as subject to wide swings in biomass and primary productivity over space and time. This stability may be attributed to the low light and low nutrient adaptation of PP, allowing the community to grow and photosynthesize at more consistent levels than larger species more sensitive to such changes.

The results of the second model, examining the combined effects of NO_x and PO_4^{3-}, are more difficult to interpret due to the nature of NO_x distribution in the NRE. Nitrate + nitrite were below detection in the estuary for most of the summer months downstream of station 30 and were always below detection at stations 120 and 180. Nutrient pulses from river flow are often important in determining NO_x distribution in the NRE (Christian et al. 1991, Mallin et al. 1993), and it is not uncommon for NO_x pulses to extend into the mid and lower estuarine environment. However, persistent drought resulting in low river flow combined with rapid uptake in the upper estuary, likely limited the downstream movement of NO_x. As a result, NO_x concentrations for more than half of all sampling dates and locations

were below detection, which made clear relationships with Chl *a* and productivity difficult to interpret.

Mallin et al. (1991) observed an inverse relationship between nitrate and salinity in the NRE, which was also observed in the current study. Mallin and colleagues also observed a strong positive correlation ($r = 0.82$) between total phytoplankton productivity and nitrate, and they concluded that nitrate was an important factor controlling primary productivity in the NRE. However, Boyer et al. (1993) found no significant correlation between total productivity and nitrate. The second model used in the current study found no significant correlation between NO_x and Chl *a* of both size fractions, and a negative correlation between NO_x and productivity in the < 3µm fraction. Lack of correlation does not necessarily indicate that NO_x is unimportant in phytoplankton dynamics in the NRE. Pulses of allochthonous DIN, primarily from winter and spring runoff events, directly precede spring and summer peaks in Chl *a* and productivity (Paerl et al. 1995, 1998). Biomass and productivity may be positively correlated with NO_x during initial bloom onset; however, as nutrients are rapidly taken up by the increasing biomass, an inverse relationship between NO_x and biomass and productivity would likely develop. This inverse relationship was predicted by model 2 for the < 3µm fraction of productivity, indicating a lagged response to NO_x in the PP size fraction.

Previous research suggests that the ammonium fraction of the DIN pool is not as strongly determined by river flow as nitrate and nitrite. Rather, the largest sources of NH_4^+ in the NRE are generally believed to be "internal," that is, in the sediments (Christian et al. 1991, Paerl et al. 1995). In the current study, however, NH_4^+ concentrations in near-surface

water samples were highest in the winter and spring, concurrent with periods of increased river flow. It may be that NH_4^+ from runoff events was more pronounced during the current study than in previous years, or it is possible that spring rain events helped to mix NH_4^+-rich bottom waters with surface water.

On average, NH_4^+ decreased from station 30 through 120, although concentrations increased slightly at station 180 (Fig. 5). Ammonium is the preferred form of DIN taken up by phytoplankton (Boyer et al. 1994, Twomey et al. 2005) and it was negatively correlated with both size fractions of Chl *a* and the smaller fraction of primary productivity. The results of the third model indicate that NH_4^+ may be more influential than NO_x in determining biomass and productivity of both size fractions. However, another source of recycled NH_4^+ comes from phytoplankton cell exudates or release during bloom senescence. Thus, this additional source of NH_4^+ could influence the correlation predicted by the third model.

Dissolved inorganic phosphate distribution, like NH_4^+, is regulated more by release from bottom sediments and recycling in the water column rather than river discharge (Christian et al. 1991, Paerl et al. 1995). Periods of highest PO_4^{3-} concentrations were concurrent with high temperatures (Figs. 2, 6) and increased during periods of low bottom water oxygen (data not shown). Hypoxic bottom water is known to contribute to increased PO_4^{3-} release from the sediments (Paerl et al. 1995). Between models 2 and 3, PO_4^{3-} was positively correlated with both large and small size fractions of Chl *a* and primary productivity. This is somewhat surprising, since DIP is rarely a limiting nutrient in estuaries, although microbially-mediated recycling of PO_4^{3-} (similar to recycling of NH_4^+) may help to explain this relationship. Pinckney et al. (1999) demonstrated the adequate availability of

PO_4^{3-} in the NRE when results of nutrient addition experiments elicited a response by cyanobacteria (indicated by zeaxanthin) to nitrate but not PO_4^{3-}. Additionally, it is possible that the strong correlation between PO_4^{3-} and temperature is partially responsible for the results of models 2 and 3.

It is clear that the influencing effects of nutrients on phytoplankton biomass and primary productivity are compounded by an array of physical factors, including temperature and river flow. Additionally, models 2 and 3 assumed nutrient sources were independent of phytoplankton biomass, when in actuality the release of DIN and DIP from cells may complicate nutrient interactions, making model results difficult to interpret. While the results of this study indicate that DIN and DIP may influence PP growth and productivity dynamics, the scope of this investigation was not broad enough to identify all responsible environmental factors. Size-fractionated nutrient limitation bioassays could be conducted to help address the influencing roles of specific nutrients. Picophytoplankton is an excellent competitor in low nutrient environments, thus in the high nutrient waters of the NRE, these bioassays may indicate that unlike larger phytoplankton, the PP component in fact may rarely be nutrient limited.

One caveat of the models used to analyze the data set is that the influence of high and low nutrient concentrations occurred concurrently with high and low biomass and productivity. A previous study in the NRE show that increases in phytoplankton biomass and productivity may occur after loading events, when nutrient concentrations have been rapidly depleted by the phytoplankton community (Paerl et al. 1998). Although the statistical methods used in the current study do not account for a time lag effect, lag patterns may be evident in seasonally averaged Chl *a* and primary productivity of both size fractions.

Figures 3 a and b show different seasonal trends between the > 3µm and < 3µm size fractions. While Chl *a* and productivity is lowest in the winter for both size fractions, the onset of the spring bloom occurs earlier for the larger size fraction than the smaller fraction. Chlorophyll *a*-specific biomass of the > 3µm fraction increases in the spring of 2008 and stays relatively constant throughout the following summer and fall, while rates of productivity for this size fraction increase in the spring, only to decline again in the summer. This rapid response, likely due to warming temperatures and DIN loading from spring discharge events, is not matched by the PP size fraction. Picoplankton biomass and productivity increase gradually from the winter of 2008 through the following summer and early fall. Considering the possibility that PP are not as severely nutrient limited as the larger size fraction, their response to nutrient loading may not be as intense, and is thus lagged further behind the response time of larger species. Temperature rather than nutrients, therefore, is likely a more important control of PP biomass and productivity than of the larger fraction.

Thus far, discussion of factors influencing PP growth and abundance has been limited to physical environmental parameters and "bottom-up" controls. Another important factor involves "top-down" control by microzooplankton grazing (Joint 1986, Weisse 1993, Stockner et al. 2000). Tight coupling between growth rates and loss by grazing have helped explain why PP do not appear to respond as strongly as larger cells when growth conditions are favorable for both size fractions (Barber and Hiscock 2006). While the results of the current study show that PP likely do respond to warmer temperatures, and perhaps to a limited extent nutrient loading, the strength of this response may be affected by grazing pressure. Studies indicate that PP in estuaries responds with increased biomass when

released from grazing pressure (Lewitus et al. 1998, Juhl and Murrell 2005). The larger size fraction is less affected by grazing than PP because their higher rates of nutrient uptake allow biomass to increase faster than grazers can consume them (Riegman et al. 1993).

4.6 Directions for future research

The results of this study indicate that PP is an important, and at times dominant, component of the total phytoplankton biomass and primary productivity in the Neuse River Estuary. Variation of the < 3µm size fraction is correlated with several physical and chemical environmental factors (temperature, salinity, and nutrient concentration), although further research is necessary in order to address the additional factors known to influence PP growth and abundance, such as river flow, water column stratification, and zooplankton grazing.

It has been hypothesized and observed that two general patterns of productivity exist in marine and aquatic environments. Cushing (1989) compared production in strongly stratified systems, such as the oligotrophic open ocean, and weakly stratified systems, like those found in upwelling zones. The former is characterized by longer food chains, supported by the microbial loop, and favors the dominance of smaller cells. However, weakly stratified upwelling systems support the traditional shorter food chain with larger cells, and include some of the most productive fisheries locations on the planet. The NRE is a highly dynamic environment that shows characteristics of both systems, depending on the time and location of observation (Paerl et al. 1995). While upwelling is not a significant mixing mechanism in the NRE (although cross-channel down- and upwelling does occur under certain conditions (Luettich et al. 2000)), small-scale climatic events such as wind and

rainfall do promote vertical mixing and sediment resuspension, as do much larger events such as hurricanes (Peierls et al. 2003). Based on the observations and conclusion of Cushing (1989) and depending on the duration of stratified versus non-stratified conditions in the NRE, fluctuations between a traditionally accepted, large cell dominated food chain and the microbial loop influenced, small cell dominated food chain may be expected.

Long residence time is a trait shared by the NRE and several other microtidal and lagoonal systems (e.g. Florida Bay, Laguna Madre, Texas), in which frequent PP dominance has been reported. Periods of low or moderate river discharge can also contribute to increased residence times and periods of strong stratification (Luettich et al. 2000, Buzzelli et al. 2002). The relationship between these two events may help to explain why some estuaries, such as the NRE and Florida estuaries, are more prone to PP dominance, while PP dominance is rarely observed in other, shorter residence time systems like San Francisco Bay. Understanding when and if water column stratification occurs may help researchers predict the ratio of picoplankton to larger phytoplankton in a given estuary.

The results of the current study provide evidence that no single pattern of productivity dominates the NRE throughout space and time. On average, the < 3µm size fraction contributes approximately half of the total primary productivity, and over a third of the Chl *a*-specific biomass in the NRE. This proportion is greater than the conservative (< 10%) estimate of Iriarte and Purdie (1994), although less than the average contribution by PP in the oligotrophic open ocean, which often exceeds 50% (Bell and Kalff 2001, Marañón et al. 2001). Mesocosm experiments have demonstrated that cyanobacteria (as indicated by zeaxanthin) favor mixed, turbid, high-nitrate conditions (Pinckney et al. 1999), while other sources indicate that warm and strongly stratified waters are optimal for cyanobacterial

growth (Paerl and Huisman 2008). However, harmful cyanobacterial blooms referenced by the latter study are likely different from the cyanobacteria in Pinckney et al. (1999). Size-fractionated HPLC pigments indicate that most of the cyanobacterial signal falls into the PP size fraction, while harmful cyanobacterial blooms are often larger filaments or colonial species. However, blooms of these nuisance species have been documented in the NRE (Paerl 1983; 2006) and may have been a small component of the total phytoplankton community during the course of the study. Longer-term monitoring of size-fractionated biomass and productivity will help to clarify the conditions most favorable for cyanobacteria and the PP size fraction.

As mentioned previously, top-down control by microzooplankton grazing is likely a significant factor affecting PP growth and abundance in the NRE, and was not addressed in the current study. However, dilution experiments similar to those of Lewitus et al. (1998) and Juhl and Murrell (2005) are currently underway to test the effects of grazing on PP. Preliminary results indicate that PP in the NRE is heavily grazed, as indicated by an increase in PP biomass when released from microzooplankton grazing pressure (Dr. Michael Wetz, personal communication).

This study has shown that in the NRE, there is strong evidence that PP primary productivity is an important and dominant component of total phytoplankton carbon fixation. However, the fate of that carbon is yet unclear. Despite recent mitigation efforts, the NRE is adversely affected by nutrient-enhanced eutrophication. Total phytoplankton biomass and productivity are closely tied to eutrophication potentials, and PP is likely an important contributor. Thus, the fate of PP carbon is of particular interest. Grazing by zooplankton may help to transfer a portion of PP carbon up the food chain, supporting the productive

nursery habitats characteristic of this and other estuaries. Another loss factor, sinking, has long been discounted because the small size of PP is not conducive to sinking, particularly in a well-mixed system (Raven 1986, Joint 1986). Recent research has suggested that aggregation can enhance the sinking of small cells in the open ocean and thus the export of carbon from surface waters (Richardson and Jackson 2007, Barber 2007). However, recent studies in the NRE suggest that PP sinking in estuaries is relatively minimal compared to the > 3µm size fraction (Wetz, unpublished data). Finally, viral lysis of PP cells may contribute to the release of carbon and nutrients into the water column, contributing to water column regeneration and microbial loop activity. However, relatively little is yet known regarding the interactions of viruses and PP (Brussaard 2004).

Since the discovery of their ubiquitous and abundant presence in the late 1970s, picophytoplankton was believed to dominate only in low nutrient, oligotrophic environments. This research demonstrates that despite this paradigm, PP is a significant component of the NRE phytoplankton community over space and time. Patterns of size-fractionated biomass and productivity suggest that while PP exhibit substantial spatial and temporal variation, it is a more consistent component of the NRE than the larger size fraction. Both physical (temperature, salinity) and chemical (DIN, DIP) factors appear to influence PP biomass and productivity, although additional research would provide a more complete understanding of these and other influential parameters (water column stratification, residence time, zooplankton grazing). Picoplankton carbon is likely to be an important component of eutrophication potentials in the NRE, thus further research into the fate of this carbon is essential to our more complete understanding of phytoplankton dynamics in estuaries.

Table 1. Differences in total Chl *a* at all stations when filtered immediately following collection (T1 – June 9, 2008) versus after overnight storage (T2 – June 10, 2008). Percent loss in biomass varied between stations, and averaged 15.1%.

	Total Chl a ($\mu g\ L^{-1}$)		Percent Loss
Station	**T1**	**T2**	**((T1-T2)/T1)*100**
30	28.4	23.3	18.1
50	9.5	8.7	8.1
70	15.3	14.4	6.1
120	16.6	12.4	25.0
180	6.8	5.5	18.2

Table 2. Biomass retention, as indicated by Chl *a* concentration, on 3.0 µm Poretics ("old") and Millipore Isopore™ ("new") membrane filters, measured in triplicate at all stations on July 9, 2008. The Wilcoxon rank sum test, run in the statistical program, R, was used to show that there is no significant difference between biomass retention by the two filter types.

	Biomass retention (µg Chl *a* L^{-1})		
Station	**"old"**	**"new"**	**p value**
	9.7	9.2	
30	9.0	10.1	$p > 0.1$
	11.3	10.9	
	26.2	25.2	
50	25.2	21.8	$p > 0.1$
	27.2	30.6	
	15.6	14.1	
70	14.7	14.2	$p > 0.1$
	15.9	17.1	
	9.8	9.7	
120	8.4	10.1	$p > 0.1$
	10.3	13.5	
	7.2	6.9	
180	7.4	6.3	$p > 0.1$
	7.0	7.3	

Table 3. Correction factors calculated for estimating Chl *a* concentrations and rates of primary productivity from June 12, 2007 to October 31, 2007 for all stations. Values obtained using GF/D glass fiber filters were entered as "*x*" in the equations above to obtain the corrected Chl *a* and productivity values, "*y*", used in all figures, tables, and calculations hereafter.

	Correction Factors	
Station	**Chlorophyll *a***	**Primary Productivity**
30	$y = 0.73x - 1.0$	$y = 0.85x - 9.22$
50	$y = 0.84x - 2.57$	$y = 0.81x - 5.19$
70	$y = 0.74x - 0.03$	$y = 0.82x - 11.13$
120	$y = 0.75x - 1.54$	$y = 0.83x - 9.75$
180	$y = 0.69x + 0.30$	$y = 0.73x - 3.64$

Table 4: Chl *a* concentrations, rates of productivity, and relative PP contribution at all stations, averaged over the entire study period.

	Station	30	50	70	120	180
Chl *a* (µg L^{-1})	**Total**	31.7	26.6	20.8	20.9	9.2
	> 3 µm	19.6	18.8	14.9	12.3	5.3
	< 3 µm	12.1	7.8	6.7	8.7	4.0
	% PP	40.6	35.3	36.7	43.7	40.2
Productivity (mg C m^{-3} h^{-1})	**Total**	113.5	84.8	78.2	74.4	46.6
	> 3 µm	75.1	49.4	41.2	40.2	22.2
	< 3 µm	38.4	35.4	37.0	34.2	25.7
	% PP	41.8	44.1	53.5	55.4	55.3

Table 5. Model results for factors influencing phytoplankton biomass and productivity. Significance indicated by asterisks (* * *, $p < 0.001$; * *, $p < 0.01$; *, $p < 0.05$), and positive or negative correlations indicated by + and -, respectfully. Shaded spaces indicate no significant interaction between parameter and phytoplankton size fraction.

		Chlorophyll *a*		Primary Productivity	
Model	**Parameter**	**> 3 µm**	**< 3 µm**	**> 3 µm**	**< 3 µm**
1	**Temperature**	* * +	* * * +		* * * +
	Salinity	* * –		* * –	
	Interaction				
2	**Nitrate+Nitrite**				* * * –
	Phosphorus		* * +		* * * +
	Interaction	* –			
3	**Ammonium**	* * –	* * –		* * * –
	Phosphorus	* * * +		* * * +	* * * +
	Interaction		* * +		

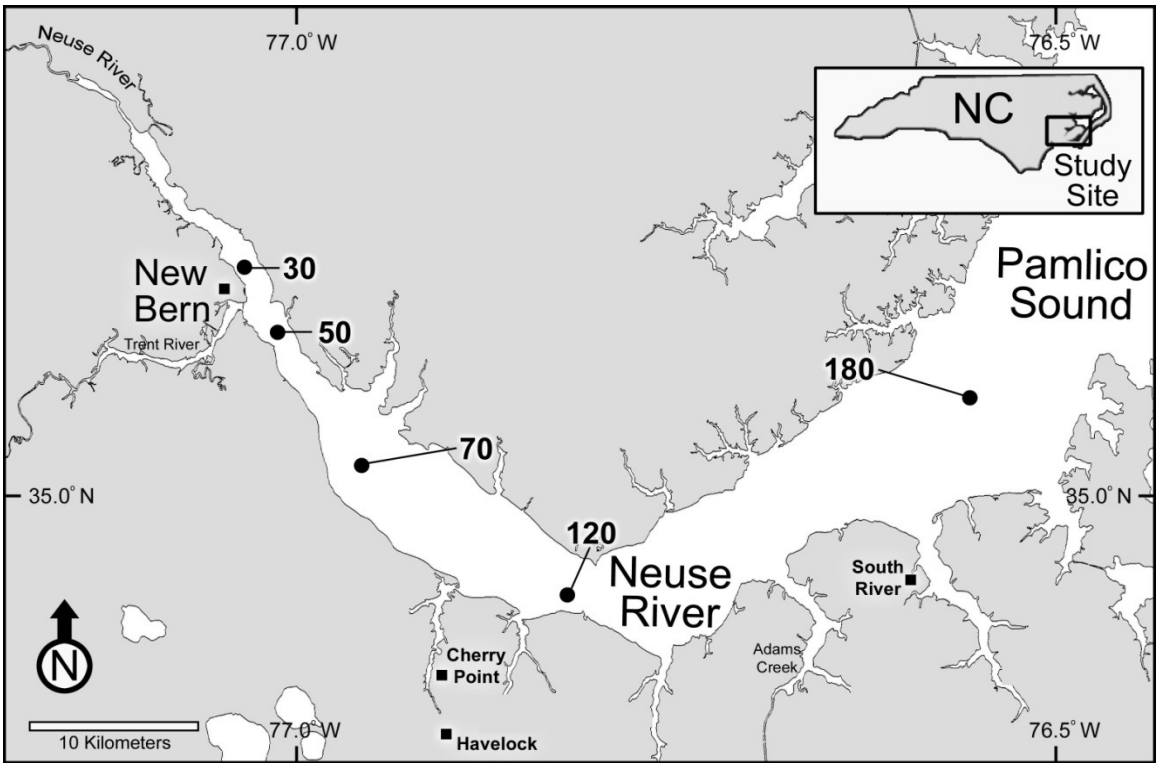

Figure 1. The Neuse River Estuary, North Carolina, USA, with points indicating the 5 stations sampled in this study.

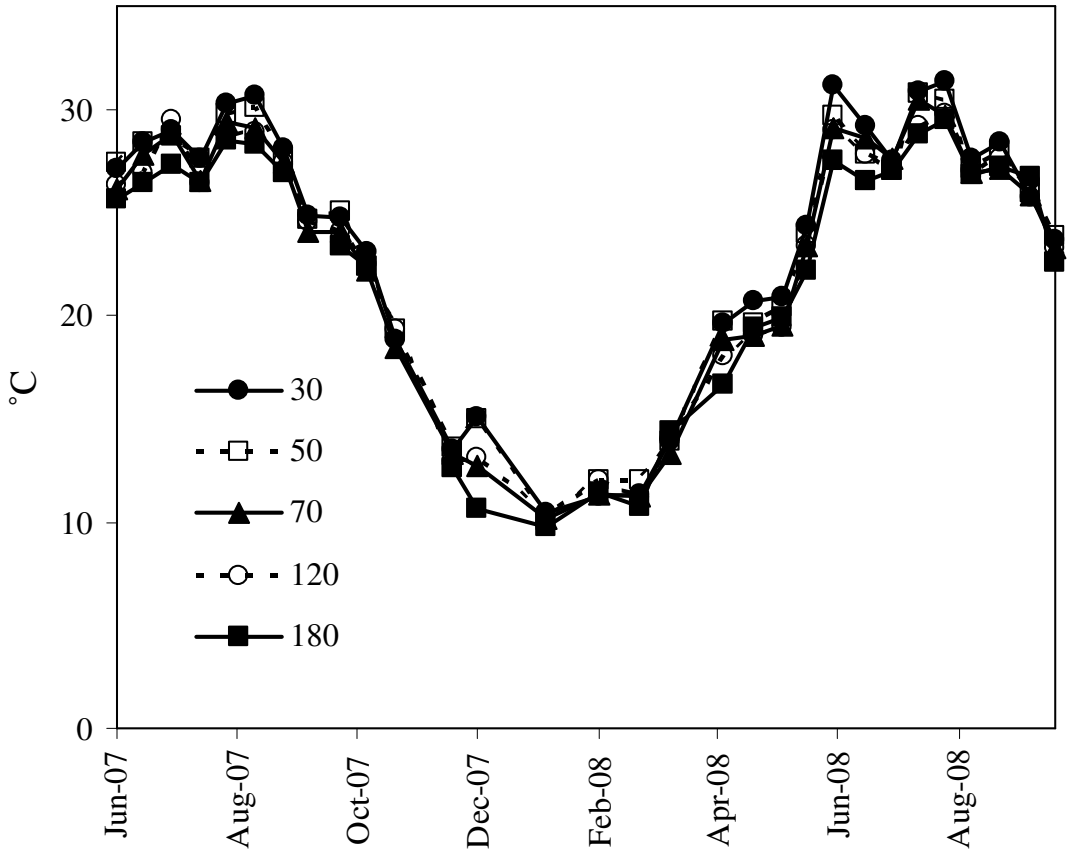

Figure 2. Time series plot of temperature measured at stations 30, 50, 70, 120, and 180 though the entire time period of the study (June 12, 2007 – September 29, 2008).

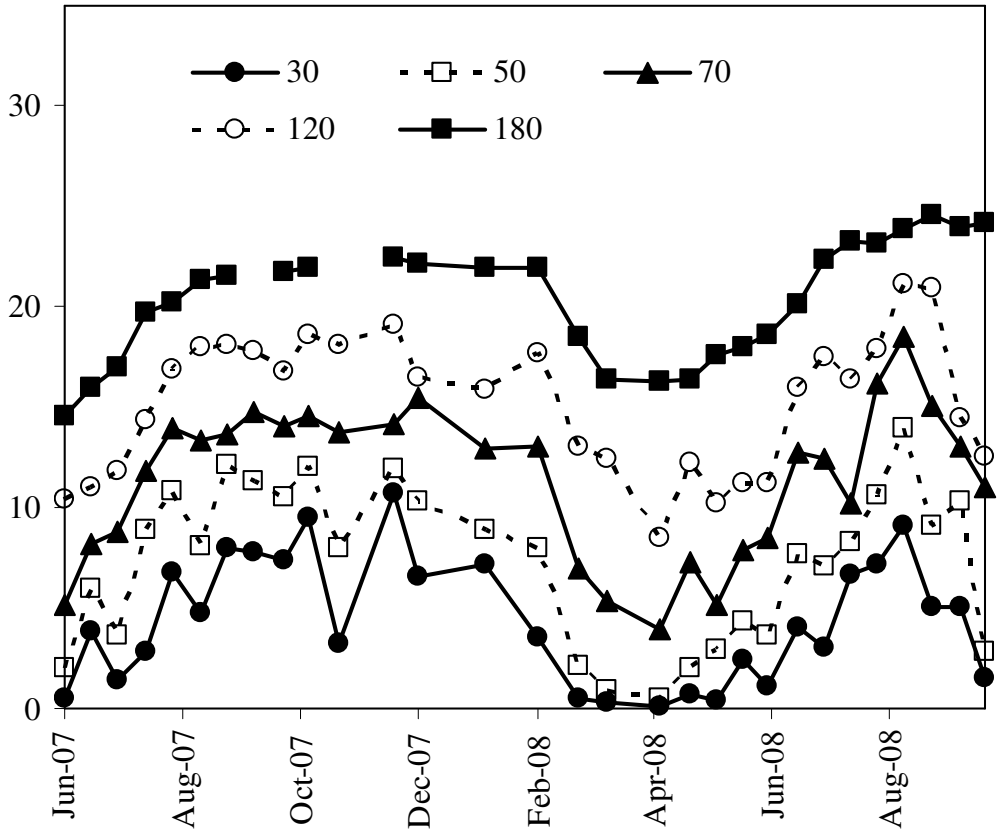

Figure 3. Time series plot of salinity measured at stations 30, 50, 70, 120, and 180 though the entire time period of the study (June 12, 2007 – September 29, 2008).

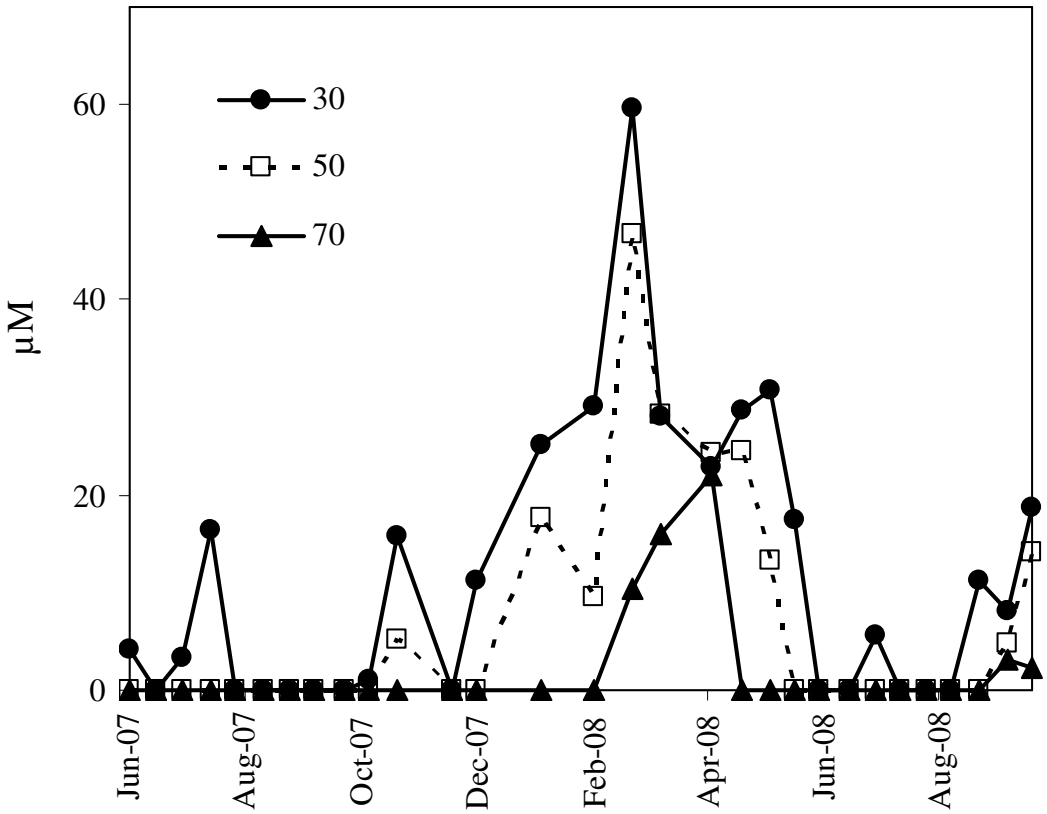

Figure 4. Time series plot NO_x measured at stations 30, 50, 70, 120, and 180 though the entire time period of the study (June 12, 2007 – September 29, 2008). NO_x was below detection at stations 120 and 180 for all dates sampled.

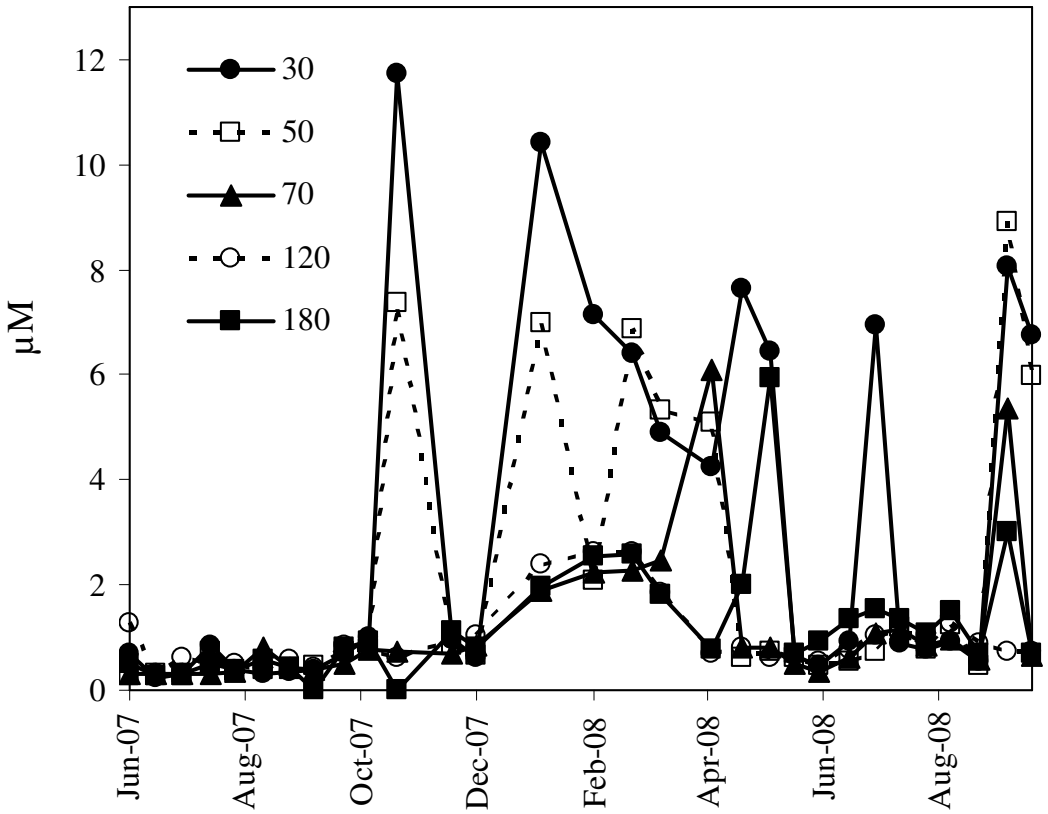

Figure 5. Time series plot NH_4^+ measured at stations 30, 50, 70, 120, and 180 though the entire time period of the study (June 12, 2007 – September 29, 2008).

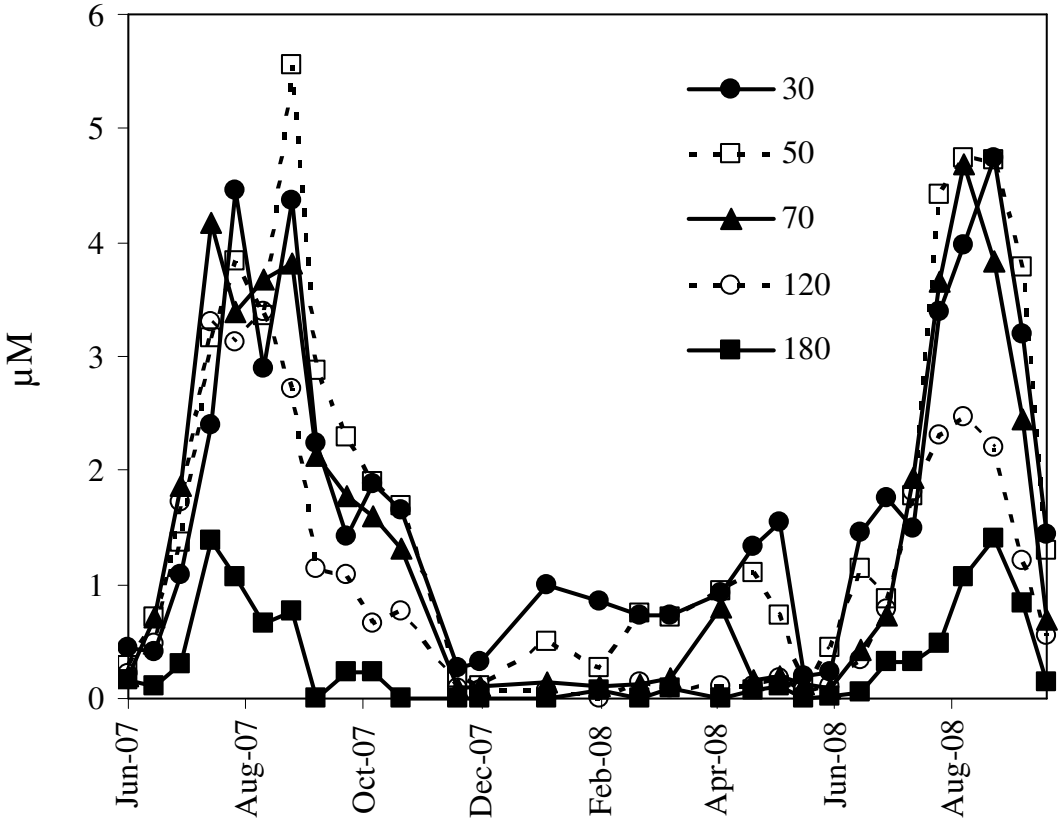

Figure 6. Time series plot PO_4^{3-} measured at stations 30, 50, 70, 120, and 180 though the entire time period of the study (June 12, 2007 – September 29, 2008).

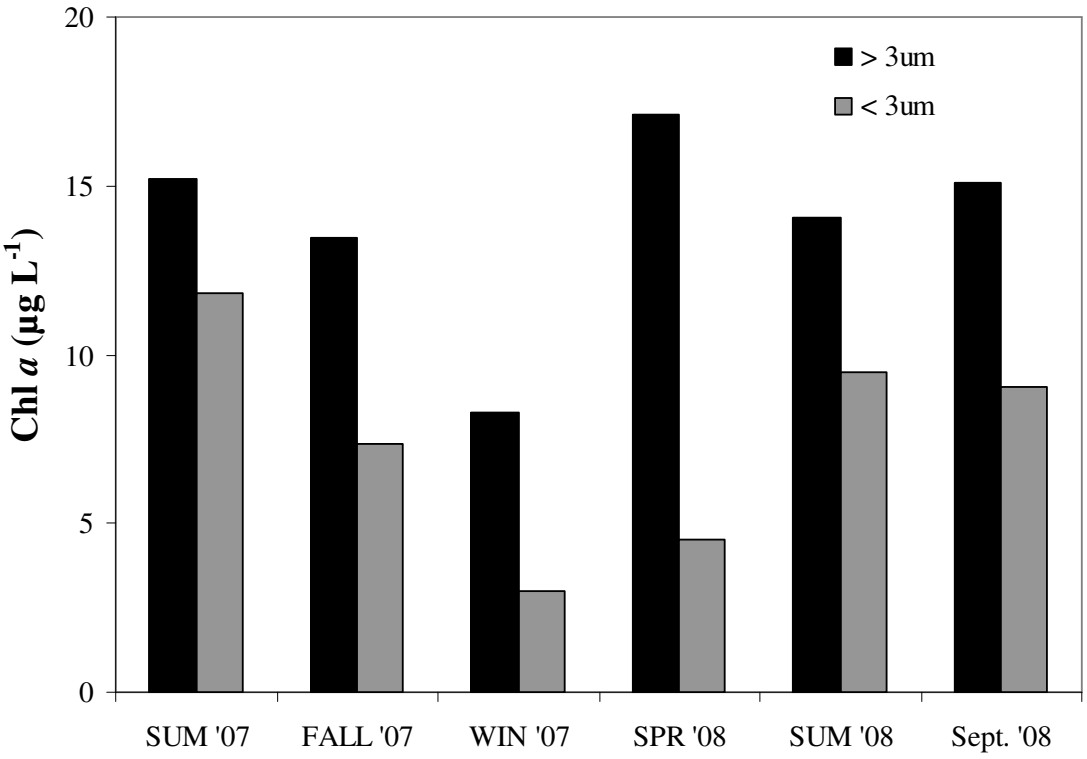

Figure 7. Chl *a* concentrations averaged for all stations by season. Black bars represent the > 3µm size fraction and gray bars represent the < 3µm size fraction. Summers (June-August), fall (September-November), and spring (March-May) include 6 sampling dates at each of 5 stations, while winter (December-February) and Sept. 08 each include 3 sampling dates.

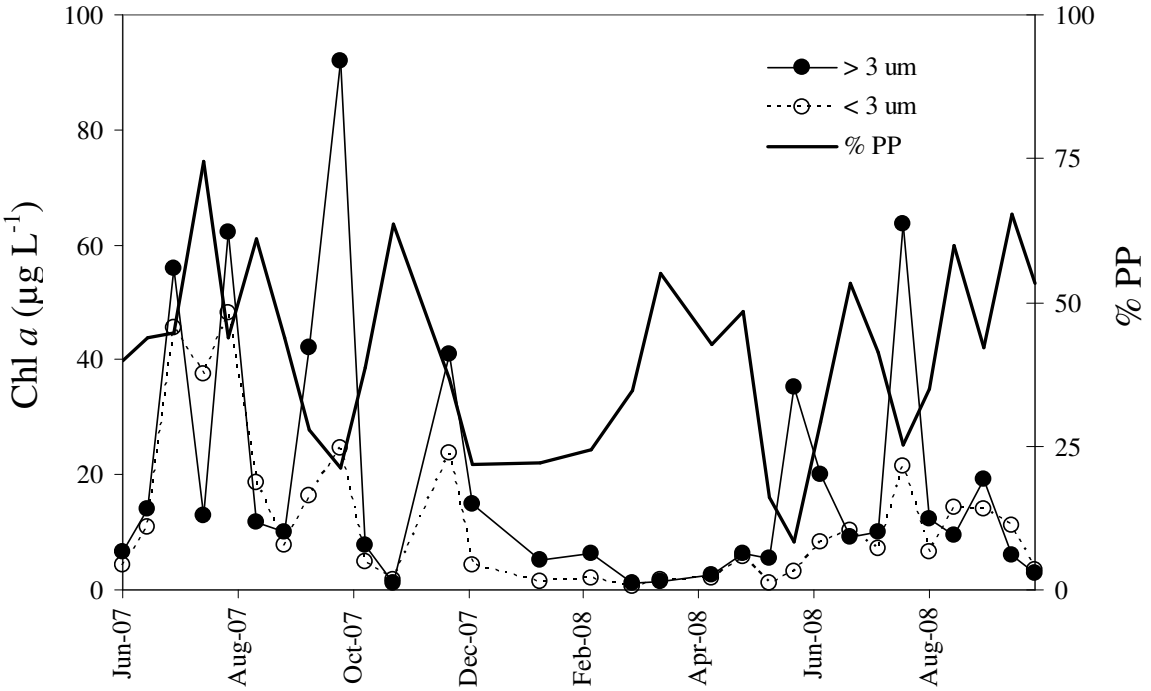

Figure 8. Size-fractionated chlorophyll *a*, station 30, in the Neuse River Estuary.

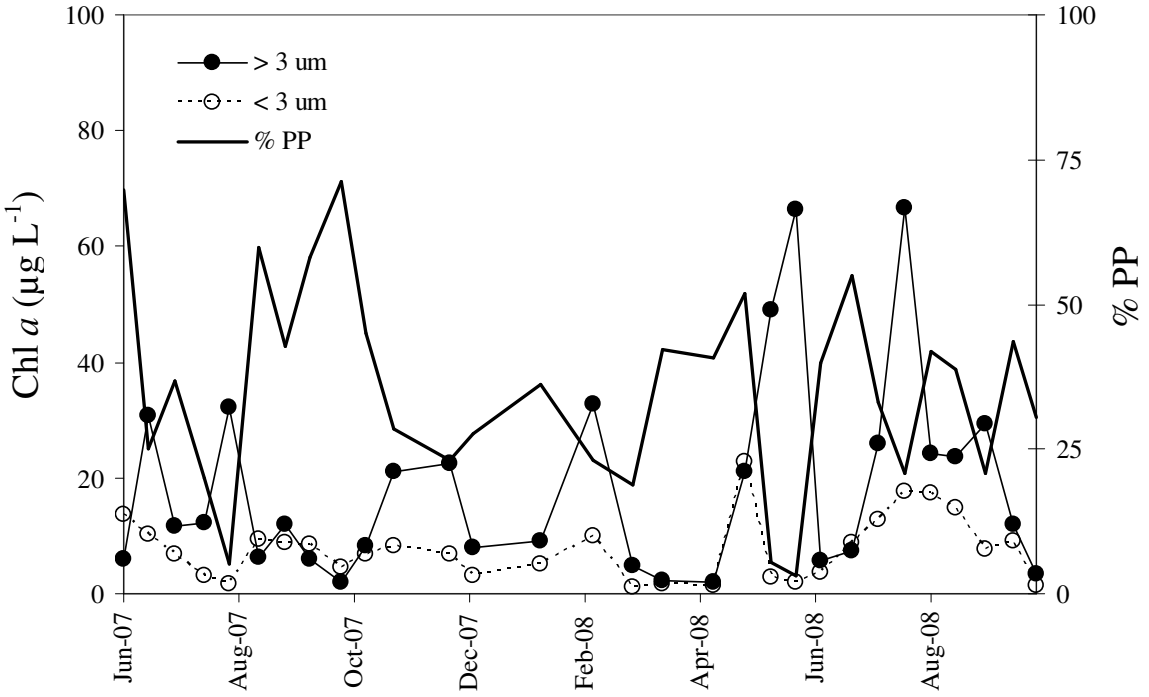

Figure 9. Size-fractionated chlorophyll *a*, station 50, in the Neuse River Estuary.

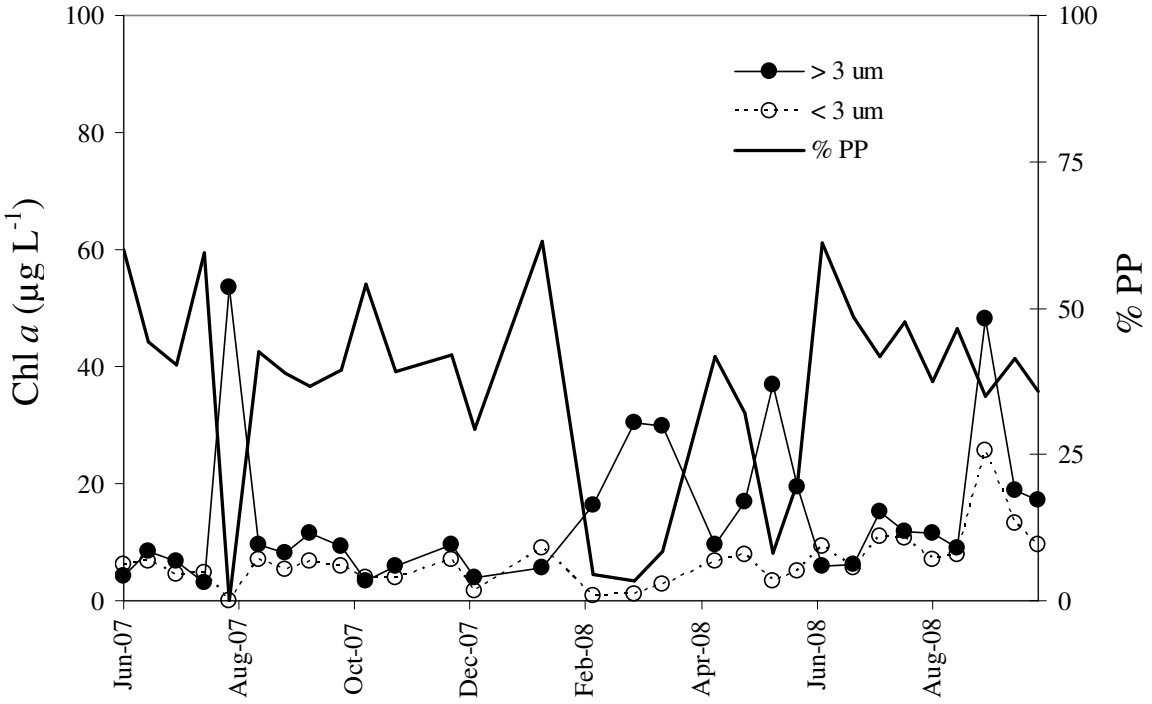

Figure 10. Size-fractionated chlorophyll *a*, station 70, in the Neuse River Estuary.

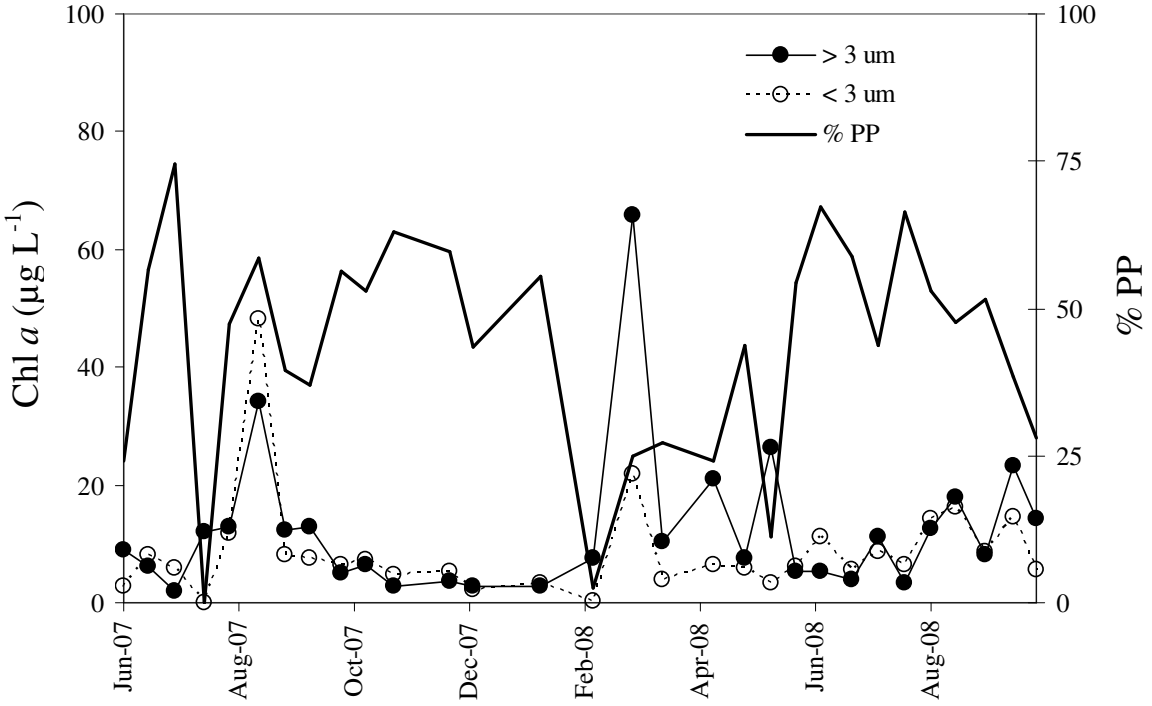

Figure 11. Size-fractionated chlorophyll *a*, station 120, in the Neuse River Estuary.

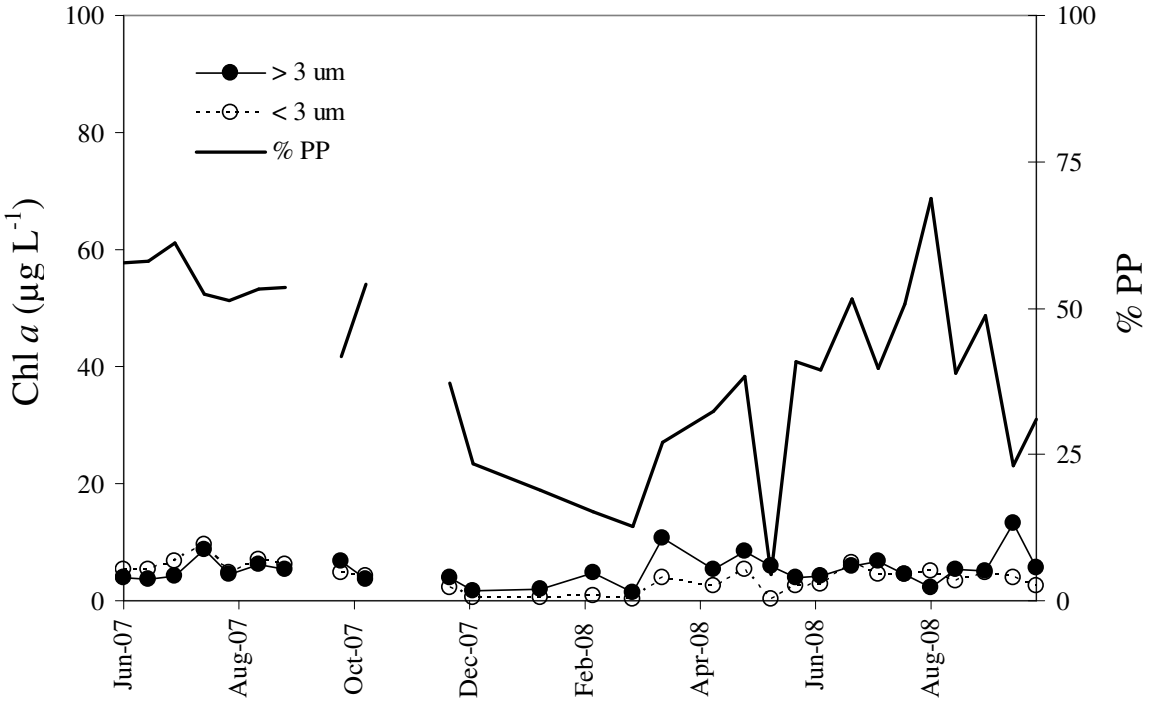

Figure 12. Size-fractionated chlorophyll *a*, station 180, in the Neuse River Estuary.

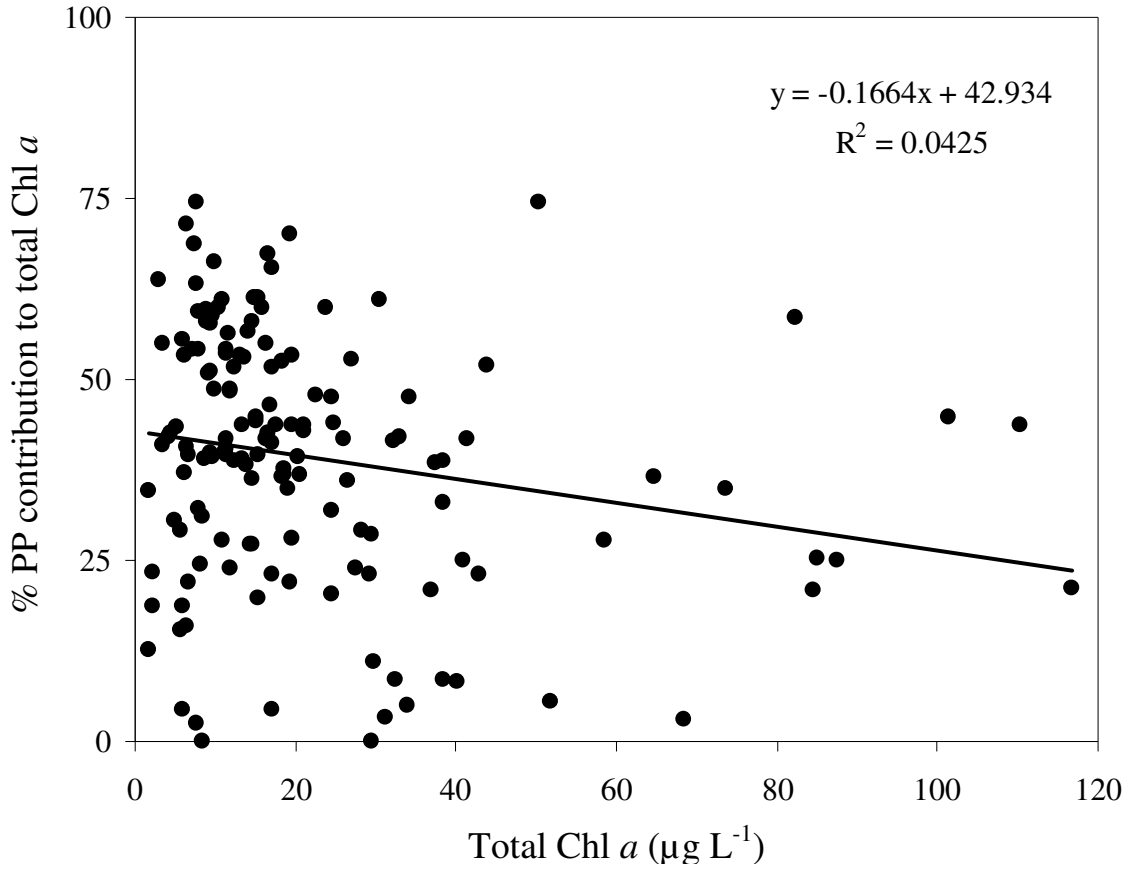

Figure 13. Relationship between total phytoplankton biomass, as chlorophyll *a*, and % PP contribution to biomass.

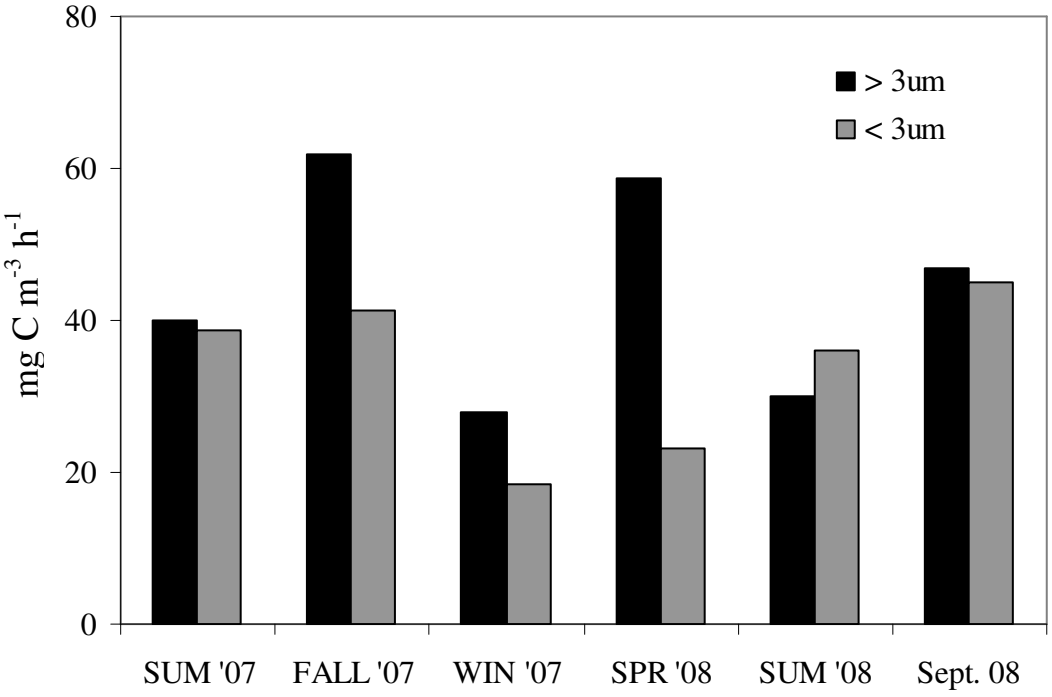

Figure 14. Rates of primary productivity averaged for all stations by season. Black bars represent the > 3µm size fraction and gray bars represent the < 3µm size fraction. Summers (June-August), fall (September-November), and spring (March-May) include 6 sampling dates at each of 5 stations, while winter (December-February) and Sept. 08 each include 3 sampling dates.

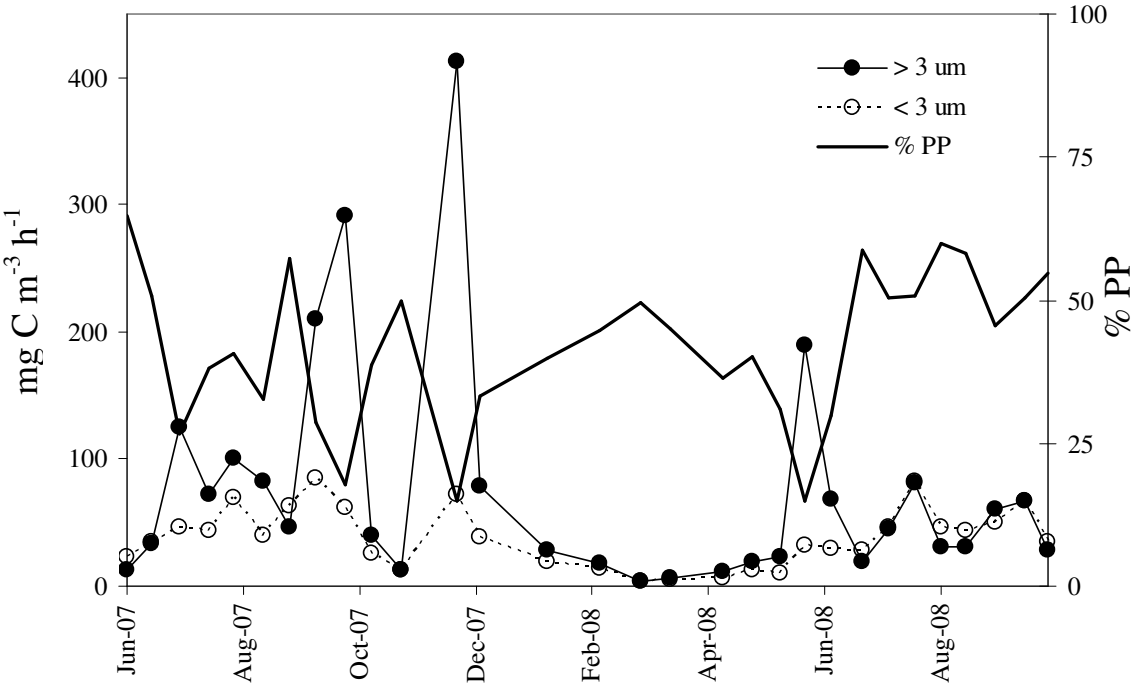

Figure 15: Size-fractionated primary productivity, station 30, in the Neuse River Estuary.

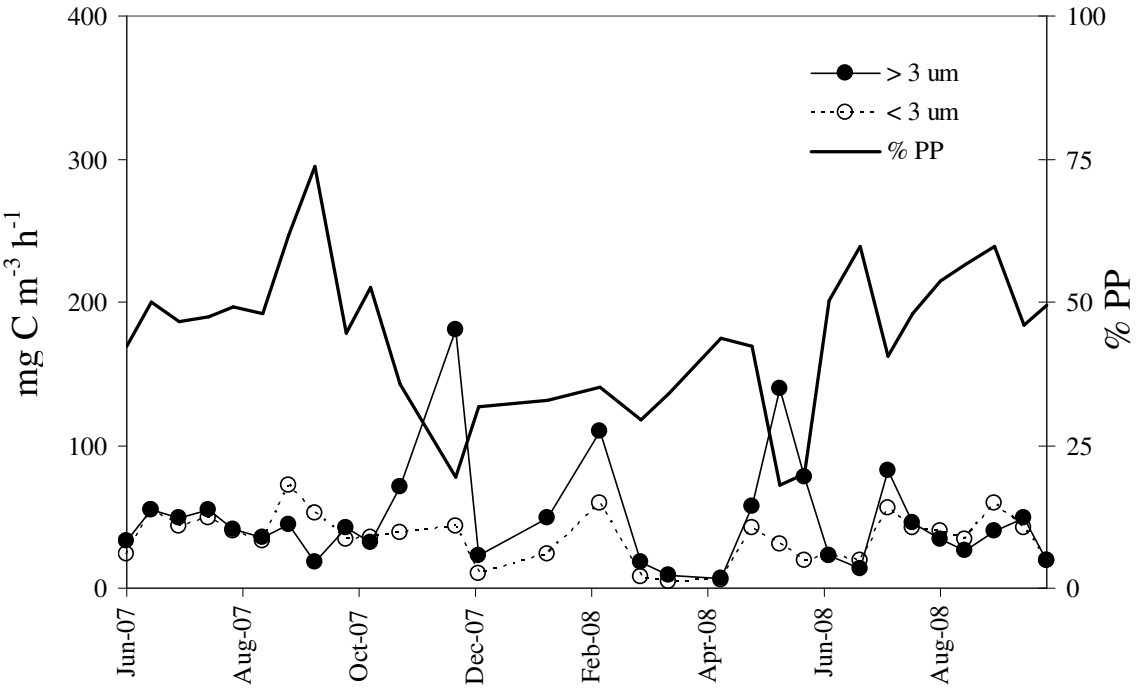

Figure 16: Size-fractionated primary productivity, station 50, in the Neuse River Estuary.

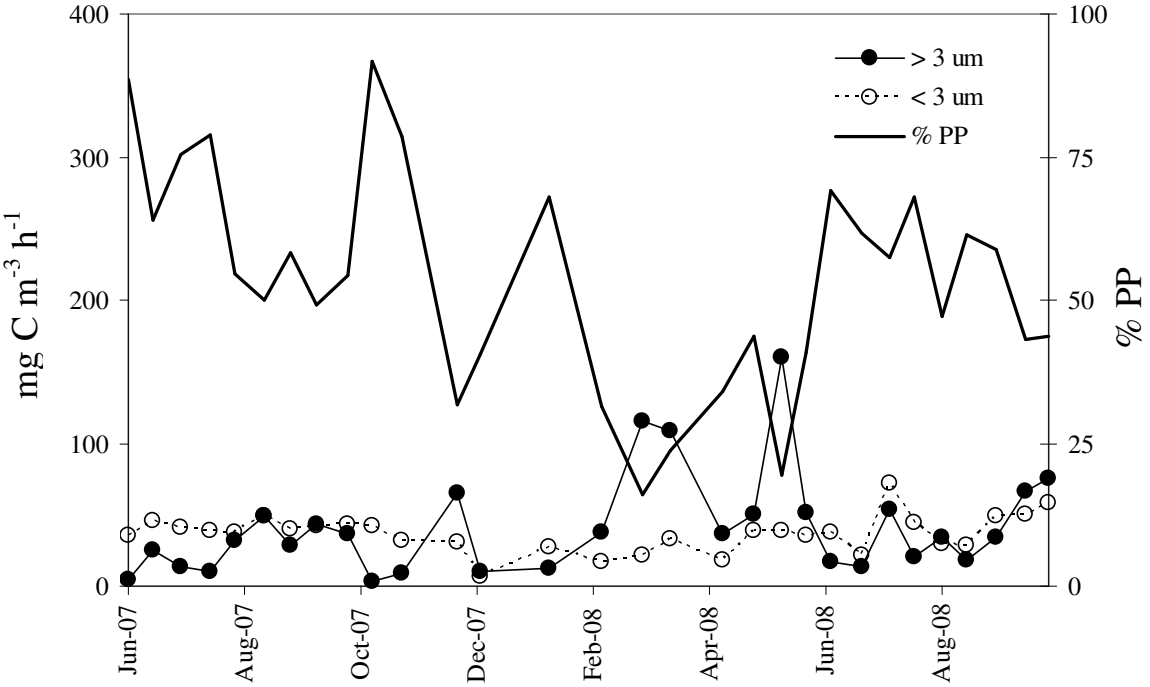

Figure 17: Size-fractionated primary productivity, station 70, in the Neuse River Estuary.

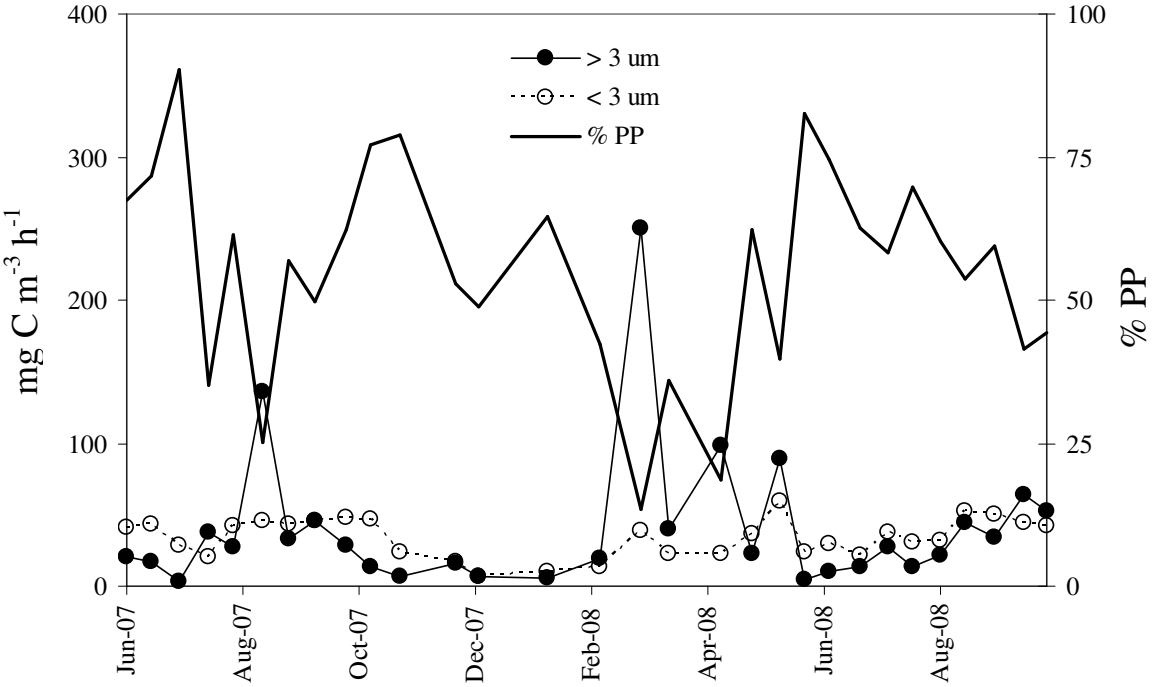

Figure 18: Size-fractionated primary productivity, station 120, in the Neuse River Estuary.

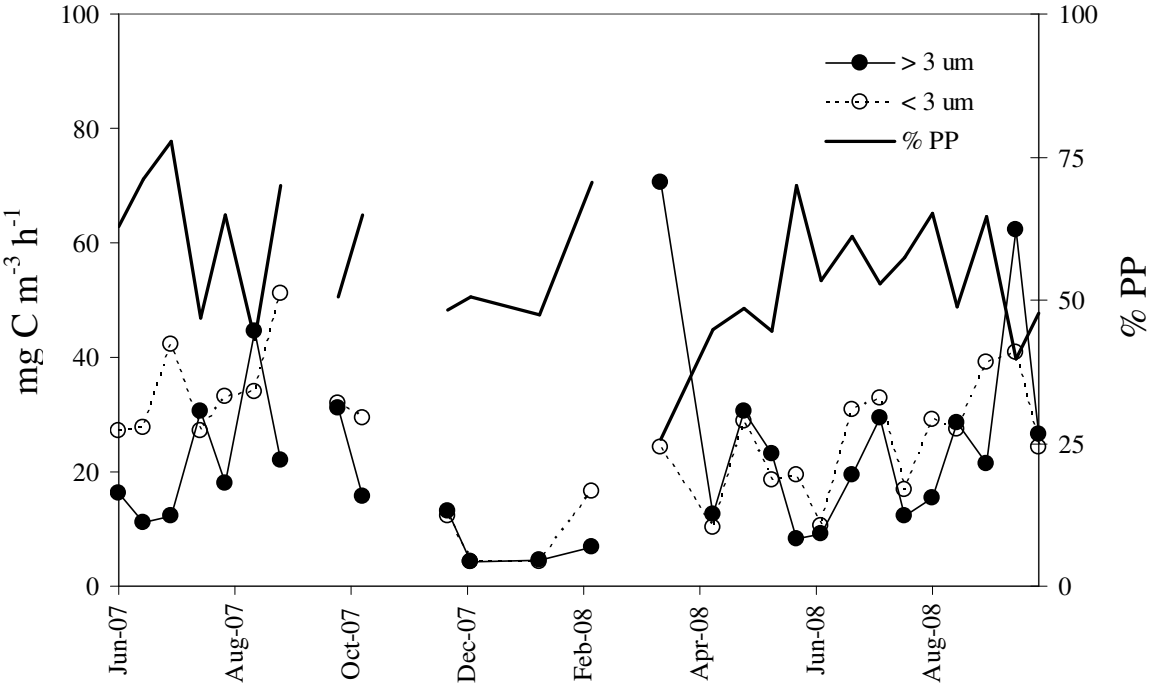

Figure 19: Size-fractionated primary productivity, station 180, in the Neuse River Estuary.

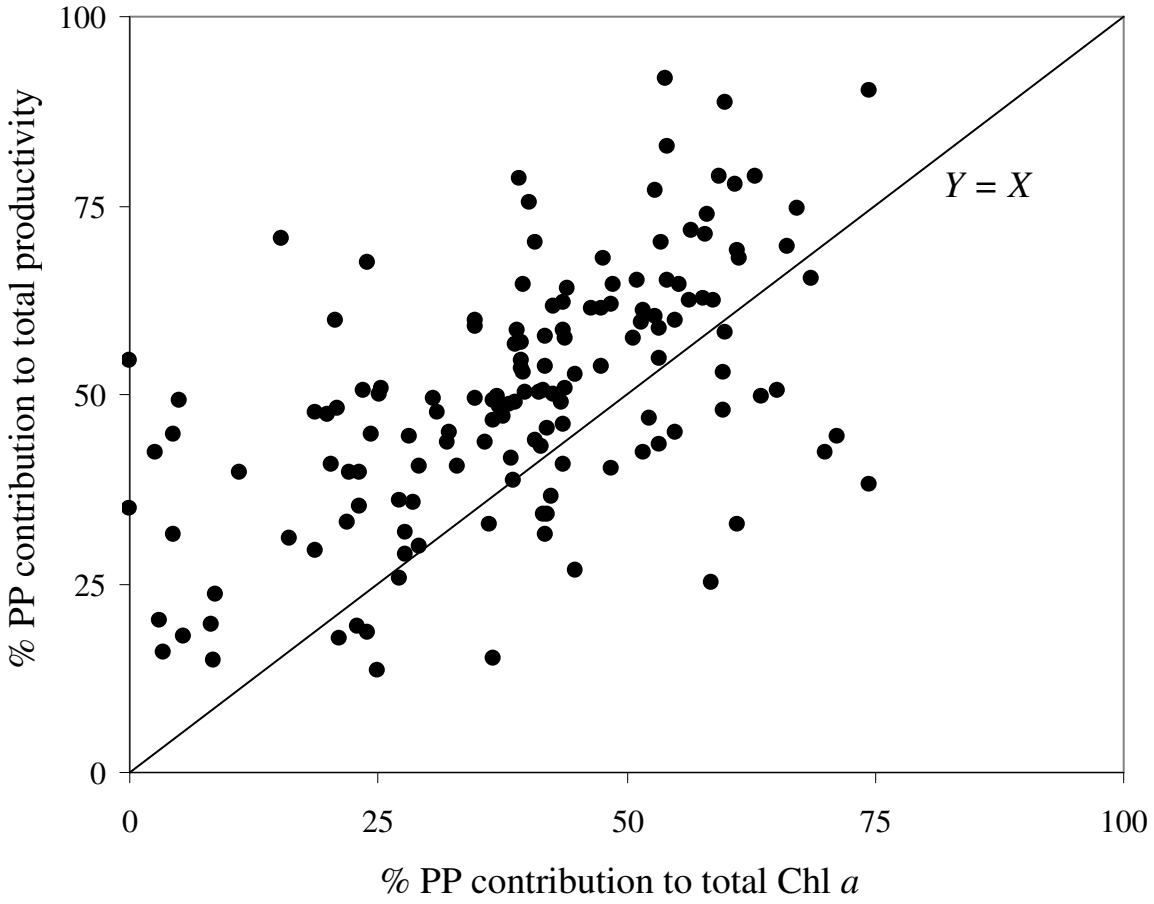

Figure 20: Relationship between % PP contribution to total production and total Chl *a* for all stations and sampling points.

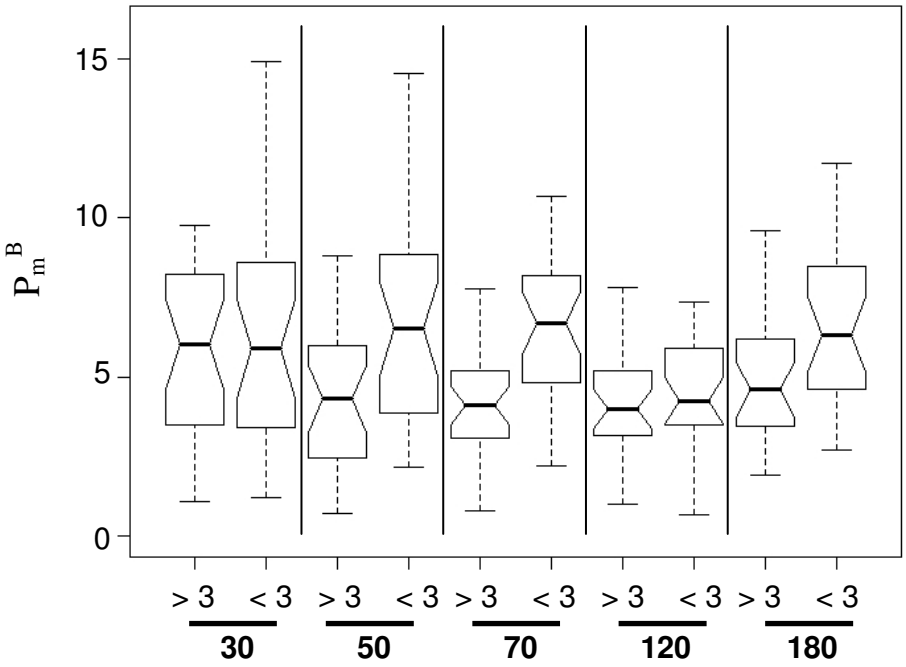

Figure 21. Boxplot of P_m^B values for the > 3µm and < 3µm size fractions, by station. Notches that do not overlap indicate that the medians (horizontal lines within notches) are significantly different ($p < 0.05$). Outliers are not included.

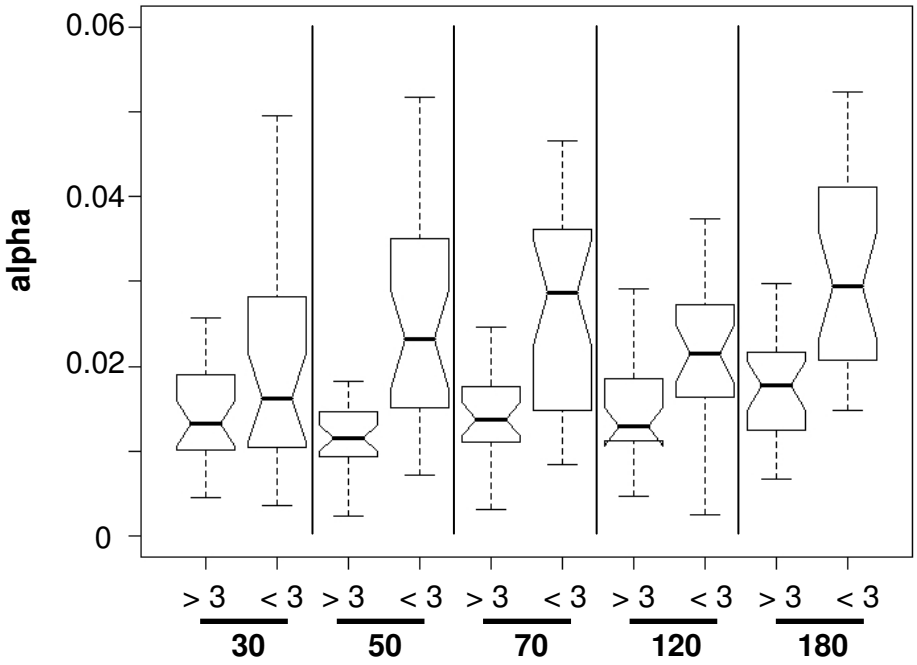

Figure 22. Boxplot of alpha values for the > 3µm and < 3µm size fractions, by station. Notches that do not overlap indicate that the medians (horizontal lines within notches) are significantly different ($p < 0.05$). Outliers are not included.

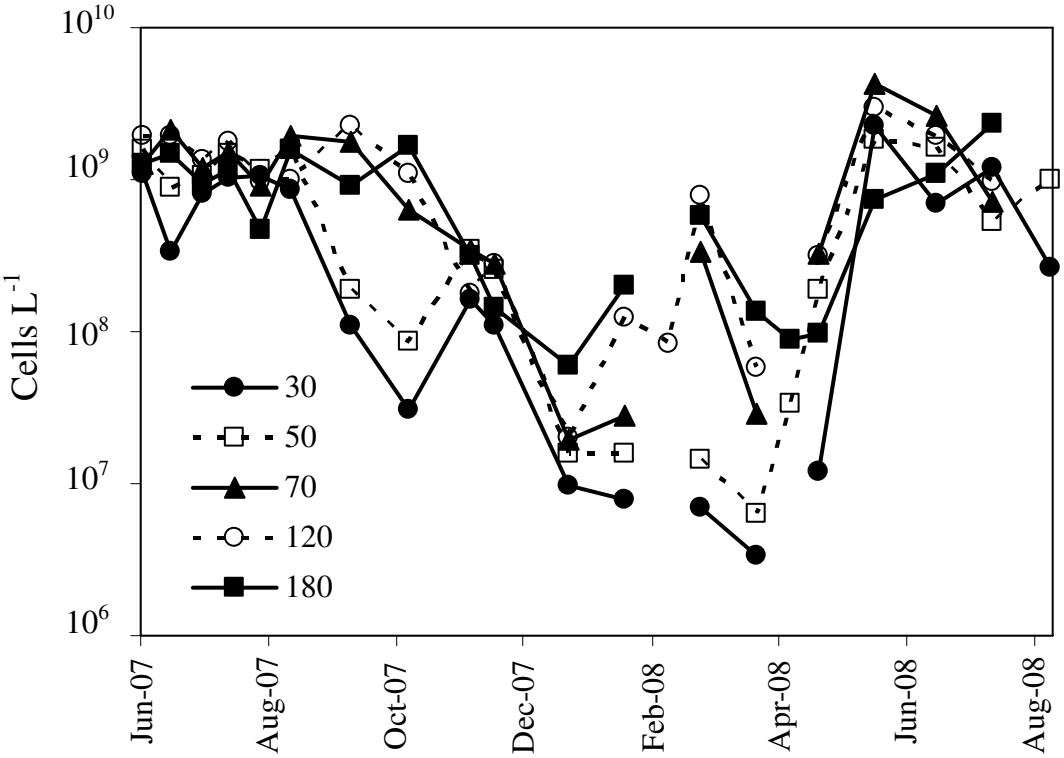

Figure 23. PP cell abundance (from epifluorescent counts) over the study period at all stations.

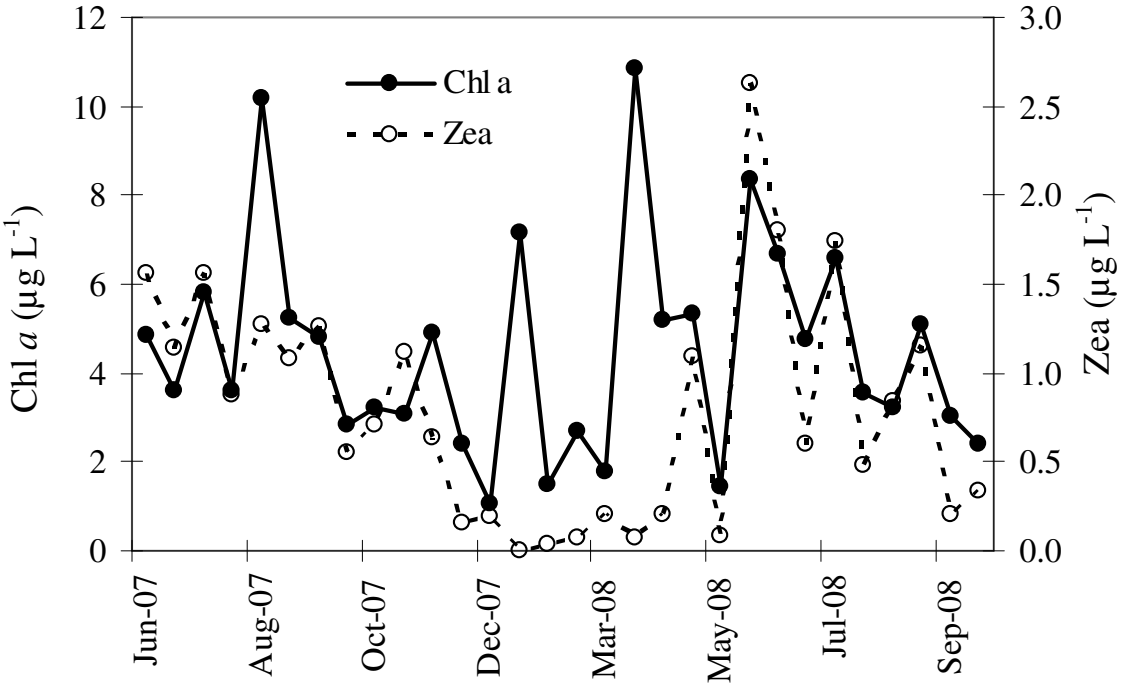

Figure 24. Zeaxanthin and Chl *a* concentrations for the < 3µm size fraction at mid-estuarine station 70 in the NRE.

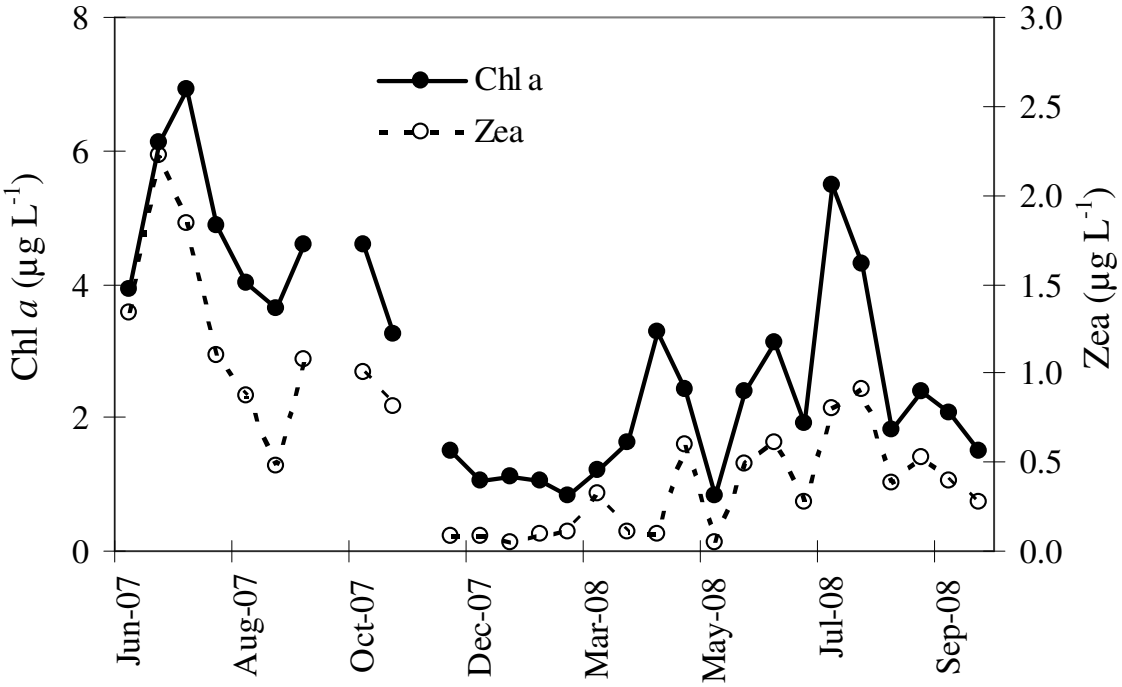

Figure 25. Zeaxanthin and Chl *a* concentrations for the < 3µm size fraction at lower-estuarine station 180 in the NRE. Missing points indicate no samples collected on that date.

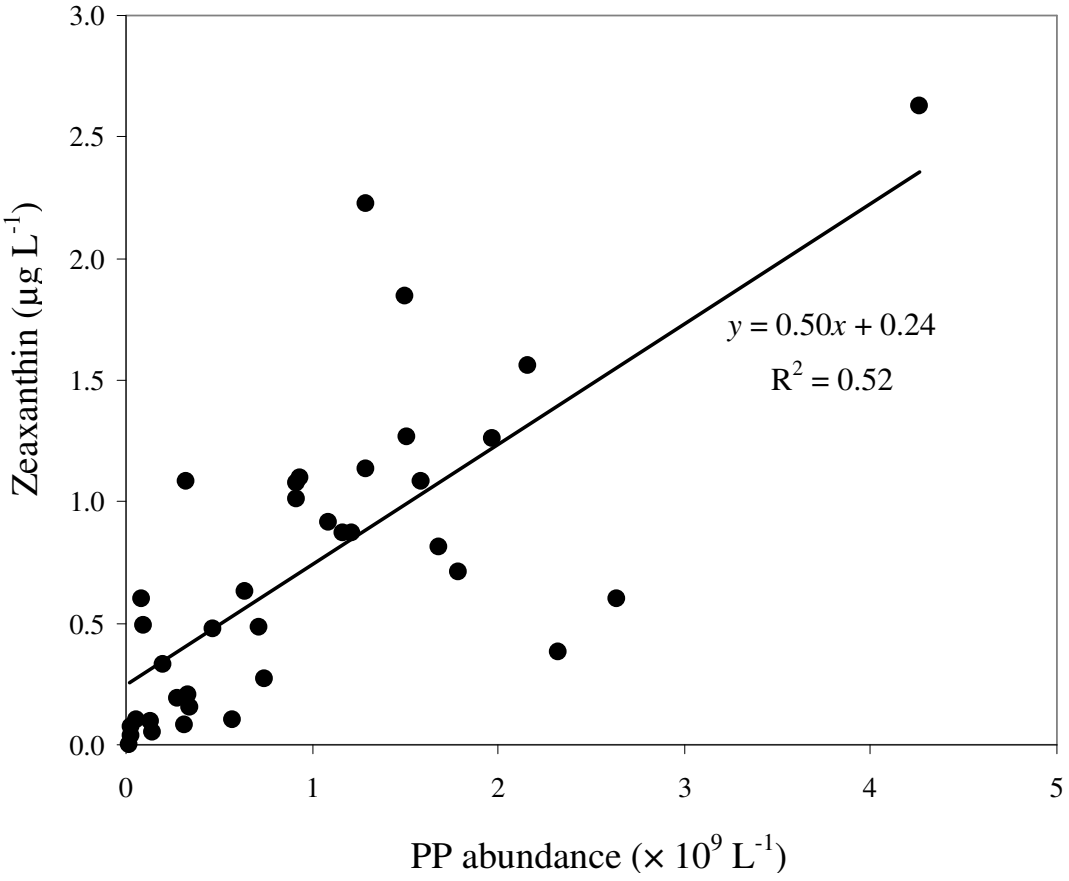

Figure 26. Relationship between < 3µm zeaxanthin concentrations and picoplankton abundance (cell counts) at stations 70 and 180. While cell counts were conducted at all stations, size-fractionated zeaxanthin was only measured at these two stations.

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