Vol. 63: 133–143, 2011
doi: 10.3354/ame01488

AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol

Published online March 31

Environmental controls upon picophytoplankton growth and biomass in a eutrophic estuary

Michael S. Wetz[1,*], Hans W. Paerl[2], J. Christopher Taylor[3], Jeremy A. Leonard[2]

[1]Texas A&M University — Corpus Christi, Department of Life Sciences, Corpus Christi, Texas 78412, USA
[2]Institute of Marine Sciences, The University of North Carolina — Chapel Hill, Morehead City, North Carolina 28557, USA
[3]NOAA Ocean Service, Center for Coastal Fisheries and Habitat Research, Beaufort, North Carolina 28516, USA

ABSTRACT: We assessed the role of top-down versus bottom-up factors in regulating picophytoplankton (PicoP) growth and overall phytoplankton size structure in a eutrophic estuary. PicoP biomass reached an annual maximum in summer/fall and was positively correlated with temperature. Ephemeral blooms (chlorophyll *a* > 20 µg l^{-1}) of PicoP were observed in the upper and middle regions of the estuary despite inorganic nitrogen concentrations <1 µmol l^{-1}. Nutrient-amended PicoP growth rates were similar to *in situ* growth rates in the upper estuary, and PicoP biomass was negatively correlated with river-derived inorganic nitrogen concentrations, indicating that regenerated nutrients are a major source of nitrogen supporting PicoP growth. Microzooplankton grazing rates routinely exceeded PicoP growth rates during summer; therefore, grazing must have become uncoupled from PicoP growth on timescales shorter than the interval between grazing experiments (i.e. 2 to 4 wk) for PicoP to have bloomed. Field data point to the possibility of trophic cascades involving copepods, protistan grazers, and phytoplankton as a mechanism for this growth–grazing uncoupling. These and other recent findings indicate that bottom-up factors alone cannot explain the PicoP blooms observed in some estuarine systems and emphasize the need for grazing control studies to better understand the regulation of primary production.

KEY WORDS: Picophytoplankton · Estuary · Microzooplankton · Mesozooplankton · Trophic cascade · Nutrients

INTRODUCTION

Phytoplankton community size structure is a critical determinant of the magnitude and direction of carbon flux and the efficiency of trophic transfer in aquatic ecosystems (Legendre & Rassoulzadegan 1996). It has long been assumed that larger phytoplankton should dominate in shallow coastal systems typified by high nutrient levels. Thus, the role of small phytoplankton, such as picophytoplankton (PicoP), in estuarine food webs and biogeochemical cycles has largely been overlooked, and understanding of environmental controls upon estuarine PicoP is limited. Recent studies have shown that PicoP can represent a seasonally, if not annually, dominant component of the estuarine phytoplankton community in some estuaries (e.g. Phlips et al. 1999, Murrell & Lores 2004, Gaulke et al. 2010). For instance, in North Carolina's Neuse River Estuary (NRE), a highly eutrophic system, PicoP accounted for an average of ~40% of total phytoplankton biomass year-round (Gaulke et al. 2010). Also, PicoP frequently reached high biomass levels exceeding 20 µg l^{-1} chlorophyll *a* (chl *a*).

Most studies on controls upon PicoP growth in estuarine and coastal waters have focused on the role of bottom-up environmental factors, such as temperature and nutrient availability (e.g. Agawin et al. 2000). Yet microzooplankton are capable of rapidly consuming PicoP production (e.g. Sherr et al. 1986a, Caron et al. 1991, Juhl & Murrell 2005), and it has been suggested that microzooplankton grazing limits PicoP biomass accumulation under otherwise favorable environmental conditions in marine systems (Barber & Hiscock 2006). Given recent observations of PicoP blooms in

*Email: michael.wetz@tamucc.edu

estuaries, however (e.g. Phlips et al. 1999, Gaulke et al. 2010), microzooplankton grazing obviously does not always prevent net PicoP biomass accumulation. One possibility is that trophic cascades create opportunities for net PicoP population growth through ephemeral reductions in grazers of the PicoP (e.g. Liu & Dagg 2003, Jing et al. 2010). Studies of this phenomenon in coastal waters have yielded conflicting results regarding the ability of trophic cascades, operating at the zooplankton to microzooplankton level, to influence phytoplankton population growth, especially that of small phytoplankton (e.g. Sipura et al. 2003, Bouvy et al. 2006, Jing et al. 2010). Nonetheless, it is clear that the role of top-down factors such as grazing must be considered along with bottom-up factors in order to better understand PicoP population dynamics.

One could argue that because the role of PicoP in estuarine food webs and the overall effects of grazing have largely been ignored, our fundamental understanding of factors that shape coastal phytoplankton size structure is incomplete. Estuaries and coastal waters worldwide will continue to experience human modification of nutrient loadings and trophic structure for the foreseeable future (Kennish 2002, Bricker et al. 2008), and superimposed on these changes will be a predicted long-term warming of coastal waters that may promote cyanobacterial PicoP dominance (Li & Harrison 2008). In order to understand why PicoP bloom in some estuaries, and to better predict how PicoP and overall phytoplankton community size structure will respond to impending environmental changes, it is important to first understand what controls estuarine PicoP growth and phytoplankton size structure. A complementary paper (Gaulke et al. 2010) describes the spatial–temporal distribution of PicoP biomass and productivity on an annual basis in the NRE, emphasizing the role of bottom-up factors. Here, field and experimental data were used to assess the role of top-down versus bottom-up factors in regulating PicoP growth and overall phytoplankton size structure on a seasonal basis in the NRE.

MATERIALS AND METHODS

Study site. North Carolina's NRE is a shallow (2.2 m average depth), microtidal estuary. Residence time is relatively long (several weeks to months), ensuring that nutrients entering the system are effectively utilized and retained (Paerl et al. 1998). Over the last 4 decades, the NRE's 16 000 km^2 watershed has undergone rapid urban and agricultural development, which has contributed to a steep increase in point and nonpoint sources of nitrogen (N) and phosphorus (P) (Stow et al. 2001).

Sample collection. Since 1994, the NRE Modeling and Monitoring Program (ModMon, www.unc.edu/ims/neuse/modmon/) has generated a continuous dataset aimed at assessing water quality trends within the estuary. In the present study, 4 ModMon stations spanning the NRE salinity gradient were selected for hydrographic and nutrient measurements, size-fractionated chl *a*, and ciliate and mesozooplankton abundance determinations (Fig. 1). Field sampling efforts were conducted biweekly to monthly from June 2007 to September 2008. Grazing experiments were

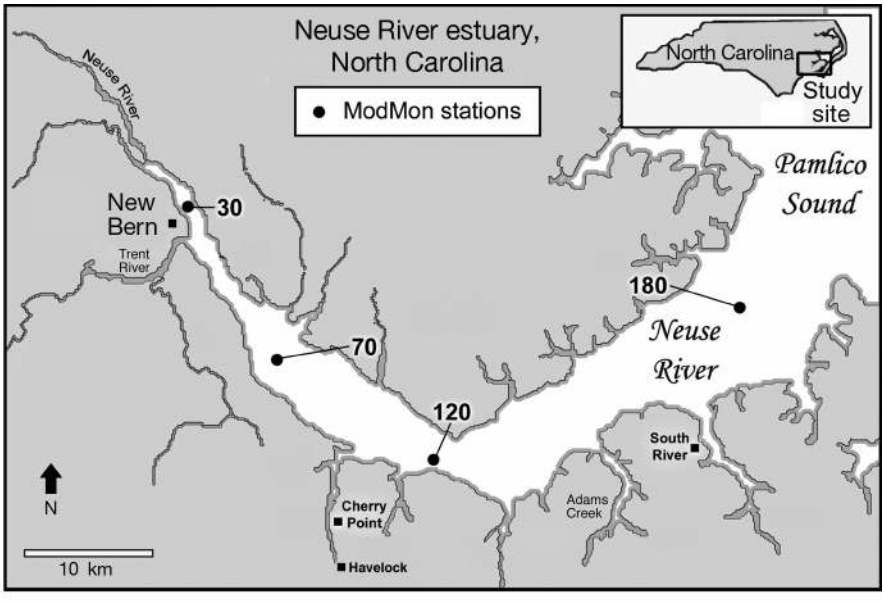

Fig. 1. Neuse River Estuary, North Carolina, USA, indicating the location of 4 stations (30, 70, 120, 180) from the present study

conducted using surface water collected at Stns 70 and 180 on 4 dates in the summer of 2008. Samples for discrete measurements or grazing experiments were collected in 1 or 20 l polyethylene containers, respectively, and stored in coolers for transport to a local field laboratory.

Field measurements. At each field site, a YSI sonde was deployed to measure water column temperature and salinity. Size-fractionated chl *a* was determined from subsamples of water collected at each location. After gentle mixing, duplicate 50 ml subsamples were vacuum filtered (<150 mm Hg) onto Whatman GF/F filters (0.7 µm nominal porosity) for determination of total chl *a*. Then, the <3 µm chl *a* fraction (i.e. PicoP plus nanoplankton between 2 and 3 µm; hereafter PicoP) was determined by subtracting chl *a* collected on 3.0 µm porosity polycarbonate membrane filters from the total (i.e. GF/F) chl *a*. Glass fiber and membrane filters were homogenized, extracted overnight in 90% acetone, and analyzed according to EPA Method 445.0 without acidification using a Turner Designs TD-700 fluorometer. Dissolved inorganic nitrogen (DIN; sum of nitrate, nitrite, ammonium) concentrations were determined using a Lachat QuikChem 8000 flow injection autoanalyzer. Ciliate abundances (from field and experimental samples) were determined from samples preserved immediately upon collection with 2% acidified Lugol's solution. In the case of field samples, ciliates were enumerated via the Utermöhl (1958) settling method within 24 h of collection. Experimental samples were analyzed within 1 mo of collection. Reported here are the combined abundances of oligotrich and unidentified naked ciliates. We recognize that heterotrophic nanoflagellates (HNAN) and heterotrophic dinoflagellates can also be significant consumers of PicoP production (e.g. Caron et al. 1991, Strom 1991), but, due to time and monetary constraints, we had to limit our efforts to the enumeration of ciliates. Samples for mesozooplankton abundance counts were collected via a submersible diaphragm pump at 0.5 m from the surface and 0.5 m from the bottom. Mesozooplankton were sampled from each station and depth by filtering water through a 65 µm mesh zooplankton net. Organisms were transferred to 100 ml bottles, fixed with 3% buffered formalin, and stored in the dark until further analysis in the laboratory. Mesozooplankton were enumerated and identified using a Leica Zoom 2000 dissecting microscope equipped with a rotating counting wheel.

Grazing experiments. Phytoplankton growth rates and microzooplankton grazing rates were determined using a modification of the dilution method (Landry & Hassett 1982). For each experiment, 5 dilution treatments were prepared in duplicate; 5% whole water, 10% whole water, 33% whole water, 100% whole water (without nutrients added), and 100% whole water (with nutrients added). Site water was flushed through sterile filter capsules (0.2 µm pore size), and the filtrate was mixed with unfiltered site water to make the dilutions noted above. During summer, NRE phytoplankton productivity peaks despite inorganic nutrient concentrations that are typically at or below detection limits (Paerl et al. 1998, Twomey et al. 2005). So as to not expose the ambient phytoplankton community with unnaturally high nutrient concentrations, we chose not to add the large quantities of nutrients as is sometimes done in dilution experiments (Andersen et al. 1991). Instead, each of the diluted treatments and 1 set of 100% whole water samples were amended with 4 µmol l^{-1} NH_4Cl and 0.7 µmol l^{-1} KH_2PO_4. The target DIN:DIP addition (~6:1) is typical of NRE surface waters during summer, when N strongly limits productivity (Rudek et al. 1991, Piehler et al. 2002). Water from each treatment was incubated in duplicate 1 l transparent Cubitainers (filled to capacity to eliminate headspace) and incubated for 24 h in an outdoor pond that is continuously flushed with water from adjacent Bogue Sound, thereby approximating *in situ* temperature and surface light conditions. Samples were collected at 0 and 24 h for ciliate abundance and size-fractionated (<3 µm, >3 µm) chl *a* determination. Phytoplankton nutrient-amended growth rates and microzooplankton grazing rates were calculated according to Landry & Hassett (1982) when linear phytoplankton growth responses were detected or according to Moigis & Gocke (2003) when non-linear responses were detected. The *in situ* (i.e. nutrient limited) phytoplankton growth rate was calculated by adding the apparent phytoplankton growth rate from unamended whole water treatments to the microzooplankton grazing rate. In addition to calculating growth and grazing rates by using the dilution fraction as the independent variable, these rates were also calculated by substituting the geometric mean ciliate abundance for the dilution fraction (e.g. First et al. 2007). This was done to determine if potential microzooplankton growth/mortality during dilution experiments affected rate measurements (e.g. Dolan et al. 2000), using ciliates as a proxy for microzooplankton. Techniques proposed by Redden et al. (2002) were used to estimate chl *a* levels at which microzooplankton grazing became saturated.

RESULTS

Abiotic environmental parameters

Temperature varied seasonally in the NRE, ranging from a low of 9.7°C in winter to a high of 31.7°C in

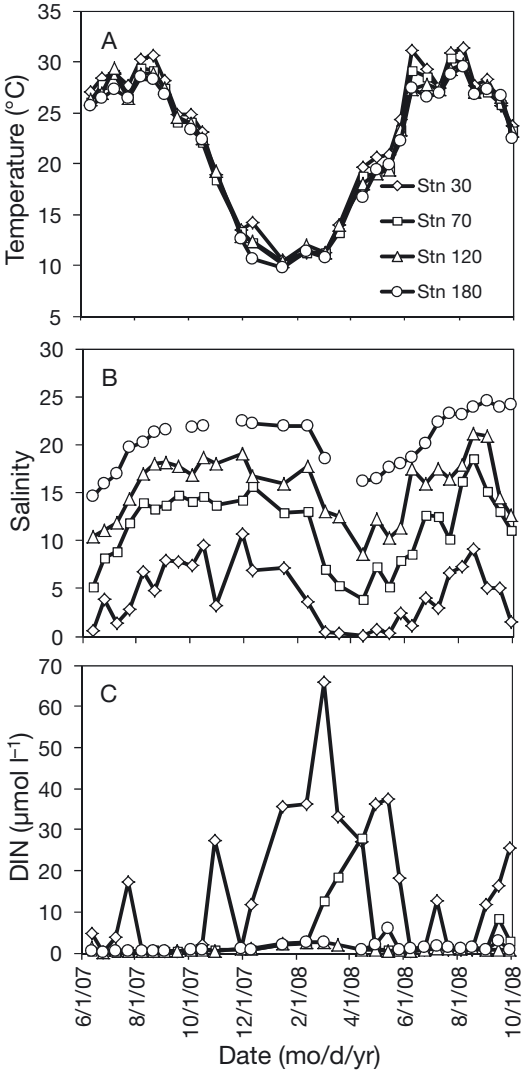

Fig. 2. (A) Temperature, (B) salinity, and (C) dissolved inorganic nitrogen (DIN) at Stns 30 (◇), 70 (□), 120 (△), and 180 (○) over the course of the present study. Note that some data for Stn 180 are missing due to a lack of sample collection resulting from poor weather conditions

summer (Fig. 2A). The average surface temperature varied little between stations (Fig. 2A). Surface salinity was consistently lowest at Stn 30 and always increased with distance downstream (Fig. 2B). Persistent drought conditions from July 2007 to February 2008 resulted in lower Neuse River freshwater discharge and relatively high salinities upstream (Fig. 2B). By spring 2008, rainfall increased and salinity sharply decreased throughout the estuary (Fig. 2B). However, moderate drought conditions returned through fall 2008, resulting in elevated salinities (Fig. 2B). DIN concentrations tended to be highest at Stn 30 and decreased in a downstream direction (Fig. 2C). During summer, when PicoP biomass was maximal in the system, surface DIN concentrations were generally 1 µmol l^{-1} or less (Fig. 2C).

Spatial-temporal distribution of phytoplankton, ciliates, and mesozooplankton

During summer/fall of 2007, highest chl *a* levels were predominantly found at Stn 30 (Fig. 3). Persistent blooms (chl *a* > 20 µg l^{-1}) of PicoP and >3 µm phytoplankton were observed at Stn 30 through late fall (Fig. 3A). Further downstream, ephemeral blooms of PicoP (Stn 120) and >3 µm phytoplankton (Stns 70 and 120) were also noted during summer 2007. Although phytoplankton biomass was generally lowest during winter throughout the estuary, sizeable late winter (February and March 2008) blooms of >3 µm phytoplankton (Stns 70 and 120) and PicoP (Stn 120) were observed (Fig. 3). In general, the annual PicoP biomass accumulation appeared to begin between mid-March and early May, depending on location in the estuary (Fig. 3). The exception was at Stn 30, where high river discharge left surface waters completely fresh through late May 2008—conditions not suitable for brackish PicoP (Figs. 2B & 3A). After river discharge increased between January and March 2008 and the estuary subsequently freshened, the zone of elevated PicoP and >3 µm phytoplankton biomass spread downstream (Fig. 3A–C). During 2008, PicoP biomass was persistently high in summer/fall at Stns 30 to 120 (Fig. 3; see also Gaulke et al. 2010), and PicoP blooms were noted at Stns 30 and 70, while blooms of >3 µm phytoplankton occurred from spring through fall at Stns 30 to 120 (Fig. 3).

Relatively high ciliate abundances were observed at Stn 30 during summer/mid-fall 2007, decreasing downstream in the estuary (Fig. 4). Ciliate abundances began to decline in mid-fall, reaching an annual minimum in winter (Fig. 4). During spring, the largest net increase in ciliate abundances occurred towards the end of May at the 2 uppermost stations (30, 70), when abundances increased from 2 to 9×10^3 to 55 to 164×10^3 cells l^{-1} (Fig. 4A,B). Within 2 wk of reaching peak abundance in late May, ciliate abundances declined rapidly. Further downstream at Stns 120 and 180, a large net increase in ciliate abundance occurred earlier than at the upper stations, this time in mid-April and reaching 76 to 89×10^3 cells l^{-1} (Fig. 4C,D). At the upper 2 stations, ciliate abundances were elevated from spring through early fall of 2008, although they were quite variable on the timescale (biweekly) of field sampling efforts (Fig. 4A,B). At the downstream stations, maximum ciliate abundances were found during spring and were relatively low during summer, except for a small net increase at Stn 120 in late summer (Fig. 4C,D).

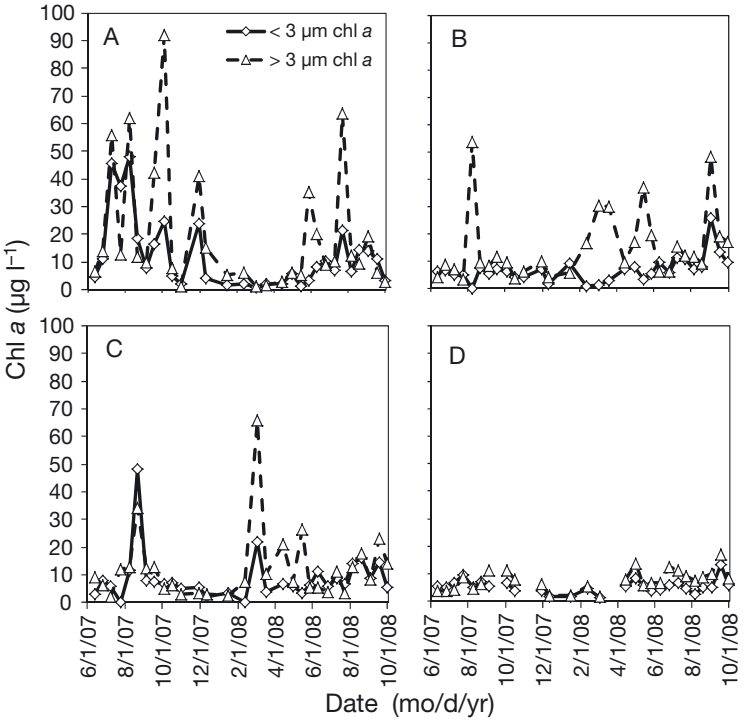

Fig. 3. Levels of the <3 µm chlorophyll *a* (chl *a*) and >3 µm chl *a* size fractions at (A) Stns 30, (B) 70, (C) 120, and (D) 180 over the course of the present study. Note that some data for Stn 180 are missing due to a lack of sample collection resulting from poor weather conditions

Mesozooplankton were not prevalent during the winter months (Fig. 4). During spring, copepod nauplii were the most abundant zooplankters in the system, averaging from 4 to 21 ind. l^{-1} (Fig. 4). In summer through fall of both 2007 and 2008, maximum mesozooplankton abundances tended to be found in the lower NRE, at Stns 120 to 180, possibly due to its optimal salinity regime (Fig. 4; Leonard et al. unpubl. data). The copepod *Acartia* sp. and copepod nauplii dominated the mesozooplankton community in terms of abundance during summer. At Stns 120 and 180, copepods averaged from 16 to 45 ind. l^{-1} in summer 2007 and from 6 to 11 ind. l^{-1} at those stations in summer 2008 (Fig. 4). Copepod abundances were <1 ind. l^{-1} during both summers at Stn 30 (Fig. 4) and were intermediate between the 2 estuarine regions at Stn 70. At Stns 120 and 180, nauplii averaged from 32 to 91 ind. l^{-1} during summer 2007 and from 13 to 16 ind. l^{-1} during summer 2008 (Fig. 4). Nauplii abundances were from 2 to 5 ind. l^{-1} during both summers at Stn 30 (Fig. 4) and were intermediate between the 2 estuarine regions at Stn 70.

Total chl *a* was positively correlated with water temperature (Spearman's rank correlation: $r_S = 0.31$, $p < 0.01$) and ciliate abundance ($r_S = 0.30$, $p < 0.01$) and negatively correlated with salinity ($r_S = -0.26$, $p = 0.01$) and copepod abundance ($r_S = -0.22$, $p = 0.04$) (Table 1). The >3 µm chl *a* fraction was positively correlated with ciliate abundance ($r_S = 0.25$, $p = 0.01$) and negatively correlated with salinity ($r_S = -0.26$, $p = 0.01$) and copepod abundance ($r_S = -0.32$, $p < 0.01$) (Table 1). The <3 µm chl *a* fraction was positively correlated with temperature ($r_S = 0.51$, $p < 0.001$) and ciliate abundance ($r_S = 0.27$, $p < 0.01$) and negatively correlated with DIN concentration ($r_S = -0.21$, $p = 0.04$) (Table 1). The >3 µm chl *a* and <3 µm chl *a* fractions were positively correlated ($r_S = 0.47$, $p < 0.001$), but the percentage of chl *a* in the <3 µm size fraction was negatively correlated with total chl *a* ($r_S = -0.25$, $p = 0.02$) (Table 1). Ciliate abundances were negatively correlated with salinity ($r_S =$

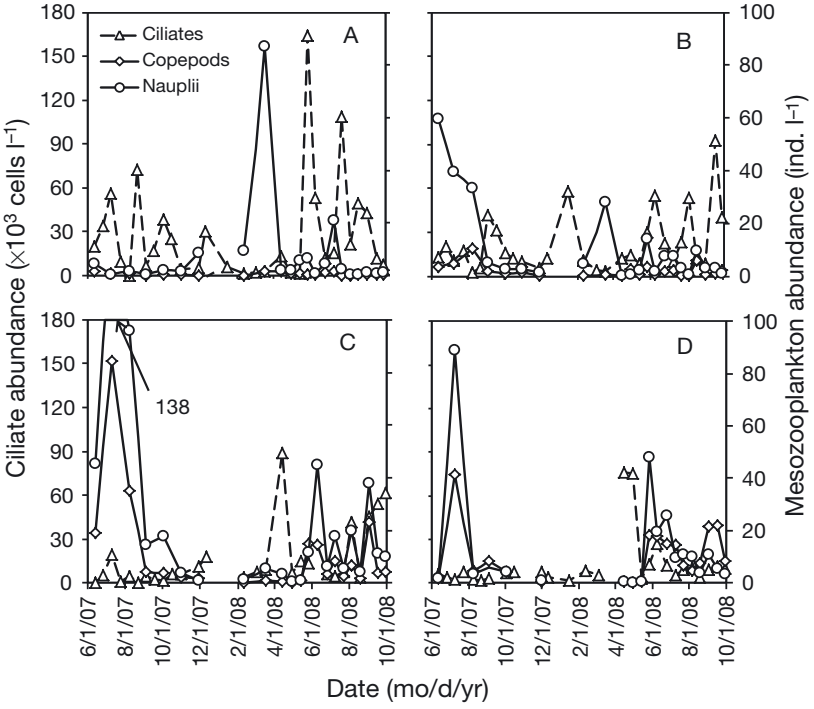

Fig. 4. Ciliate and mesozooplankton abundances at (A) Stns 30, (B) 70, (C) 120, and (D) 180 over the course of the present study. Note that some data for Stn 180 are missing due to a lack of sample collection resulting from poor weather conditions

Table 1. Results from Spearman rank sum correlation analysis of ciliate abundance and size-fractionated phytoplankton biomass (estimated by chlorophyll *a* [chl *a*]) and percentage of total chl *a* in the <3 µm size fraction. Data are from Stns 30, 70, 120, and 180 from 12 June 2007 to 29 September 2008. **Bold** values indicate statistically significant ($p < 0.05$) relationships. DIN: dissolved inorganic nitrogen; r_S: Spearman's correlation coefficient; p: significance of the correlation; N: number of samples

	Temp. (°C)	Salinity	DIN	Ciliates	Total chl *a*	>3 µm chl *a*	<3 µm chl *a*	<3 µm chl *a* (%)	Copepods	Nauplii
Ciliates										
r_S	0.174	**–0.237**	–0.009		**0.301**	**0.253**	**0.271**	–0.087	–0.151	**–0.215**
p	0.088	0.019	0.929		0.003	0.013	0.007	0.397	0.168	0.049
N	97	97	97		97	97	97	97	85	85
Total chl *a*										
r_S	**0.306**	**–0.262**	–0.116	**0.301**		**0.934**	**0.693**	**–0.245**	**–0.223**	–0.094
p	0.002	0.010	0.259	0.003		0.000	0.000	0.016	0.041	0.390
N	97	97	97	97		97	97	97	85	85
>3 µm chl *a*										
r_S	0.145	**–0.259**	–0.024	**0.253**	**0.934**		**0.474**		**–0.317**	–0.159
p	0.156	0.010	0.814	0.013	0.000		0.000		0.003	0.145
N	97	97	97	97	97		97		85	85
<3 µm chl *a*										
r_S	**0.512**	–0.124	**–0.214**	**0.271**	**0.693**	**0.474**			–0.070	–0.091
p	0.000	0.226	0.035	0.007	0.000	0.000			0.523	0.408
N	97	97	97	97	97	97			85	85
<3 µm chl *a* (%)										
r_S	**0.357**	0.076	–0.190	–0.087	**–0.245**				**0.296**	0.167
p	0.000	0.461	0.063	0.397	0.016				0.006	0.128
N	97	97	97	97	97				85	85

–0.24, p = 0.02) and nauplii abundance (r_S = –0.22, p = 0.05) (Table 1).

Phytoplankton growth and microzooplankton grazing rates

When comparing dilution plots using either the dilution factor or geometric mean ciliate abundance as the independent variable, for only a single date did the use of the latter result in the shape of the dilution plot changing from non-linear to linear (Stn 70, >3 µm fraction, 26 June 2008 experiment; data not shown). Further, there was no statistically significant difference in growth- or grazing-rate estimates from either approach (data not shown). Therefore, reported here are the rate estimates obtained using the dilution factor as the independent variable. Environmental conditions at the start of each experiment are presented in Table 2.

In situ PicoP growth rates were variable in time and space, ranging from 1.7 to 3.1 d^{-1} at Stn 70 and from 0.7 to 1.7 d^{-1} at Stn 180 (<3 µm, Table 3). PicoP growth was not nutrient limited at Stn 70, as nutrient-amended growth rates were generally similar to *in situ* growth rates (Table 3). At Stn 180, nutrient-amended PicoP growth rates were higher than *in situ* rates on 3 of 4 dates (Table 3), though no statistically significant differences were detected. Microzooplankton grazing rates exceeded PicoP growth rates on all 4 dates at Stn 70, ranging from 2.1 to 3.7 d^{-1} (<3 µm, Table 3). There was upward curvature in the dilution plot toward the more highly diluted treatments on all 4 dates, indicative of saturated feeding kinetics (data not shown). The estimated PicoP chl *a* concentration at which grazing became saturated was between 6.2 and 8.3 µg l^{-1} (Table 3), and the *in situ* PicoP biomass frequently exceeded this level at Stn 70 throughout the summer (Fig. 3, Table 2). At Stn 180, microzooplankton grazing rates also exceeded *in situ* PicoP growth rates on all 4 dates, ranging from 0.7 to 2.0 d^{-1} (Table 3). There was no evidence of saturated feeding on PicoP in any of the experiments run at Stn 180.

As with the PicoP, *in situ* growth rates of >3 µm phytoplankton were variable in time and space, ranging from 1.4 to 2.9 d^{-1} at Stn 70 and from 0.9 to 1.8 d^{-1} at Stn 180 (>3 µm, Table 3). Nutrient-amended growth rates of >3 µm phytoplankton exceeded *in situ* growth rates on 3 of 4 sampling dates at Stn 70, though this difference was only significant (*t*-test, $p < 0.05$) on a single date. Nutrient-amended growth rates exceeded *in situ* growth rates on all 4 sampling dates at Stn 180 (statistically different on 2 of 4 dates; *t*-test, $p < 0.05$), indicative of growth being nutrient limited (Table 3). Microzooplankton grazing rates upon the >3 µm

Table 2. Environmental conditions at the start of dilution experiments. DON: dissolved organic nitrogen; ND: not determined

Date 2008	Temp. (°C)	Salinity	NH_4^+ (µmol l^{-1})	DON (µmol l^{-1})	PO_4^{3-} (µmol l^{-1})	<3 µm chl *a* (µg l^{-1})	>3 µm chl *a* (µg l^{-1})
Stn 70							
9 Jun	29.11	8.53	0.35	26.06	0.09	7.68 ± 0.52	4.02 ± 0.00
25 Jun	28.59	12.70	0.61	21.66	0.43	6.94 ± 0.53	2.78 ± 0.66
9 Jul	27.57	12.46	1.09	24.82	0.73	10.21 ± 5.07	8.47 ± 0.15
5 Aug	29.71	16.22	0.85	23.28	3.65	7.26 ± 2.08	8.48 ± 1.85
Stn 180							
9 Jun	27.48	18.65	0.93	19.77	ND	1.39 ± 0.09	3.32 ± 0.11
25 Jun	26.55	20.17	1.36	21.33	ND	3.98 ± 0.60	6.07 ± 0.89
9 Jul	26.98	22.31	1.56	15.65	0.31	5.55 ± 1.14	4.20 ± 0.30
5 Aug	29.52	23.15	1.09	17.39	0.47	3.48 ± 0.42	4.04 ± 0.74

phytoplankton exceeded growth rates on all 4 dates at Stn 70, ranging from 1.8 to 3.7 d^{-1} (Table 3). There was upward curvature in the dilution plot toward the more highly-diluted treatments on all 4 dates, indicative of saturated feeding kinetics (data not shown). The >3 µm chl *a* concentration at which grazing became saturated was between 3.2 and 8.1 µg l^{-1} (Table 3), and *in situ* >3 µm chl *a* frequently exceeded this level at Stn 70 throughout summer (Fig. 3, Table 2). At Stn 180, microzooplankton grazing rates also exceeded *in situ* growth rates on all 4 dates and ranged from 1.4 to 2.3 d^{-1} (Table 3). On 2 dates (25 June, 5 August), there was evidence of saturated feeding on the >3 µm phytoplankton at Stn 180.

Table 3. Results, according to size fraction and station, from microzooplankton grazing experiments conducted during summer 2008. Indicated are the nutrient-amended phytoplankton growth rates (µ, +nutrients), the *in situ* phytoplankton growth rates (µ, *in situ*), the microzooplankton grazing rate (*g*), and the estimated chl *a* concentration at which microzooplankton grazing became saturated. Values are means ± SD. *: statistically significant differences ($p < 0.05$) between nutrient-amended and *in situ* phytoplankton growth rates based on 2-tailed *t*-tests

Date 2008	µ, +nutrients (d^{-1})	µ, *in situ* (d^{-1})	*g* (d^{-1})	Chl $a_{saturation}$ (µg l^{-1})
<3 µm, Stn 70				
9 Jun	1.75 ± 0.18	1.70 ± 0.21	2.12 ± 0.14	6.76 ± 0.56
25 Jun	2.28 ± 0.03	2.45 ± 0.04*	3.06 ± 0.01	7.15 ± 0.31
9 Jul	2.02 ± 0.61	2.35 ± 1.23	2.66 ± 0.68	8.32 ± 4.10
5 Aug	3.01 ± 0.41	3.10 ± 0.65	3.73 ± 0.66	6.20 ± 1.17
<3 µm, Stn 180				
9 Jun	2.51 ± 0.13	1.74 ± 0.38	2.04 ± 0.33	
25 Jun	1.63 ± 0.40	1.64 ± 0.54	2.01 ± 0.44	
9 Jul	1.18 ± 0.08	0.69 ± 0.21	1.35 ± 0.14	
5 Aug	1.28 ± 0.06	0.84 ± 0.15	0.67 ± 0.35	
>3 µm, Stn 70				
9 Jun	1.82 ± 0.24	1.35 ± 0.14	1.85 ± 0.36	4.00 ± 0.16
25 Jun	2.22 ± 0.17	1.64 ± 0.08*	1.76 ± 0.33	3.22 ± 0.22
9 Jul	2.44 ± 0.40	2.29 ± 0.44	2.61 ± 0.46	8.08 ± 0.03
5 Aug	2.65 ± 0.42	2.86 ± 0.44	3.74 ± 0.21	7.37 ± 1.24
>3 µm, Stn 180				
9 Jun	2.13 ± 0.00	1.48 ± 0.17*	2.08 ± 0.08	
25 Jun	1.31 ± 0.29	0.85 ± 0.31	1.68 ± 0.42	5.25 ± 0.55
9 Jul	1.57 ± 0.08	1.20 ± 0.04*	1.37 ± 0.08	
5 Aug	1.87 ± 0.93	1.83 ± 1.22	2.26 ± 1.21	3.61 ± 0.28

DISCUSSION

PicoP are an important component of the phytoplankton community in this eutrophic estuary, especially during the warmer months of the year and averaged ~40% of the total phytoplankton biomass. These findings are consistent with those from other temperate to subtropical microtidal estuaries (e.g. Phlips et al. 1999), but contrast with those of tidally flushed estuaries where PicoP are a minor component of the phytoplankton community (e.g. Iriarte & Purdie 1994). Given the numerical importance of PicoP and other small phytoplankton in many estuaries and their potential roles in food web dynamics and biogeochemical cycling, this raises the question: What controls PicoP growth and overall phytoplankton community size structure in estuaries?

The combined effects of high temperature and regenerated nutrients are known to favor PicoP growth (Agawin et al. 2000, Murrell & Lores 2004). Laboratory and field-based studies have noted a strong relationship between cyanobacterial PicoP growth and temperature (Agawin et al. 2000, Murrell & Lores 2004, Fu et al. 2007), and our findings are consistent with those observations. The PicoP were negatively correlated with DIN, which in

the NRE primarily exists as the nitrate and ammonium that is derived from riverine inputs of freshwater (Paerl et al. 1998). A relationship was not observed between the >3 µm phytoplankton and DIN, consistent with the findings of Boyer et al. (1993), and this finding, along with the negative correlation between the PicoP and DIN, indicates that internally produced/regenerated nutrients are a major source of nitrogen for NRE phytoplankton (Twomey et al. 2005). During summer at Stn 70, PicoP growth rates were not enhanced by nutrient addition in the dilution experiments despite *in situ* DIN concentrations of ≤1 µmol l^{-1}, providing more evidence that intense regeneration of nutrients supports PicoP growth. Seasonally high water temperatures enhance degradation of phytodetritus in estuarine bottom waters, promoting hypoxia/anoxia formation and regenerated nutrient release to the water column, especially in eutrophic, long-residence time systems (Cowan & Boynton 1996, Rizzo & Christian 1996). Because of their small size, the PicoP would undoubtedly be efficient competitors for these regenerated nutrients (Raven 1986).

During summer, it was occasionally observed that nutrient additions stimulated >3 µm phytoplankton growth rates. However, most of the observed cases of nutrient stimulation were centered on Stn 180, which is strongly influenced by water exchange with the oligotrophic Pamlico Sound. In this geographic region of the estuary, Rudek et al. (1991) found that phytoplankton can be strongly nutrient limited. Yet from our field data encompassing multiple seasons and stations along the NRE salinity gradient, no overall relationship was observed between >3 µm chl *a* and DIN, indicating that internally produced/regenerated nutrients were sufficient to maintain growth of this size fraction for much of the year. Diatoms, which are mostly >3 µm in size and efficient users of nitrate, usually represent <20% of total phytoplankton biomass in the NRE (Pinckney et al. 1998). In contrast, naked flagellates, which are also mostly >3 µm, are important members of the NRE phytoplankton community (Pinckney et al. 1998) and can efficiently utilize and grow on regenerated nutrients (Lewitus et al. 1998, 2000). These factors may explain the lack of correlation between >3 µm chl *a* and DIN. It is important to note that very high dissolved organic nitrogen concentrations (>20 µmol l^{-1}; Table 2, ModMon unpubl. data) are routinely observed throughout the NRE and may serve as an additional source of nitrogen that fuels growth of phytoplankton in both size fractions (reviewed by Bronk et al. 2007).

In addition to the role of temperature and nutrients, findings presented here highlight the importance of grazing in controlling estuarine PicoP growth and phytoplankton size structure during warmer seasons. Microzooplankton grazing rates exceeded *in situ* PicoP and >3 µm phytoplankton growth rates in nearly all summer experiments. Thus, for PicoP to have bloomed, grazing must have become uncoupled from PicoP growth on timescales shorter than the interval between experiments (i.e. 2 to 4 wk). Several authors have observed very high temporal variability in the ratio of grazing to phytoplankton growth in estuaries (e.g. Iriarte et al. 2003, Wetz et al. 2006), attributed in part to variable hydrographic conditions. The relatively high growth rates of both phytoplankton size fractions at Stn 70 in the NRE (1.4 to 3.1 d^{-1}), where many of the blooms occurred, would allow for rapid accumulation of biomass if grazing were reduced.

How could phytoplankton growth become uncoupled from micro- and mesozooplankton grazing? While the role of physical forcing certainly warrants further evaluation, trophic cascades are another mechanism by which growth and grazing can become uncoupled. There is increasing recognition of the importance of trophic cascades in aquatic ecosystems, although studies centered in estuarine and coastal waters have yielded conflicting results on the effects of cascades on lower trophic levels (i.e. phytoplankton and bacteria) (e.g. Sipura et al. 2003, Bouvy et al. 2006, Zöllner et al. 2009, Jing et al. 2010). Statistically significant relationships were observed between various trophic levels in the NRE planktonic food web, suggesting that trophic cascades may have played a role in the uncoupling of phytoplankton growth from grazing. For instance, copepod nauplius abundances were inversely correlated with ciliate abundances. Ciliates are an important food source for copepods (Sherr et al. 1986b, Rollwagen Bollens & Penry 2003, Calbet & Saiz 2005), including juvenile copepod life stages (Merrell & Stoecker 1998). Ciliate abundances in the NRE were positively correlated with both PicoP and >3 µm chl *a*. The positive correlation between ciliate abundance and PicoP chl *a* is particularly interesting, as it suggests that trophic interactions operating at the zooplankton to microzooplankton level may ultimately influence phytoplankton size structure. We speculate that the correlation between ciliates and PicoP was actually a result of ciliate–HNAN and HNAN–PicoP interactions rather than a direct relationship between ciliates and PicoP. HNAN are both an important prey item for ciliates (Zöllner et al. 2009) and a primary consumer of PicoP (Sherr et al. 1986a, Caron et al. 1991, Chen & Liu 2010). Zöllner et al. (2009) observed significant increases in HNAN abundance when ciliates were removed via copepod feeding. If representative of trophic interactions in the NRE and other estuaries, strong copepod feeding on ciliates would theoretically lead to elevated HNAN abundances and grazing on PicoP and reduced PicoP population growth. On the other hand, reduced copepod grazing on ciliates would

theoretically lead to reduced HNAN abundances and grazing on PicoP, enhanced PicoP population growth, and the observed positive correlation between ciliates and PicoP.

Aside from the indirect effects (i.e. trophic cascading) on the phytoplankton size structure outlined above, total chl *a* and >3 µm chl *a* were both negatively correlated with copepod abundances, pointing to a direct grazing effect by copepods. This is not surprising considering that estuarine copepods such as *Acartia* sp. (the dominant taxa in our study) can readily consume nano- and microplankton (Berggreen et al. 1988, Rollwagen Bollens & Penry 2003). Mallin & Paerl (1994) also observed substantial copepod grazing pressure on larger phytoplankton in the lower NRE during summer months. Consumption of larger phytoplankton by copepods may lead to shifts in the overall phytoplankton community towards smaller sized organisms, as denoted by the positive correlation between copepod abundance and the percent of total chl *a* in the <3 µm size fraction.

Few studies have examined the role of grazing in PicoP bloom dynamics in estuaries, instead focusing on bottom-up factors such as light, nutrients, and temperature. Recently, Goleski et al. (2010) argued that lack of microzooplankton grazing allowed for cyanobacterial PicoP blooms in Florida Bay. It was suggested that production of grazing-inhibitory compounds by PicoP may have been responsible for the lack of grazing, although trophic cascades driven by mesozooplankton omnivory could not be discounted (Goleski et al. 2010). Buskey et al. (1997) also found that the picoplanktonic brown tide organism *Aureococcus anophagefferens* was able to proliferate in Laguna Madre, Texas, due to lack of grazing pressure. Those authors suggested that production of grazing-inhibitory compounds or mesozooplankton consumption of the microzooplankton may have been responsible for the lack of grazing on the bloom. Our findings point to the presence of trophic cascades from mesozooplankton to protistan grazers and ultimately to phytoplankton, consistent with the hypothesis that top-down controls on small prostistan grazers allow the PicoP to bloom during summer. We cannot, however, rule out the possibility that cyanobacterial toxins played a role in microzooplankton grazing inhibition, which should be assessed in future studies of microzooplankton–PicoP trophodynamics in aquatic systems.

The findings presented here and in other recent studies indicate that a complex suite of top-down and bottom-up environmental factors interact to control PicoP growth and to shape the overall estuarine phytoplankton community size structure. Because temperature and grazing both have a strong impact on estuarine PicoP growth, what does this mean for estuarine planktonic food web structure in a future where water temperatures are predicted to be warmer than at present? In other words, will increased water temperatures result in increased cyanobacterial PicoP population growth and shifts toward dominance of estuarine planktonic food webs by small phytoplankton? The answer to this question is not straightforward, since coastal ecosystems will continue to be modified by human impact on food webs (through fish removal) and nutrient loadings. These changes will interact to modify planktonic food web structure in a complex manner, emphasizing the need for sophisticated food web models (and supportive data collection and synthesis) incorporating direct and indirect (trophic cascade) pathways.

Acknowledgements. We thank Dr. A. Lewitus and 4 anonymous reviewers for constructive comments on the manuscript. We thank P. Wyrick, J. Braddy, M. Leonard, K. Rossignol, L. Kelly, N. Hall, and B. Peierls for technical assistance. M.S.W. was supported by a National Research Council postdoctoral fellowship. This research was funded by North Carolina Department of Environment and Natural Resources and the Lower Neuse River Basin Association (ModMon), the National Science Foundation (Projects OCE 0726989, 0825466, 0812913), and the North Carolina Sea Grant (Project R/MER-58).

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Editorial responsibility: Robert Sanders, Philadelphia, Pennsylvania, USA

Submitted: June 4, 2010; Accepted: December 30, 2010
Proofs received from author(s): March 16, 2011