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Phytoplankton and zooplankton seasonal dynamics in a subtropical estuary: importance of cyanobacteria

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A seasonal study of phytoplankton and zooplankton was conducted from 1999 to 2001 in Pensacola Bay, Florida, USA, to further the understanding of pelagic food webs in sub-tropical estuaries. Monthly measurements included size-fractionated chlorophyll (whole water, <5 μm, <20 μm), net- and picophytoplankton composition analyzed using microscopy, flow cytometry, and HPLC pigment analysis. Additionally, zooplankton abundance and dry weight were determined from net tows. The results show a phytoplankton community dominated by the small size fraction (<5 μm), especially during the warm periods. The <5 μm chlorophyll fraction was strongly correlated with cyanobacterial abundance and zeaxanthin. Cyanobacteria (cf. Synechococcus) abundance peaked during summer in the upper estuary, typically exceeding 3×10^9 L^{-1}, and was strongly correlated with temperature. Cyanobacteria abundance at the freshwater end of the Bay (in the Escambia River) was very low, suggesting that cyanobacteria were not delivered via freshwater. Two pigmentation types of cyanobacteria were observed. Phycoerythrin-containing cells (PE-rich) were more abundant at the marine end, while phycocyanin-containing cells (PC-rich) were more abundant in the upper estuary. The larger algae (>5–10 μm) were predominantly composed of diatoms, followed by chlorophytes, cryptophytes and dinoflagellates. The three most abundant genera of diatoms were Thalassiosira, Pennales and Cyclotella. Zooplankton biomass averaged 12.2 μg C L^{-1}, with peak biomass occurring during May (~30 μg C L^{-1}). Zooplankton abundance averaged 16.7 ind. L^{-1}, peaking at 30 ind. L^{-1} during May. During the summer, the zooplankton community shifted from the ubiquitous Acartia tonsa towards Oithona sp. The increase in Oithona coincided with increases in picophytoplankton and may reflect the changing food resources available to zooplankton. Thus, the trophic implications of cyanobacterial dominance in sub-tropical estuaries need to be more fully assessed.

INTRODUCTION

There is growing evidence that human activities are changing the distribution and movement of major nutrient elements in the landscape resulting in increased nutrient loading to receiving waters (NRC, 2000). Bottom-up controls, such as changes in nutrient loading, can alter the species composition of primary producers (Stolte *et al.*, 1994; Ingrid *et al.*, 1996; Roelke *et al.*, 1999). Top-down grazing controls can also alter phytoplankton community composition (Micheli, 1999) and size structure (Hansen *et al.*, 1994; Mousseau, 1998; Cottingham, 1999; Sipura *et al.*, 2003). Such shifts in the primary producer community are likely to cascade through the food web, altering consumer food-web dynamics, and thus the flow of carbon and energy through a system. It is therefore critical to understand better the linkages between nutrient loading to coastal waters and the ecosystem response.

The environmental forcings that control plankton dynamics in sub-tropical Gulf of Mexico estuaries are little-studied compared with temperate Atlantic estuaries. Sub-tropical waters have a warmer temperature regime, including long periods of warm temperatures (>28°C for 3–5 months), and are more frequently impacted by large rainfall events such as tropical storms and hurricanes (Solis and Powell, 1999). Except for very large river systems (e.g. Mississippi–Atchafalaya),

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freshwater flows tend to have a muted seasonality; high flow events can occur almost any time of year. These characteristics tend to make sub-tropical estuarine hydrodynamics less predictable than temperate estuaries.

There are few data documenting the phytoplankton community composition in Gulf coast estuaries (e.g. Livingston, 2001, 2003). One study examined the abundance and species composition of larger phytoplankton (>5 μm) in Pensacola Bay (Livingston, 2001), concluding that the community exhibited relatively low species richness and tended to be dominated by bloom-forming species (*Cyclotella choctawhatcheeana*, *Prorocentrum minimum* and *Heterosigma akashiwo*). That study was restricted to the larger phytoplankton community, and therefore did not take into account the contribution of picophytoplankton, despite growing evidence from other estuaries of the numerical and functional importance of cyanobacteria (Pinckney *et al.*, 1998; Phlips *et al.*, 1999).

Cyanobacteria have not been enumerated in this region of the Gulf of Mexico coast. While the importance of cyanobacteria and other picophytoplankton are well documented in the open oceans, their importance in estuaries is less well studied. Many studies have found high abundances of cyanobacteria, but their contribution to the total phytoplankton community is usually relatively small (Pinckney *et al.*, 1998; Ning *et al.*, 2000). A notable exception to this pattern was reported from Florida Bay, where cyanobacterial abundances peaked at 5×10^9 cells L^{-1} and appeared to dominate the phytoplankton community (Phlips *et al.*, 1999).

The complex nature of the trophic linkages between phytoplankton and zooplankton communities is of broad and sustained interest to the scientific community, yet little is known of the zooplankton in Gulf of Mexico estuaries in general and Pensacola Bay in particular. One study (Lores *et al.*, 2002) reported zooplankton abundance in Pensacola Bay averaging 3.1 L^{-1}, and found higher abundances in the bay proper compared with the adjacent tidal bayous. An earlier study (Dye, 1987) reported much higher average zooplankton abundances of 38 L^{-1} in Pensacola Bay. Both studies noted the predominance of *Acartia tonsa*; other taxa included the cyclopoid copepod, *Oithona colcarva*, the cladocerans *Podon* and *Evadne*, the rotifers *Brachionus* and *Synchaeta*, and the larvacean *Oikopleura sp.* In nearby Perdido Bay, zooplankton abundances averaged ~2 L^{-1} and ranged from <1.0 to 28 L^{-1} (Livingston, 2001). To our knowledge, these are the only zooplankton data from the region.

The purpose of this study was to examine seasonal patterns in phytoplankton and zooplankton in Pensacola Bay to develop a more comprehensive picture of the biomass of the major plankton groups, and to give insight into likely trophic pathways. This study augments previous and ongoing process-oriented studies, and will help to develop a clearer understanding of the processing of nutrients and organic matter in such sub-tropical estuaries.

METHOD

Study area

Pensacola Bay is a moderately sized (370 km^2), shallow (mean depth 3 m) system located in northwestern Florida, USA (Figure 1), classified as a micro-tidal, partially-stratified, drowned river valley estuary (Schroeder and Wiseman, 1999). Tides are predominantly diurnal with a mean amplitude of 0.5 m. About 80% of the freshwater flows into the western side of the system from the Escambia River into the Escambia Bay, with an annual mean discharge of ~200 m^3 s^{-1}. The remaining 20% of the surface flow comes from the Blackwater, Yellow, and East Rivers, all emptying into the East Bay. The Escambia River is alluvial with a watershed area of 9900 km^2 draining a landscape of pine forests (74%), croplands (12%), pastures (7%) and urban development (2%). River concentrations of total N averages 39 μM (range 14–107) and total P averages 1.2 μM (range 0.3–4.8) (Alexander *et al.*, 1996). Escambia and East Bays converge into Pensacola Bay proper, which exchanges with the Gulf of Mexico through a narrow deep pass at the western end of Pensacola Bay and Santa Rosa Sound. The mean water residence time for the entire system is ~25 days (Solis and Powell, 1999), although this estimate is not well constrained. Unlike many temperate estuaries, phosphorus appears to frequently limit phytoplankton growth (Murrell *et al.*, 2002a), a frequently observed feature of Gulf coast estuaries (Myers and Iverson, 1981; Flemer *et al.*, 1998; Mortazavi *et al.*, in preparation).

Field collection

This study was conducted from June 1999 through November 2001 at five stations oriented along the salinity gradient from Escambia River to Pensacola Bay proper (Figure 1). Nutrient and hydrodynamic conditions during that period are detailed elsewhere (Murrell *et al.*, in preparation). Samples were collected from surface waters using a Van Dorn bottle, and stored in acid-cleaned polyethylene bottles for laboratory processing within 2–3 h. As stratification was frequently evident, surface samples cannot be considered representative of the water column at a given site but of the salinity regime.

Chlorophyll

Samples for size-fractionated chlorophyll were prescreened through 5 or 20 μm Nitex mesh before collecting onto filters. Whole water and size fractionated chlorophyll

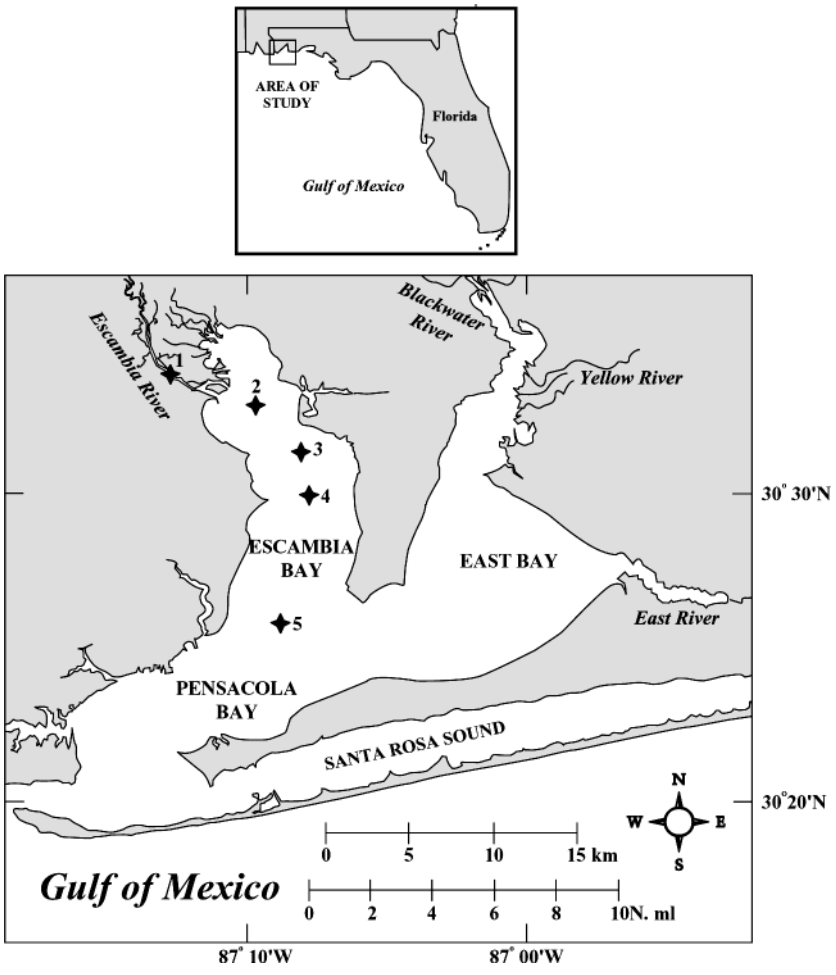

Fig. 1. Map of Pensacola Bay system showing sampling locations.

concentrations were determined by filtering 100–200 mL of water onto 25 mm Whatman GF/F filters using gentle vacuum (<250 mm Hg). The filters were frozen at −70°C until analysis. Chlorophyll was extracted in 10 mL of buffered methanol (Jeffrey *et al.*, 1997), sonicated with a micro-probe sonicator (50 W, 30 s); fluorescence was measured with a Turner Designs TD 700 fluorometer equipped with excitation and emission filters developed by Welschmeyer (Welschmeyer, 1994). The fluorometer was calibrated using commercially available chlorophyll *a* standards (Sigma Chemicals).

HPLC pigments

Samples for phytoplankton accessory pigments were collected as described for total chlorophyll except that larger volumes (200–500 mL) were filtered. The filtered cells were extracted in 2 mL of acetone and micro-probe sonicated (50 W, 30 s). The extracts were analyzed on a Hewlett Packard 1090 HPLC with a tertiary gradient elution system, a 250 × 4 mm Spherisorb 5 μm ODS 2 column, and absorbance (350–500 nm) was measured using a photodiode array detector (Wright *et al.*, 1991). The nominal detection limit for each pigment was 0.05 μg mL^{-1} in the extract. The instrument was calibrated using standards for chlorophyll *a*, chlorophyll *b*, chlorophyll *c*1 and *c*2, alloxanthin, butanoyloxyfucoxanthin, diadinoxanthin, diatoxanthin, divinyl pheophorphyrin *a*5, fucoxanthin, 19′-hexanoyloxyfucoxanthin, lutein, monadoxanthin, myxoxanthophyll, neoxanthin, nostoxanthin, peridinin, prasinoxanthin, violaxanthin, and zeaxanthin. The instrument was calibrated using commercial pigment standards (Sigma Chemicals and VKI, Denmark). Given the difference in extraction protocols, the fluorometric method for Chl *a* (described above) was considered quantitative, while the HPLC method provided a qualitative measure of the pigment composition.

Phytoplankton taxonomy

For phytoplankton taxonomic analysis, 100 mL samples were fixed with 2% formaldehyde, followed by 1% acid

Lugols. The samples were concentrated by settling in Utermöhl chambers for 1 h mm^{-1} of column depth. Typically, 5–10 mL of water sample was settled; phytoplankton were counted and identified at 450× to the lowest possible taxon using appropriate keys (e.g. Tomas, 1997). A minimum of 100 microscope fields, or 100 individuals of the most dominant taxa were counted, whichever came first. This method was considered quantitative for the larger (>5–10 μm) phytoplankton taxa.

Cyanobacteria abundance

Water samples (20 mL) were fixed in 2% final concentration formaldehyde (pre-filtered through 0.2 μm syringe filter). Within several days of collection, 1–2 mL was filtered onto black, 0.2 μm polycarbonate membrane filters and mounted on microscope slides sandwiched between layers of low-fluorescing immersion oil (Hobbie *et al.*, 1977). The cells were enumerated with a Nikon Microphot epifluorescence microscope at 1250× equipped with a green excitation (510–560 nm) and red emission (590 nm LP) filter combination. A minimum of 300 cells was counted distributed over at least 10 microscope fields (typically 20–40). Duplicate samples were periodically counted and the coefficient of variation among replicates averaged 7.1% (n = 15). Cyanobacteria were distinguishable based upon their fluorescence characteristics: phycoerythrin-containing cells (PE-rich) fluoresced orange while phycocyanin-containing cells (PC-rich) fluoresced a deep red color. The relative abundance of PE-rich and PC-rich cells was measured on 20 samples from July to Oct 2000 using flow cytometry (Beckman FACSCaliber) excited with blue argon (488 nm) and red diode (635 nm) lasers, and emission measured in the orange (564–606 nm) and red (653–669 nm) wavelengths. Additionally, six of these samples were visually scored as PE-rich or PC-rich using epifluorescence microscopy.

Cyanobacteria isolation and culture

Cyanobacterial strains were isolated from Pensacola Bay waters of salinity 15–20 p.s.u. as follows. Bay water was pre-screened through a 5 μm filter to exclude larger phytoplankton, enriched with nutrients [SN media, salinity 20 p.s.u. (Waterbury *et al.*, 1986)], and placed in a lighted incubator (25°C, ~100 μmol quanta m^{-2} s^{-1}, 24 h light). After several days of growth, 200 μL aliquots of this mixed assemblage was distributed into 12 wells of a 72 well micro-titre plate. The aliquots were serially diluted through seven series for a maximum dilution of $\sim 10^{-9}$ and replaced into the incubator for several weeks. Growth was observed (by change in color) in 10 of 12 wells of series 6, no growth was evident in any well of series 7. The ten positive series 6 wells were transferred into 10 mL of fresh media. After growth, all isolates were examined microscopically and by flow cytometry; two isolates contained pure PE-rich cells, and one isolate contained pure PC-rich cells. The chlorophyll content of one of the PE-rich isolates and the PC-rich isolate was further characterized, by simultaneous chlorophyll and cell abundance analysis, of three replicate cultures of each strain grown in 500 mL Pyrex Erlenmeyer flasks, and harvested during exponential growth.

Zooplankton

Zooplankton net tows were collected from November 1999 to November 2001 at four sites with a flow-meter equipped (General Oceanics, Miami, FL), 0.5 m diameter, 153 μm net. The net was towed for 3 min at ~1 m s^{-1} at 0.5–1 m below the surface. To examine replicability, a second tow was taken at a single randomly-chosen site on each date. Net contents were washed down and strained through a 5 mm sieve to remove ctenophores and placed on ice for return to the laboratory. For dry weight analysis, a 10–50 mL sub-sample was removed with a Hensen-Stempel pipette, desalted by washing with deionized water over a 100 mm mesh, and transferred into pre-weighed drying tins. The samples were dried at 70°C for 24–48 h and re-weighed. The remaining sample was preserved with 10% formalin for taxonomic identification and abundance measurements (see below). Zooplankton dry weight was converted to carbon biomass using a factor of 0.4, and secondary production was calculated using the temperature-dependent relationship of Huntley and Lopez (Huntley and Lopez,1992),

$$P = B \times 0.0445\ e^{0.111 \times T}$$

where P is zooplankton production in μg C L^{-1} day^{-1}, B is zooplankton biomass in μg C L^{-1} and T is water temperature in °C.

Zooplankton total abundance and taxonomic analysis was conducted on samples collected from November 1999 to November 2001, including 10 samples from replicate tows. The formalin-fixed samples were stained with Rose Bengal, washed over a 63 μm mesh to remove fixative, and re-diluted into sufficient water to obtain a workable counting density. One mL aliquots were pipetted from the shaken sample, transferred into a counting chamber, and examined using a compound microscope. This process was repeated until a minimum of 200 individuals was counted and identified to the lowest possible taxon. In order to make zooplankton data comparable with measures of other variables, abundance and biomass are reported as individuals (ind.) L^{-1}

and μg C L^{-1}, respectively, rather than the more traditional units of ind. m^{-3} and mg C m^{-3}.

RESULTS

Chlorophyll

During this study, chlorophyll concentrations (measured fluorometrically) ranged from 1 to 26 μg L^{-1}, averaging 6.8 ± 5.8 μg L^{-1} (mean ± SD) (Figure 2). Peak concentrations occurred during summer. In general, chlorophyll was lower during 1999–2000 than in 2001. The minimum chlorophyll concentrations were typically observed at the Escambia River site (station 1). The size-fractionation data suggest that, on average, over 70% of the chlorophyll was associated with cells of <5 μm, however, the seasonal peaks also included increases in the larger size fractions. The <5 μm size fraction exhibited strong seasonality being highest during summer (June–October), often 70–90% of total chlorophyll.

Cyanobacteria

Cyanobacteria were enumerated from June 1999 to November 2001 on a total of 105 samples. Cyanobacterial abundance exhibited a striking seasonality (Figure 3A) that correlated strongly with water temperature ($r = 0.61$, $P << 0.0001$, $n = 105$). The cyanobacteria observed were clearly of estuarine origin given that, in the Escambia River, abundances were one or more orders of magnitude lower than at the nearest estuarine site (~2 km away). Cyanobacterial abundance also correlated with the <5 μm chlorophyll size fraction ($r = 0.77$, $P << 0.0001$, $n = 105$); the slope of Model II geometric mean regression was 3.42 and the intercept was near zero (Figure 3B). Using this slope factor suggests that cyanobacterial chlorophyll ranged from 1.1 to 15.7 μg L^{-1}, averaging >70% of the total

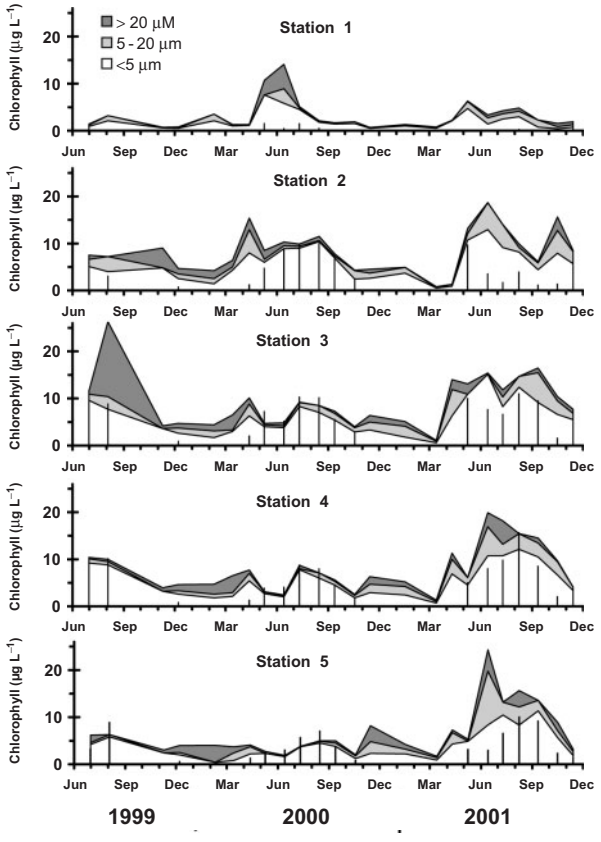

Fig. 2. Size fractionated chlorophyll concentrations measured in Pensacola Bay at five sites, from June 1999 through November 2001. The >20 μm fraction was calculated as the difference between the total and the <20 μm fractions; the 5–20 μm fraction was calculated as the difference between the <20 μm and the <5 μm fractions. The vertical lines indicate the estimated contribution of cyanobacteria to total chlorophyll, based on cell counts (see Figure 3).

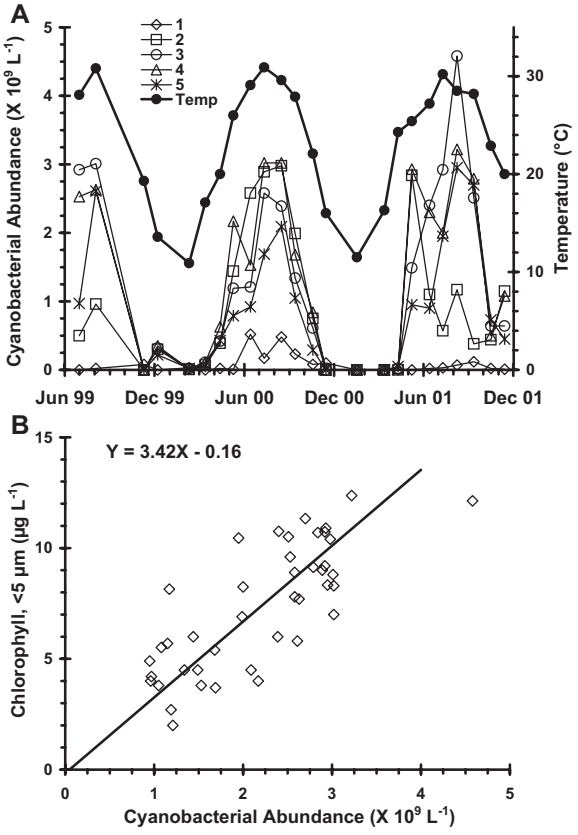

Fig. 3. (**A**) Cyanobacterial abundance in Pensacola Bay measured from June 1999 to Nov 2001 at the five sampling sites (see legend). Note the strong coherence of abundance with water temperature (heavy solid line), and the relatively low abundances in the Escambia River (site 1, diamonds). (**B**) Regression of the <5 μm chlorophyll fraction and cyanobacterial abundance, when cyanobacterial abundances reached a threshold of 1 × 10^9 L^{-1}. The regression (Model II, geometric mean) equation was used to calculate cyanobacterial contribution to bulk chlorophyll (see Figure 2).

chlorophyll (Figure 2). At times, the estimated cyanobacterial contribution to bulk chlorophyll exceeded the small size fraction.

There were clear differences in fluorescence characteristics of the cyanobacteria detectable with both epifluorescence microscopy and flow cytometry. With epifluorescence microscopy under green excitation, cells rich in phycocyanin or allophycocyanin (PC-rich) fluoresced a deep red color, whereas cells rich in phycoerythrin (PE-rich) fluoresced orange. This distinction in cell types was also evident using flow cytometry when excited with blue argon (488 nm) and red diode lasers (635 nm) and emission measured in the orange (564–606 nm) and red (653–669 nm). A clear spatial pattern was evident in the distribution of PC-rich and PE-rich cells, which when plotted against salinity (Figure 4), showed that PC-rich cells were 8 to 10-fold more abundant in the upper estuary, while PE-rich cells were more abundant in the lower estuary. There was a sharp transition in dominance from PC-rich to PE-rich at salinities of ~20–25 p.s.u., a pattern less pronounced with flow cytometry than with microscopy.

Cultured isolates of PE-rich and PC-rich cyanobacteria were analyzed for chlorophyll content and cell abundance under exponential growth conditions. Both strains were small (1–2 μm), unicellular, and coccoid to rod-shaped, similar to open-ocean *Synechococcus* sp. and the majority of cyanobacteria observed in Pensacola Bay water samples. The cultures were very distinctive in color; the PE-rich culture had a red–orange tint while the PC-rich culture had a green tint. However, both strains had similar cellular chlorophyll content; PC-rich cells contained 3.1 ± 0.3 fg Chl cell^{-1} (n = 3) and PE-cells contained 3.2 ± 0.2 fg Chl cell^{-1} (n = 3).

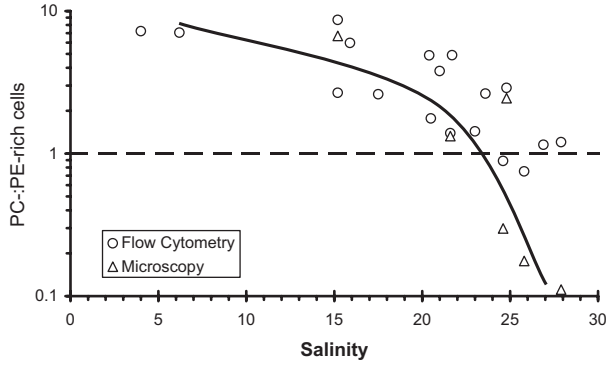

Fig. 4. Ratio of PC-rich to PE-rich cyanobacteria with respect to salinity in Pensacola Bay from July to October 2000. The curve was fitted by eye. PE-rich cells were distinguished from PC-rich cells by their characteristic orange fluorescence. Samples were analyzed by flow cytometry (circles) and/or epi-fluorescence microscopy (triangles).

HPLC pigments

HPLC pigments were analyzed from 66 samples collected at all fives sites from June 1999 to Nov 2000 (Figure 5). The most frequently measured pigments were Chl *a* (100%), fucoxanthin (74%), zeaxanthin (47%), Chl *c* (39%), diadinoxanthin (30%), and peridinin (8%). Zeaxanthin concentration was correlated with cyanobacterial abundance (r = 0.71, P < 0.001, n = 31) (Figure 6), increasing in the spring/summer and declining in the autumn. Chl *c*, diadinoxanthin and fucoxanthin increased in the fall and decreased in the spring. Alloxanthin, representing cryptophytes, was found occasionally, though always in low concentration, and exhibited no consistent temporal or spatial patterns.

Phytoplankton taxonomy

A total of 38 samples from two stations (2 and 5) were analyzed for phytoplankton taxonomy from July 1999 to June 2001. The abundance and taxonomic patterns were very similar at the two sites, so the data were averaged for each sampling date (Figure 7). Diatoms were the most abundant taxa (Figure 7A), generally

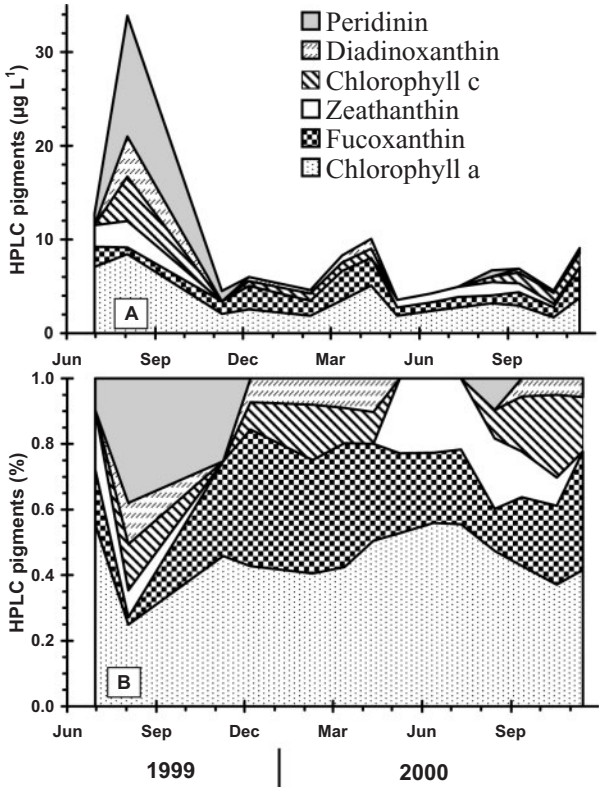

Fig. 5. Accessory pigments determined by HPLC analysis from June 1999 to Nov 2000, showing the six pigments in highest concentration. Each point represents a mean of four analyses, one from each of the four estuarine stations (stations 2–5), shown as (**A**) absolute concentration in μg L^{-1}, and (**B**) relative contribution to total pigments.

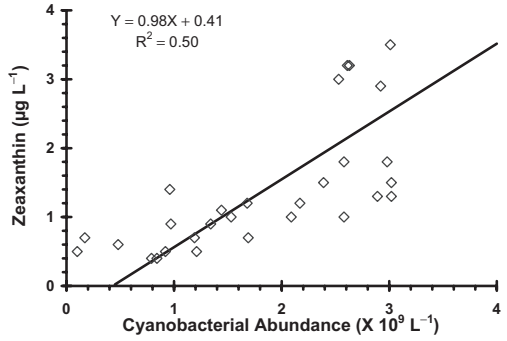

Fig. 6. Relationship between zeaxanthin and cyanobacterial abundance. The Model II (major axis) regression line is plotted. The slope suggests zeaxanthin content of cyanobacteria of 1 fg cell^{-1}.

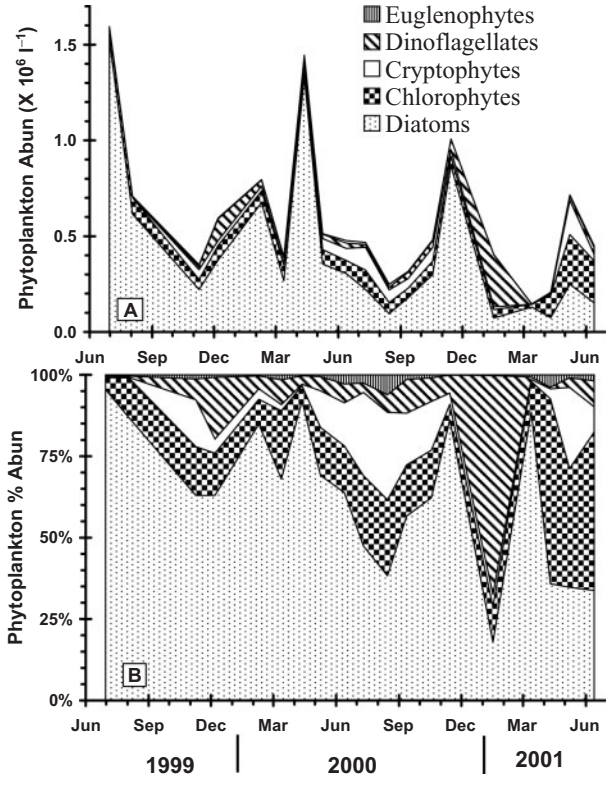

Fig. 7. Net phytoplankton abundance by major taxonomic groups. Values plotted are the mean abundance from two sites, including the upper bay (station 2) and lower bay (station 5). Values are plotted as (**A**) total abundance and (**B**) percentage abundance.

representing over 50% of total abundance (Figure 7B), and accounted for the three most abundant taxa (Table I). Total abundances averaged $0.60 \pm 0.39 \times 10^6$ L^{-1} (mean $\pm$ SE), peaking at 1.6×10^6 L^{-1} during June 1999. In January 2001, when total abundance was low ($<0.5 \times 10^6$ L^{-1}), dinoflagellates comprised 70% of total abundance, mostly *Prorocentrum minimum*. Chlorophytes (cf. *Chlorella*) represented ~50% in the spring of 2001, with abundances of 0.2×10^6 L^{-1}. Cryptophytes were most abundant during spring and summer, with abundances averaging about 0.05×10^6 L^{-1}.

Zooplankton

A total of 107 zooplankton samples were collected from November 1999 to November 2001 at the four estuarine sites (2, 3, 4 and 5). Six samples were excluded from analysis due to a malfunctioning flowmeter, but for the remainder of the samples, the tow volume averaged 21.6 ± 5.8 m^3 (mean $\pm$ standard deviation). The variability among replicates was relatively small; the average percentage difference among duplicate tows was 16% (n = 18) for dry weight and 13% (n = 9) for total abundance. Within-date variability in zooplankton biomass among stations was also relatively small with an average coefficient of variation of 32%, and only weakly correlated with salinity (r = 0.17, P = 0.12, n = 81). Based on the lack of strong spatial patterns, and to examine system-wide seasonal patterns, zooplankton biomass was averaged across the four stations for each date (Figure 8). Zooplankton biomass ranged from 1.4 to 34.1 µg C L^{-1}, averaging 12.2 ± 8.5 µg C L^{-1}. Generally, biomass minima occurred during winter (Feb–Mar) and maxima occurred during May of both years. Calculated zooplankton production ranged from 0.3 to 27.5 µg C L^{-1} day^{-1} and tracked zooplankton biomass except during winter, when production was almost nil due to low temperatures. The very low abundances observed during March and April 2001 coincided with a large rain event in the watershed, when Escambia River flow exceeded 1000 m^3 s^{-1}, about 3-fold higher than normal.

Zooplankton total abundance and taxonomic analysis was conducted on a subset of 58 samples taken from November 1999 to November 2000 (Figure 9). Total abundance ranged from 2.7 to 45.5 ind. L^{-1} and averaged 16.9 ± 9.9 ind. L^{-1} (mean $\pm$ SD). The dominant taxon was *Acartia tonsa*, which averaged 54% (range 20–96%) of total abundance over the time series. Other major taxa included the cyclopoid copepod, *Oithona* sp. (15%), larval barnacles *Balanus* sp. (7%), the larvacean *Oikopleura* sp. (5%) and the cladoceran *Podon* sp. (4%). *A. tonsa* peaked during spring in both relative (69%) and absolute abundance (16 ind. L^{-1}), while *Oithona* sp. peaked during summer when it comprised 30% of total zooplankton, and reached average abundances of 5.9 ind. L^{-1}. The larvacean, *Oikopleura* sp. was common in the summer to early fall (average 1.2 ind. L^{-1}). Another calanoid periodically observed was *Clausicalanus furcatus*, which reached high abundances during July 2000 at station 5 (Figure 9).

The relationship between zooplankton total abundance and carbon biomass showed a strong positive correlation (r = 0.70, $P < 0.01$, n = 43). In order to

Table I: List of most abundant phytoplankton identified to lowest possible taxa from Pensacola Bay

Group	Taxa	Abundance	% abundance	% occurrence	Peak abundance	Date
Diatom	*Thalassiosira*	150	25.3%	71%	1477	12-Apr-00
Diatom	*Pennales*	119	20.1%	95%	1443	30-Jun-99
Diatom	*Cyclotella*	43	7.3%	92%	195	9-Feb-00
Chlorophyte	*Chlorella*	35	5.9%	100%	82	8-May-01
Cryptophyte	*Chroomonas*	31	5.3%	79%	230	8-May-01
Dinoflagellate	*Prorocentrum*	24	4.0%	76%	287	16-Jan-01
Diatom	*Thalassionema*	23	3.8%	34%	466	30-Jun-99
Diatom	Fragilariaceae	21	3.5%	58%	191	14-Nov-00
Chlorophyte	Chlorophyceae	19	3.2%	71%	60	8-May-01
Chlorophyte	Chlorococcaceae	16	2.6%	37%	55	10-Jun-01
Diatom	*Nitzschia*	19	3.2%	87%	118	14-Nov-00
Dinoflagellate	*Gymnodinium*	12	2.1%	66%	94	7-Dec-99
Diatom	*Chaetoceros*	12	2.0%	37%	56	14-Nov-00
Diatom	*Coscinodiscus*	9.3	1.6%	66%	82	30-Jun-99
Cryptophyte	*Plagioselmis*	5.1	0.9%	34%	25	9-May-00
Chlorophyte	*Chlamydomonas*	4.3	0.7%	55%	32	8-May-01
Diatom	*Navicula*	3.2	0.5%	55%	17	30-Jun-99
Cryptophyte	Cryptophyceae	3.0	0.5%	29%	21	8-May-01
Diatom	*Skeletonema*	2.9	0.5%	13%	45	14-Nov-00
Chlorophyte	*Selenastrum*	2.8	0.5%	34%	28	3-Aug-99
Cyanophyte	*Lyngbya*	2.8	0.5%	39%	24	10-Apr-01
All other taxa		35.7	6.0%			

Average abundance ($\times 10^3$ L^{-1}), relative % abundance, % occurrence (n = 38 samples), peak abundance, and the date of peak abundance.

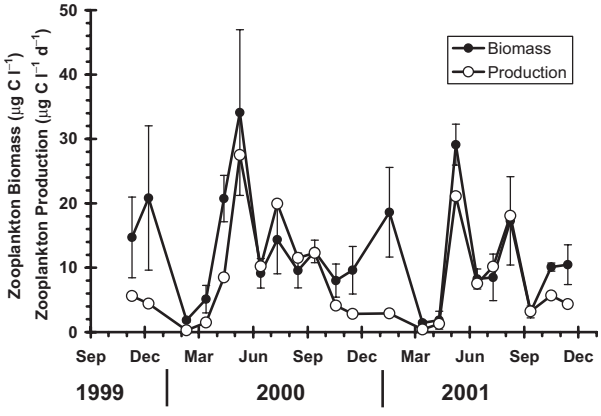

Fig. 8. Zooplankton biomass (µg C L^{-1}) based on dry weight analysis (solid circles), and estimated production (open circles). Error bars represent standard deviations of samples taken at the four estuarine stations (stations 2–5).

examine the size structure of the zooplankton community over the study period, we used the ratio of zooplankton carbon biomass to total abundance to estimate the carbon content per individual. The carbon content ranged from 0.2 to 3.2 µg C ind.$^{-1}$, with an overall median of 0.66 µg C ind.$^{-1}$ (Figure 10). There was a trend towards increasing median size, from 0.54 to 0.89 µg C ind.$^{-1}$, with increasing salinity, although the linear regression against salinity was not statistically significant (P = 0.52).

DISCUSSION

The purpose of this study was to examine seasonal patterns in phytoplankton and zooplankton in Pensacola Bay to develop a more comprehensive understanding of the biomass of the major plankton groups, and to provide insight into likely trophic pathways. Little is known of the phytoplankton or zooplankton dynamics in Gulf of Mexico estuaries in general and Pensacola Bay in particular. Previous research in Pensacola Bay has been motivated by occurrences of eutrophication-associated summer fish kills, largely attributable to industrial point sources (Olinger *et al.*, 1975). In the present study, chlorophyll concentrations averaged 6.8 ± 5.1 µg L^{-1} (mean ± SD), suggesting a system of a moderate trophic state. Similar averages of 6.7 µg L^{-1} and 6.0 µg L^{-1} in upper and lower Escambia Bay respectively were reported

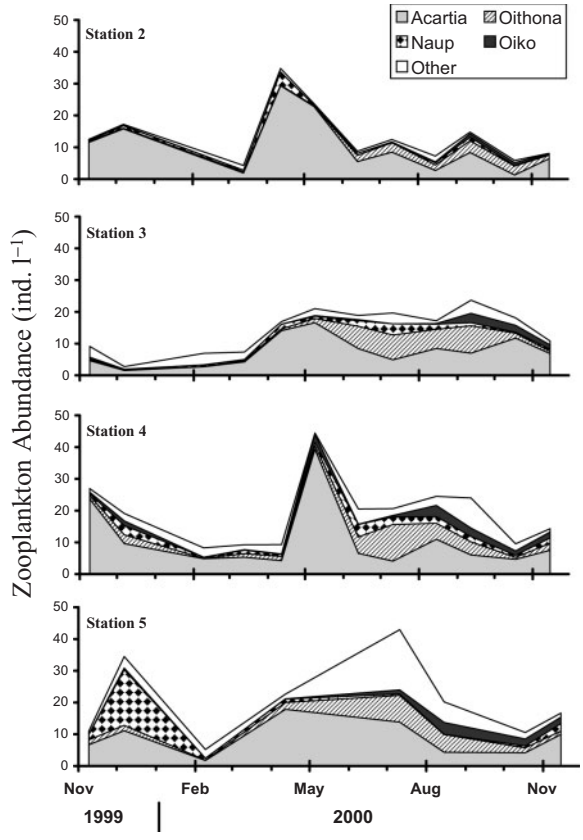

Fig. 9. Zooplankton abundance by taxa at the four estuarine sites (stations 2–5), from November 1999 to November 2000. Abundance was grouped by the four most abundant taxa plus all other taxa.

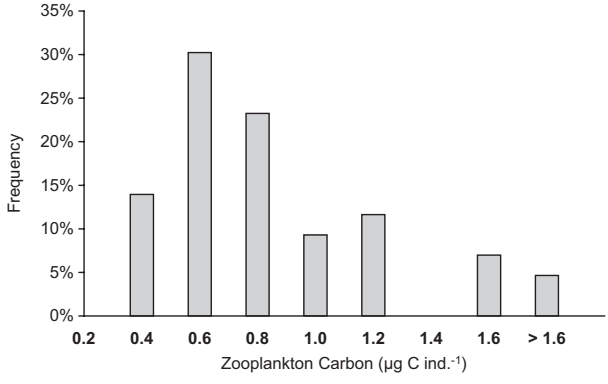

Fig. 10. Frequency distribution of the ratio of zooplankton biomass and abundance calculated at each station for which both measurements were made (n = 43).

from a 1974 survey, following a reduction in point sources (Olinger *et al.*, 1975). Also, a quarterly survey from 1996 to 2001 reported a bay wide average chlorophyll of 5.4 ± 4.8 µg L^{-1} (USEPA, unpublished data). While historical data are scant, these comparisons suggest that the trophic state of Pensacola Bay has remained relatively stable for the past 25 years.

In this study, net phytoplankton abundance averaged 6.0 ± 4.6 × 10^5 cells L^{-1}; the three most abundant taxa were the diatoms: *Thalassiosira* (25%), *Pennales* (20%) and *Cyclotella* (7%). In contrast, a previous study (Livingston, 2001) reported lower average abundance (~1 × 10^5 cells L^{-1}) during a 1997–1998 survey (estimated from Figure 6.12 in Livingston, 2001), and listed dominant taxa as the diatom *Cyclotella choctawhatcheeana* (13%), the dinoflagellate *Prorocentrum minimum* (1.5%), and the raphidophyte *Heterosigma akashiwo* (0.8%). Additionally, Livingston observed that Escambia Bay had lower phytoplankton abundance and species richness than nearby Perdido Bay, yet the dominant species in Escambia Bay are known to form harmful or nuisance algal blooms. These characteristics led Livingston (Livingston, 2001) to conclude that Escambia Bay was still recovering from past excessive nutrient loading. However, low abundances of potential bloom-forming species are common in estuarine waters, and do not necessarily indicate eutrophication. Conspicuously missing from Livingston's work are data on picophytoplankton abundance (including cyanobacteria) and their potential contribution to the phytoplankton community.

Zooplankton total abundance (Figure 9) averaged 16.9 ± 0.9 L^{-1} (mean ± SD), and *A. tonsa* was the dominant taxa, as is typical for estuarine environments. A similar survey conducted during 1973 reported higher zooplankton abundances averaging 38 L^{-1} (Dye, 1987). However, much of this discrepancy can be explained by differences in mesh size (153 versus 74 µm, respectively), which particularly affected the number of copepod nauplii sampled (mean 1.5 versus 14.0 L^{-1}, respectively). Lores *et al.* (Lores *et al.*, 2002) reported an average zooplankton abundance of 3.1 ± 2.5 L^{-1} from three sites in Pensacola Bay situated in the lower bay; this average was much lower than the average of 20.5 ± 12.8 L^{-1} from our station 5, the most comparable site. It is unclear why abundances were so much lower given that the same sampling protocol was used.

Based on predictions of the temperature driven production model (Huntley and Lopez, 1992), daily integrated mesozooplankton production averaged by date ranged from 0.3 to 27.5 µg C L^{-1} day^{-1} (Figure 8). During the peak spring and summer months (March–September), zooplankton production averaged 12.6 µg C L^{-1} day^{-1} and was nearly equivalent to the average zooplankton standing crop of 14.0 µg C L^{-1} (Figure 8), suggesting near daily turnover of zooplankton carbon. Assuming a 40% gross growth efficiency (Kiørboe *et al.*, 1985), the zooplankton community consumed on the order of 32 µg C L^{-1} day^{-1}, which translates into a demand of

about 1 μg Chl L^{-1} day^{-1}, based on a Chl:C ratio of 0.03 (Cloern *et al.*, 1995). This demand could readily be met with the observed chlorophyll concentrations of 5–10 μg L^{-1}, except that during summer, about 90% of this biomass is contained in the cyanobacterial fraction, and not directly consumable by zooplankton. Adult *Acartia* sp. cannot efficiently filter particles <4 μm, and even the naupliar stages are limited to particles >2 μm (Nival and Nival, 1976; Berggreen *et al.*, 1988; Hansen *et al.*, 1994). For *Acartia* to be successful in this sort of environment, they likely exert a strong grazing pressure on the larger, but relatively scarce, phytoplankton taxa, as well as the microzooplankton (Lonsdale *et al.*, 1996; Sipura *et al.*, 2003). Other studies conducted in Pensacola Bay have confirmed that microzooplankton are important grazers of phytoplankton (Murrell *et al.*, 2002b), including the cyanobacteria (Juhl and Murrell, in review) and that microzooplankton are an important resource to mesozooplankton (Sipura *et al.*, 2003). Thus, in estuaries dominated by picoplankton, it is likely that the trophic pathway to mesozooplankton production is relatively inefficient and it is possible that mesozooplankton become food limited during these times.

A central finding of this study was the striking summertime peak in cyanobacteria abundance, reaching 3×10^9 L^{-1} (Figure 3), which strongly covaried with the small chlorophyll size fraction (Figure 3B) and with zeaxanthin concentration (Figure 6). The cyanobacteria were small (1–2 μm) and typically were observed as single cells, but occasionally occurred in small clumps of 10–20 cells. Preliminary molecular characterization by terminal restriction fragment length polymorphism (TRFLP) analysis of PCR-amplified *rpoC* genes indicated that the cyanobacteria belong to the genus *Synechococcus* and that relatively few types of cyanobacteria occurred in ambient water samples (J. Collier, SUNY Stony Brook, personal communication).

Cyanobacterial abundances have been reported in estuaries and coastal bays, including the Florida Bay (Phlips *et al.*, 1999), the Mississippi–Atchafalaya river plume (Dortch, 1998), the Chesapeake Bay (Malone *et al.*, 1991; Marshall and Nesius, 1996), the Neuse River estuary (Pinckney *et al.*, 1998), the San Francisco Bay (Ning *et al.*, 2000), and the York River estuary (Sin *et al.*, 2000). Cyanobacteria abundances in these estuaries vary considerably, but in general warmer estuaries tend to have the higher abundances.

At present, we do not have comprehensive estimates of cyanobacterial productivity in Pensacola Bay, but results from dilution experiments show that the maximum specific growth rate of cyanobacteria is 1–1.5 day^{-1} during summer and is strongly linked to temperature (Juhl and Murrell, in review). Similar specific growth rates of 1.1 day^{-1} were observed during summer in Chesapeake Bay (Affronti and Marshall, 1994). Carpenter and Campbell (Carpenter and Campbell, 1988) reported peak specific growth rates of 1.08 day^{-1} (reported as 1.56 divisions day^{-1}) in Long Island Sound, and that cyanobacterial productivity peaked during August. Temperature dependence of cyanobacterial growth rate appears particularly strong, being repeatedly noted in estuarine (Carpenter and Campbell, 1988; Ray *et al.*, 1989; Iriarte and Purdie, 1994) and oceanic environments (Li, 1998). Based on these observations, it is clear that estuarine cyanobacteria actively grow during warm periods, and thus significantly contribute to bulk productivity, probably in proportion to their relative biomass.

Our empirical approach of estimating cyanobacterial chlorophyll content (Figure 3B) cannot account for variation caused by different growth and light conditions. For example, Kana and Glibert (Kana and Glibert, 1987) cultured *Synechococcus* in varying light conditions, and found a 3-fold variation in cyanobacterial chlorophyll content (range of 1.3–3.7 fg Chl $cell^{-1}$). Similarly, Moore *et al.* (Moore *et al.*, 1995) reported cyanobacterial chlorophyll content of 4 fg Chl $cell^{-1}$ for cultured *Synechococcus* in high light. It is also likely that the cyanobacterial chlorophyll content will vary with nutrient status; nutrient starved cells should have less chlorophyll than nutrient-replete cells. We have successfully isolated PC-rich and PE-rich cyanobacteria from Pensacola Bay and found that both PC-rich and PE-rich isolates contained about 3.1 fg Chl $cell^{-1}$, when grown in moderate light, nutrient-replete conditions. The coherence among these estimates suggests that the empirical factor of 3.5 fg Chl $cell^{-1}$ used here was probably reasonable.

We observed at least two distinct types of cyanobacteria in our samples, PC-rich and PE-rich, and there was a clear spatial gradient with PC-rich cells dominant in the upper estuary. A similar pattern of dominance has been observed in other estuaries. For example, in the York River estuary, PC-rich cells were up to 8-fold more abundant than PE-rich cells (Ray *et al.*, 1989). In the Hudson River, PC-rich cells predominated to the apparent exclusion of PE-rich cells (Collier, 2000). In Mississippi–Atchafalaya plume waters in the Gulf of Mexico, Dortch (Dortch, 1998) found PC-rich cells more abundant than PE-rich cells at lower salinities. It is currently unclear what drives this apparent salinity pattern, but it may be a physiological adaptation to lower salinity, or other variables expected to covary with salinity in estuarine environments (i.e. nutrients, light). Future studies directed at the comparative physiology of PC-rich versus PE-rich cyanobacteria using cultured or field populations would improve our understanding of these observed distributions.

While cyanobacteria abundance has been reported from many estuaries, their contribution to total phytoplankton biomass is less frequently quantified. Averaged over this time series (excluding station 1), cyanobacteria in Pensacola Bay represented 43% of total chlorophyll, and this fraction was usually well over 90% during summer (Figure 2). In other estuaries where this estimate has been made, their contribution appears to be much smaller. For example, in San Francisco Bay cyanobacteria were a relatively small component of total chlorophyll (Ning *et al.*, 2000), averaging 15% (maximum 38%). In the Neuse River estuary, cyanobacteria represented 18% of total chlorophyll based on HPLC pigment analysis (Pinckney *et al.*, 1998). In the York River estuary, picophytoplankton comprised 7% of chlorophyll over an annual cycle, peaking at 14% during summer (Ray *et al.*, 1989). In Kiel Bight, Jochem (Jochem, 1988) reported that cyanobacteria contributed up to 52% of the total chlorophyll during summer, but presented no data during other seasons. In Southampton estuary, Iriarte and Purdie (Iriarte and Purdie, 1994) found that cyanobacteria contributed 10% or less to bulk chlorophyll. Based on a survey of the available literature, they further argued that the picoplankton contribution to bulk chlorophyll is only dominant in oligotrophic environments with chlorophyll levels from 0.5 to 1 $\mu g\ L^{-1}$, and that their importance diminishes with increasing trophic state, ultimately contributing <5% when chlorophyll concentrations exceed 5 $\mu g\ L^{-1}$. While this pattern may hold for temperate estuaries, Pensacola Bay and similar sub-tropical systems such as Florida Bay (Phlips *et al.*, 1999) do not fit this pattern. Clearly, the role of cyanobacteria in tropical and sub-tropical estuaries needs further clarification.

In summary, this study described the phytoplankton and zooplankton composition in Pensacola Bay, Florida, a sub-tropical estuary in the northern Gulf of Mexico. We observed remarkably high abundances of cyanobacteria in Pensacola Bay during three summer periods from 1999 to 2001 (Figure 3A), and that cyanobacteria appeared to dominate the chlorophyll biomass during these periods (Figures 2 and 3B). The HPLC data supported this interpretation, showing high relative concentrations of the diagnostic pigment zeaxanthin (Figure 5) and a strong coherence between zeaxanthin and cyanobacterial abundance (Figure 6). The zooplankton were dominated by *Acartia tonsa*, typical of temperate estuaries, and may become food-limited during periods of cyanobacterial dominance.

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