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Nutrient quality drives differential phytoplankton community composition on the southwest Florida shelf

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Abstract

During May 2003, the dry season, the southwest Florida shelf was surveyed for nutrient concentrations and phytoplankton community composition concurrently with plankton nutritional and physiological status (tracer techniques, enzyme assay, and biomass response bioassays). Inorganic nitrogen concentrations were low throughout the region, while dissolved organic nitrogen (DON) was elevated (>30 μmol N L^{-1}) nearshore. Conversely, PO_4^{3-} and dissolved organic phosphorus concentrations were high only in northern coastal areas adjacent to estuarine outflows. Nitrogen : phosphorus (N : P) ratios in the particulate material; gradients in NO_3^- and urea uptake rates; urease and alkaline phosphatase activities; and bioassay responses were indicative of a strong gradient from N to P limitation of plankton biomass from north to south. Nitrogen limitation was apparent in the northern region ($N:P_{particulate} < 8$, Tampa to Sanibel), where PO_4^{3-} and DON inputs dominated; balanced nutrient conditions were evident in the mid-region ($N:P_{particulate} = 8$–24, Sanibel to Shark River); and P limitation was evident south of the Shark River ($N:P_{particulate} > 24$), where inorganic N input was greatest. Phytoplankton community composition varied along the same gradient, from a cyanobacteria and dinoflagellate community in the north, to a cyanobacteria-dominated community in the mid-region, and to a diatom community in the southern region. Percentages of NO_3^- uptake and diatoms in the total phytoplankton community were positively related, as were percentages of urea uptake and cyanobacteria. Inorganic and organic N and P fractions in the nearshore shelf region reflect longitudinal gradients in regional watershed characteristics, and their relative availability thus appeared to control both phytoplankton community composition and its physiological status.

The southwestern Florida coastal shelf region is a shallow, productive coastal shelf influenced by a diverse variety of river systems. Although generally characterized by oligotrophic conditions (Lester et al. 2001; Vargo et al. 2001), this region is the site of frequent and persistent algal blooms, including blooms of the toxic "red tide" dinoflagellate *Karenia brevis*, which has been associated with human health effects, fish kills, and marine mammal deaths (Steidinger et al. 1998). More recently, other algal blooms, including cyanobacterial and large-scale diatom blooms occasionally referred to as "black water" (Hu et al. 2002; Neely et al. 2004), have also been documented on this coastal shelf. Both the maintenance stages of the *K. brevis* blooms, which can last more than 21 months, and the "black water" diatom blooms occur in a coastal region in which inorganic nutrient concentrations are low. The origin of the nutrients that sustain these blooms and the role of riverine discharge in their maintenance has thus been the subject of intensive studies and debate (Heil et al. 2001; Lester et al. 2001; Vargo et al. 2004). Most of these studies have focused on the region between Tampa Bay and Charlotte Harbor, where *K. brevis* blooms almost annually (Steidinger et al. 1998). However, there has been no unifying conceptual understanding of the factors that may promote or maintain the broader suite of blooms that occur in different regions of this coastal shelf.

Tropical and subtropical oceanic areas are acutely vulnerable to nitrogen (N) pollution, particularly agricultural runoff (Beman et al. 2005). The southwestern Florida

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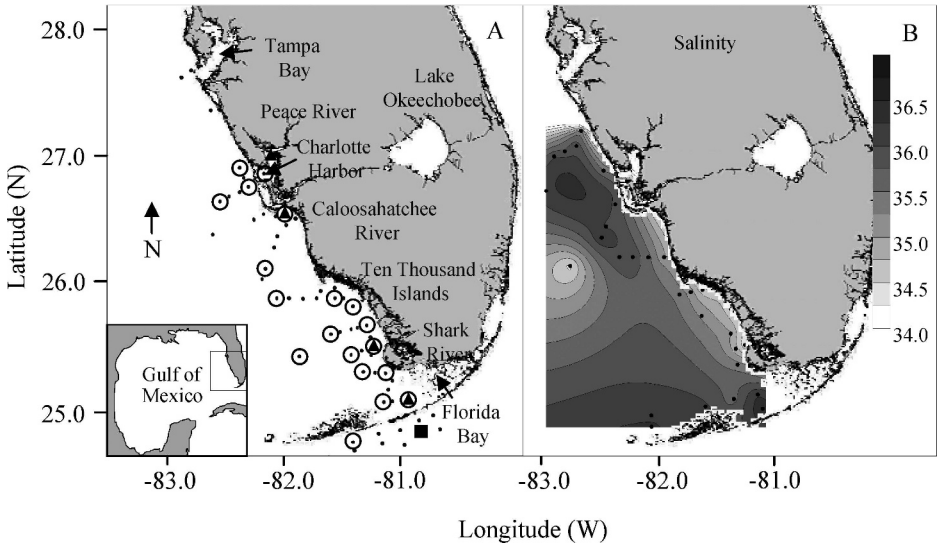

Fig. 1. (A) Locations of stations sampled in May 2003 on the southwestern Florida shelf. Station positions shown with a triangle are those at which nutrient enrichment bioassays and tracer uptake measurements were made. An additional uptake measurement was made at a fifth station on the Atlantic side of the Florida Keys, as indicated by a filled square. Circled stations were those stations included in statistical analyses and where enzymatic, HPLC, or size-fractionated Chl *a* measurements were made. (B) Isopleths of salinity.

shelf receives nutrient inputs from numerous rivers that drain watersheds that vary greatly in terms of their degree of residential development and agricultural use. Most of these rivers have natural flow, but at least one, the Caloosahatchee River, has gated flow. These riverine systems differ greatly in the amount of nutrient inputs they deliver to the coastal area, the frequency of delivery, and the form in which nutrients are supplied (Pennock et al. 1999; Turner and Rabalais 1999). The Peace River, which drains into Charlotte Harbor, lies primarily within a watershed characterized by the Hawthorne phosphatic deposits, which have been actively mined since the 1880s (Pittman 1990). This watershed also has considerable agricultural land (citrus production) and cattle ranches. Coastal areas within the Peace River/Charlotte Harbor region, already severely N limited (McPherson and Miller 1990), are also subject to increasing population growth, urban pressure, and sewage loading. To the south, the Caloosahatchee River flows onto the shelf via gated flow, with periodic releases from Lake Okeechobee. This system is heavily affected by nutrient inputs from agricultural sources, especially sugar and citrus production within the Everglades Agricultural Area. The coastal area to the west of the Everglades (e.g., Ten Thousand Island area) receives inputs from the Shark River as well as from numerous smaller river systems that drain the western Everglades (Jurado 2003). Further south is Florida Bay, a shallow, seagrass-dominated embayment that is the site of frequent algal blooms, seagrass die-offs, and coral losses (Fourqurean and Robblee 1999). Nutrient flux onto the Florida Bay/Keys region, influenced by groundwater (Corbett et al. 1999), canal inputs (LaPointe and Clark 1992), and sheet-flow inputs from the Everglades (Light and Dineen 1994) as well as inputs from the Gulf of Mexico (Brand 2002), is complex, difficult to measure, and the subject of much controversy.

Compounding the complexity of the varying riverine influences within the southwestern Florida region is the process of Everglades restoration, designed to restore natural sheet flow as opposed to current gated flow. The Everglades restoration effort is expected to alter the forms, amounts, and delivery of dissolved nutrients to the southwest shelf region. The influence of these changes on coastal phytoplankton populations, in particular the annually reoccurring *K. brevis* 'red tide' as well as other algal blooms that have been documented in this region for nearly a century (Kusek et al. 1999), is unknown.

To examine the influence of the diverse nutrient sources and inputs on downstream phytoplankton and bacterial community composition and abundance and to address some of these uncertainties, a comprehensive survey of the southwestern Florida inner and coastal shelf region was conducted in May 2003. The study was conducted during the dry season and thus represents baseline, or the minimal flow period. Conducted in a synoptic manner over a 5-d period, this survey allowed comparisons between the different riverine systems affecting the region. This survey examined the availability of both inorganic and organic nutrients, the composition of the microbial community, and the biotic responses to nutrients, as measured using enzymatic, tracer uptake, and bioassay techniques.

Methods

Sample collection—Fifty-eight stations were sampled from 15–23 May 2003 in the nearshore (0–25 km from shore) coastal region between Tampa Bay, the Florida Keys, and east of the Florida Keys reef track (Fig. 1A).

Some stations were also sampled in western Florida Bay and in deeper water to the east of the Keys reef track. Stations were located such that potential nutrient plumes from the major river systems affecting this coastal region—the Peace River, the Caloosahatchee River, the Ten Thousand Islands area, and the Shark River—were sampled. Sampling was conducted between 07:00 and 18:00 h.

At each station, a conductivity–temperature–depth (CTD) cast was used to assess vertical water column structure (temperature, salinity, fluorescence). Water samples were taken concurrently with a rosette mounted on the CTD. Where the column was stratified, near-surface (1 m) and deep chlorophyll *a* (Chl *a*) maximum samples were collected; otherwise, a single sample was taken from a bottle located 1 m beneath the surface for all chemical and biological characterizations. Only the near-surface data are reported here.

Water samples were collected at all stations with a Niskin bottle for subsequent analysis of dissolved inorganic (NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}, and SiO_4) and organic (dissolved organic nitrogen [DON], dissolved organic phosphorus [DOP], and urea) nutrients, and Chl *a* (*see below*). At selected stations (Fig. 1A), particulate N (PN) and P (PP) and other biological parameters (size-fractionated Chl *a*, accessory pigments, bacterial and phytoplankton abundance, urease and alkaline phosphatase activity [APA], and rates of nitrogen uptake) were determined, with at least one such station at the mouths of each of the riverine systems of interest.

Nutrient and biomass sample analysis—Samples (in duplicate) were filtered through precombusted (2 h, 450°C) Whatman GF/F filters and were immediately frozen and analyzed for bulk (>0.7 μm) Chl *a* according to Holm–Hansen et al. (1965) within 2 weeks of collection. At selected stations, additional water samples were filtered in duplicate through 3.0-μm Nuclepore filters and were frozen for subsequent Chl *a* analyses, as previously described. The concentration of Chl *a* in the 0.7–3.0-μm fraction was obtained by subtracting the concentration measured in the 3.0-μm filter from the bulk Chl *a* concentration. Duplicate aliquots of the GF/F filtrate were retained and stored frozen for subsequent analysis of total dissolved nitrogen (TDN) and phosphorus (TDP), urea and inorganic nutrient (NO_3^-, NO_2^-, NH_4^+ [collectively, DIN], and PO_4^{3-}) concentrations. Analyses of TDN and TDP were conducted according to the methods of Bronk et al. (2000) and Solórzano and Sharp (1980), respectively. Samples for SiO_4 analyses were filtered through 0.45-μm Millipore filters and were immediately frozen. Urea concentrations were determined in triplicate using the urease method of Parsons et al. (1984), modified for small sample volumes. Inorganic nutrient concentrations were determined by autoanalyzer techniques (Atlas et al. 1971; Gordon et al. 1994). DON and DOP concentrations were determined by subtraction of inorganic concentrations from the TDN and TDP concentrations, respectively.

From selected stations (Fig. 1A), aliquots of water samples were also filtered through precombusted (2 h, 450°C) GF/F Whatman filters and frozen in liquid N_2 for PN and PP and phytoplankton pigment analyses. Concentrations of PN were later determined using a Control Equipment CHN analyzer and PP according to the method of Solórzano and Sharp (1980). Phytoplankton accessory pigments were analyzed using a Hewlett-Packard high-performance liquid chromatography (HPLC) Model 1100 system according to the methods of Van Heukelem et al. (1994) and Van Heukelem and Thomas (2001).

Samples for bacteria enumeration were collected from the selected stations and preserved in glutaraldehyde (3%) at 4°C and subsequently analyzed using a FACS Caliber Becton Dickinson flow cytometer according to the method of del Giorgio et al. (1996). A 100-mL sample aliquot was also preserved using Lugols Preservative for phytoplankton community composition and enumeration. Additionally, concentrations of *K. brevis* were determined on unpreserved samples within 30 min of sample collections, as described in Heil et al. (2001).

Plankton nutritional status—Three types of analyses were performed at a further subset of these selected stations (Fig. 1A), primarily those located near the river mouths, to determine the nutritional status of the microbial community. These assays included enzyme characterization of N and P utilization, rates of N uptake (as NO_3^- and urea), and bioassay responses of ambient phytoplankton and bacterial assemblages to a suite of nutrient additions.

Activities of the enzymes urease and APA were determined on both bulk (unfiltered) and <3.0-μm water samples, and APA was also measured on a dissolved (<0.2-μm) fraction. All samples for urease activity were filtered onto precombusted (2 h, 450°C) GF/F filters, stored frozen in liquid N_2, and analyzed within 2 weeks of sampling according to the method of Peers et al. (2000), as modified by Fan et al. (2003) and Solomon et al. (pers. comm.). For APA analysis, whole-water samples were gently (<35 kPa) filtered through 3.0-μm Nuclepore filters, and the activity of the resulting water sample was measured. APA was also measured on the <0.2-μm fraction obtained by gentle syringe filtration through a 0.2-μm Cameo nitrocellulose filter. APA activity was measured on all samples within 10 min of sample collection, according to the method of Perry (1972).

The rates of uptake of NO_3^- and urea were determined at the same selected stations using ^{15}N tracer techniques (Glibert and Capone 1993). For this study, personnel and space limitations restricted our focus to only the anthropogenic N forms NO_3^- and urea. Incubations were done in 1-liter Nalgene polycarbonate bottles (precleaned with 10% HCl, repeated deionized water [DIW] rinses) under 60% natural irradiation (achieved by neutral density screening) and ambient temperature (achieved by flowing surface seawater) for a period of 0.5–0.7 h. All incubations were initiated in mid- to late morning by an addition of the ^{15}N label representing 10–20% of ambient concentrations and were terminated by filtration onto precombusted GF/F filters. Filters were dried and analyzed by mass spectrometry on a Sercon mass spectrometer. Uptake rates were calculated according to the method of Glibert and Capone (1993).

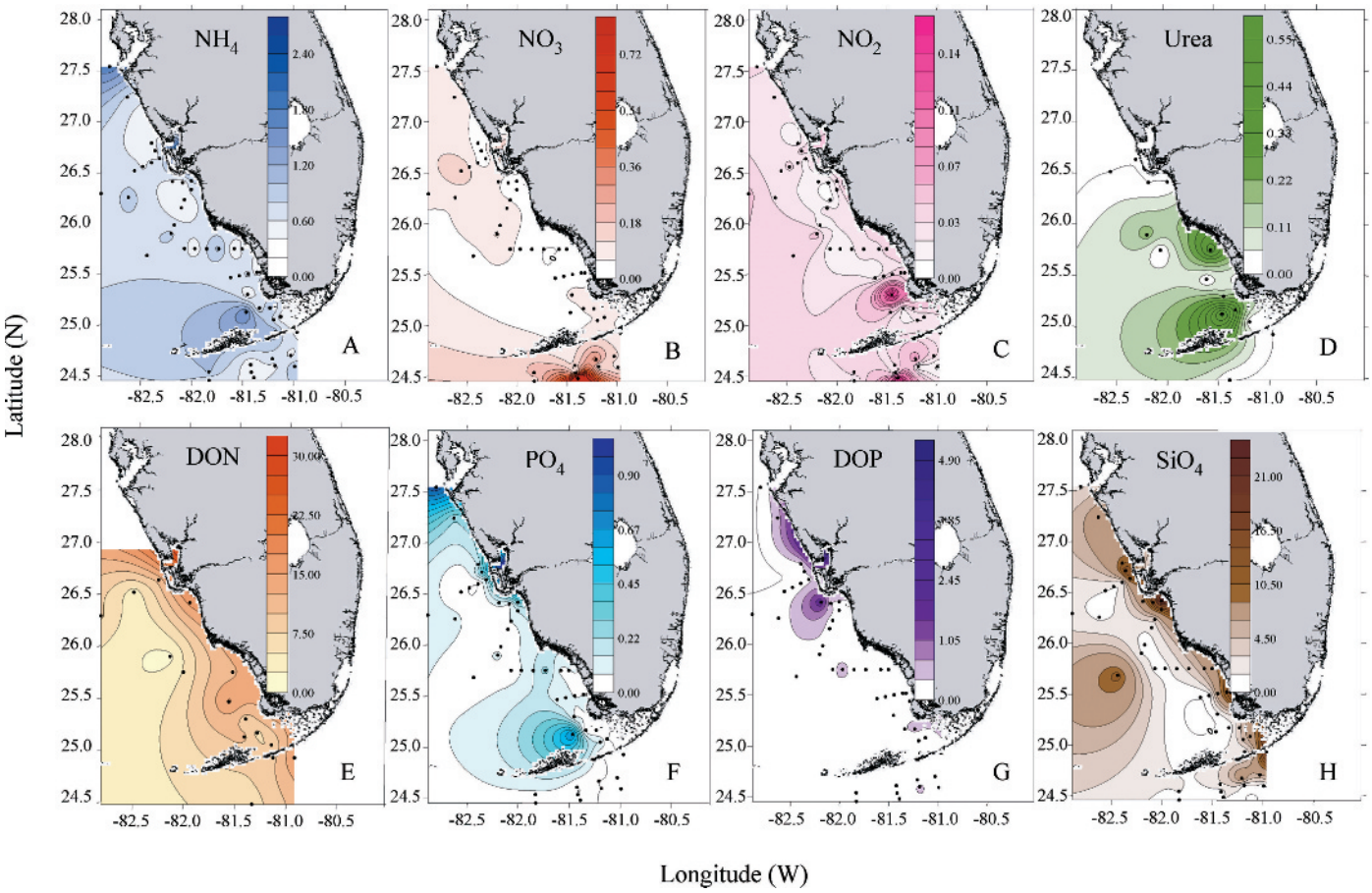

Fig. 2. Contour maps of the distribution of the dissolved nutrient in surface samples. Each nutrient is indicated at the top of each contour map. All units are given in μmol N L^{-1} or μmol P L^{-1}.

Short-term (48-h) nutrient enrichment bioassays were conducted at these stations with water that was prefiltered through 154-μm mesh to eliminate large zooplankton grazers. Nutrient treatments consisted of a control (no addition), $+NO_3^-$ (10 μmol N L^{-1} final concentration), $+PO_4^{3-}$ (2.5 μmol P L^{-1} final concentration), +DOP (prepared as 2.5 μmol P L^{-1} glycerol-phosphate, final concentration), +DON (prepared as 5 μmol N L^{-1} urea, 2.5 μmol N L^{-1} arginine, and 2.5 μmol N L^{-1} glutamine; 10 μmol N L^{-1} final concentration), and +Suwannee River humic acid (2 mg L^{-1} final concentration, International Humic Substance Society standard Suwannee River humic acid). Incubations were conducted in duplicate in 2-liter Nalgene bottles precleaned with 10%-HCl repeated DIW rinses and were maintained at 60% of natural irradiance and ambient temperature, as described above. During incubation each bottle was gently inverted several times. Each replicate was sampled after 24 h and 48 h, with the station measurement serving as a time 0 measurement. At each sampling time, subsamples were taken for determination of bacterial concentration and size-fractionated (0.7-μm and 3.0-μm) Chl *a*. Approximately 25 mL of whole water was immediately fixed with 5% glutaraldehyde and stored in a refrigerator until analysis using a FACS Caliber flow cytometer for bacterial abundance, as previously described. Chl *a* samples were analyzed according to the method of Holm–Hansen et al. (1965) within 2 weeks of the cruise.

Data analysis—Except where noted, the statistical analyses represent the selected stations at which all the variables were measured (19 stations total). Relationships between variables were determined using the Pearson correlation coefficient. Maps comprise the available data from all stations.

Results

General hydrographic parameters—All stations were well mixed except for the deeper water stations sampled to the south of the Florida Keys. Salinities within 5 km of nearshore were >34 ppt (Fig. 1B). Temperatures ranged between 30°C and 32°C in the entire study region, and there was no evidence of upwelling in any of the vertical profile data (not shown).

Nutrient distributions—The inorganic N pool (Fig. 2) was generally dominated by NH_4^+, which ranged from 0.2 to 2.7 μmol N L^{-1} (Fig. 2A). The highest concentrations of NH_4^+ were found in Charlotte Harbor, at the mouth of

Table 1. Dissolved inorganic N : P and organic N : P ratios for each of the major coastal areas sampled along a north–south gradient.

River system	DIN : DIP*	DON : DOP*
Peace River mouth	1.2	5.7
Charlotte Harbor entrance	3.2	42.7
Caloosahatchee River mouth	4.5	47.2
Ten Thousand Islands	11.0	52.7
Shark River mouth	42.4	59.9
Western Florida Bay	417.0	44.6

* DIN, dissolved inorganic nitrogen; DIP, dissolved inorganic phosphorus; DON, dissolved organic nitrogen; DOP, dissolved organic phosphorus.

Tampa Bay and at a single station west of Florida Bay. Low concentrations (<1.0 μmol N L^{-1}) were observed throughout the rest of the region. Concentrations of NO_3^- were generally higher in the southern region of the study area, peaking on the Atlantic side of the Florida Keys (Fig. 2B). Trends in NO_2^- were similar to those of NO_3^-, but concentrations were approximately an order of magnitude less (Fig. 2C). Elevated urea concentrations, from 0.2 to 0.6 μmol N L^{-1}, were detected in nearshore waters off of the Ten Thousand Island region and west of Florida Bay (Fig. 2D). Urea concentrations in all other areas were <0.2 μmol N L^{-1}. DON was always >5 μmol L^{-1} and was highest in nearshore regions, with a concentration high of 30.8 μmol L^{-1} inside Charlotte Harbor at the Peace River mouth (Fig. 2E). The concentration of DON was approximately an order of magnitude higher than that of the DIN pool throughout the study area.

Concentrations of PO_4^{3-} were below the detection limit in Florida Bay and at the Atlantic Florida Keys (Fig. 2F). Elevated levels, reaching 1.6 μmol P L^{-1}, were found at the mouth of the Caloosahatchee River and adjacent to Charlotte Harbor and Tampa Bay. Several stations west of Florida Bay and the Ten Thousand Island area also exhibited elevated PO_4^{3-} concentrations, while the remaining stations had PO_4^{3-} values of <0.1 μmol P L^{-1}. The highest concentrations of DOP, up to 5.4 μmol P L^{-1}, were also found associated with the same riverine systems as PO_4^{3-} (Fig. 2G). Silicate varied between 5 and 23 μmol L^{-1} in nearshore waters, but in offshore waters concentrations of <1.0 μmol L^{-1} were observed (Fig. 2H).

In the northern three systems (Peace River, Charlotte Harbor, and Caloosahatchee River), the average ratio of DIN to dissolved inorganic phosphorus (DIP) (Table 1) was less than half of the stoichiometric proportions of 16 : 1, the value typically suggested to be supportive of balanced growth (Redfield 1958). In the Ten Thousand Islands region, the average DIN : DIP ratio converged on a value of $\sim$16 : 1, and at the two more southern regions of the study area, Florida Bay and western Florida Keys, the average DIN : DIP ratio exceeded stoichiometric proportion by a factor of >2.

The ratio of the organic fractions of the nutrient pool yielded a somewhat different pattern (Table 1). While an increase from north to south was evident, it was only at the Peace River mouth station that the ratio was less than stoichiometric proportions. At all other sites, DON : DOP ratios exceeded 40.

Phytoplankton and heterotrophic bacteria biomass and composition—Concentrations of PN and PP ranged from 1.07 to 12.6 and from 0.02 to 0.56 μmol L^{-1}, respectively. The highest values were observed nearshore at the mouths of river systems and within the estuaries (data not shown).

Chl *a* in the 0.7–3.0-μm size fraction (Fig. 3A) was highest at the mouths of the Peace and the Caloosahatchee Rivers ($\sim$3.00 μg L^{-1}). Phytoplankton biomass in the $>$3.0-μm size fraction was also high in Charlotte Harbor and adjacent coastal waters and several coastal stations in the northwest Ten Thousand Island area (Fig. 3B), but it was highest in the offshore regions of southern shelf waters, with concentrations of up to 3.00 μg L^{-1}. In general, the smaller-size phytoplankton accounted for approximately 70% of the total phytoplankton biomass in nearshore waters, compared with only about 30% in offshore waters. At the mouth of the rivers, the percentage contribution of the smaller-size phytoplankton (0.7–3.0 μm) to the total biomass was $>$50%. However, in Florida Bay and at the western Florida Keys, phytoplankton biomass was dominated by the $>$3.0-μm fraction.

Relative to Chl *a*, fucoxanthin and zeaxanthin were the most abundant accessory pigments (Fig. 3D,E). Peridinin, the pigment signature for most dinoflagellates, was only observed in significant concentrations in Charlotte Harbor and immediately adjacent stations and at several stations offshore and southwest of the Shark River (Fig. 3F). The cyanobacterium *Trichodesmium* spp. and *K. brevis* (concentrations ranged from 0.1 to 2×10^5 cells L^{-1}; data not shown) were both noted in microscopic observations from the Caloosahatchee River mouth area. The presence of *K. brevis* from this area was also detected by the pigment gyroxanthin-diester (Millie et al. 1997; Fig. 3C). In the mouth of the Shark River, near Ten Thousand Islands and offshore, the high ratio of zeaxanthin to Chl *a* indicates the quantitative importance of cyanobacteria. In the Western Florida Bay and western Florida Keys, the relatively high fucoxanthin to Chl *a* ratios indicate an important contribution of diatoms. The diatom community near the Shark River mouth was dominated by *Rhizosolenia* spp., as found by microscopic examination.

The abundance of heterotrophic bacteria decreased offshore (Fig. 4A). Bacterial abundance ranged from 0.7 to 3.8×10^9 cells L^{-1}, with the exception of in Charlotte Harbor, where a maximum of 6.9×10^9 cells L^{-1} was observed. Concentrations did not exceed 2×10^9 cells L^{-1} at the other sites, with the lowest value observed at the Atlantic station ($<1 \times 10^9$ cells L^{-1}). Cells with high DNA content (i.e., actively metabolizing bacteria; Gasol et al. 1999) accounted on average for 46% ($\pm$13% standard deviation) of the total heterotrophic bacteria community (Fig. 4B).

Correlation analysis showed positive and significant relationships between Chl *a* (total and fractionated) and DON ($r^2 > 0.6$). Heterotrophic bacterial abundance was

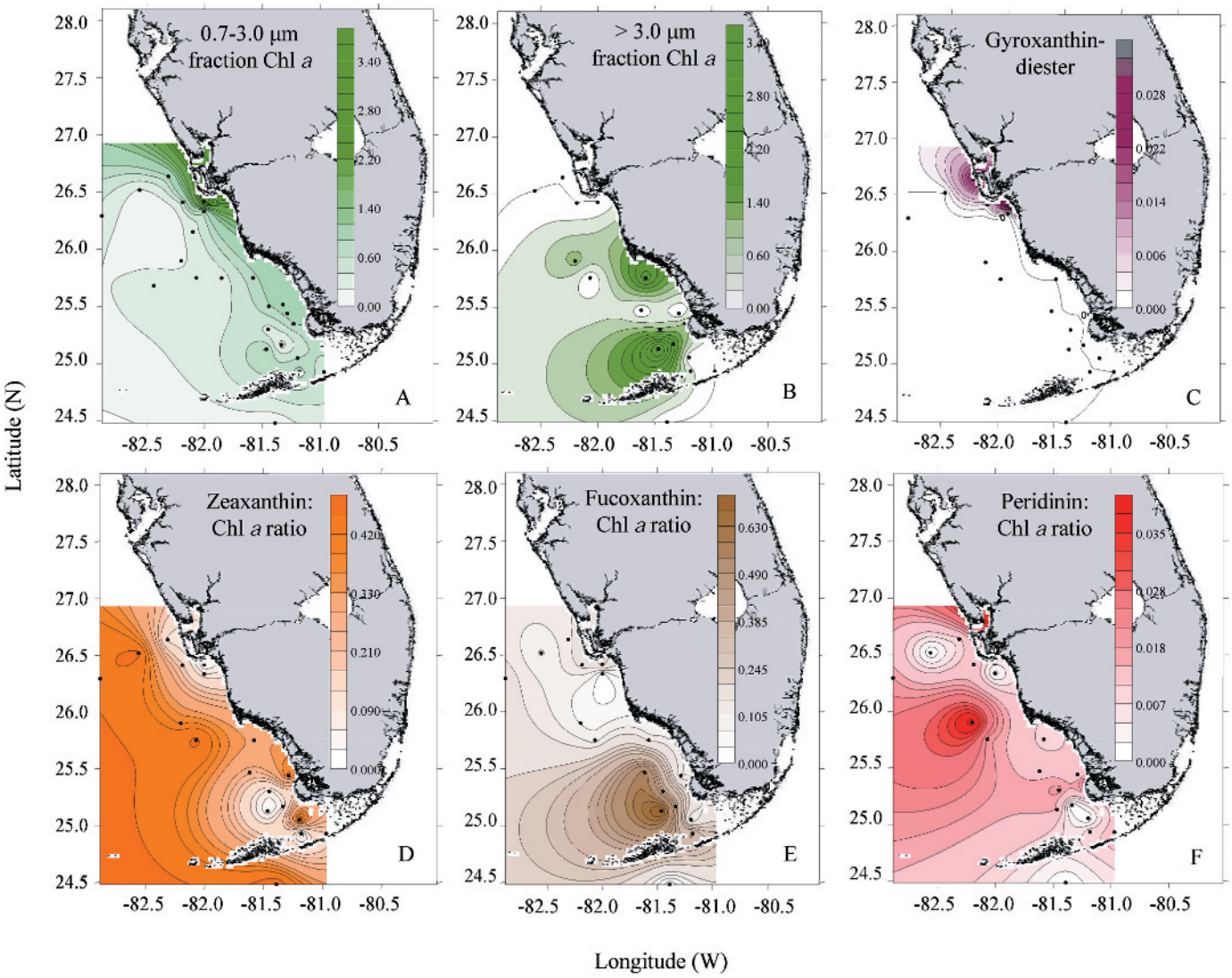

Fig. 3. (A, B) Contour maps of Chl *a* in surface water by size fraction and (C) abundance of the pigment gyroxanthin-diester. Units for A–C are given in μg L^{-1}. (D–F) Contour maps of accessory pigments in surface water normalized to Chl *a*.

highly correlated with DON and DOP ($r^2 > 0.7$; $p < 0.001$), but not with the inorganic nutrients.

Enzymatic activities—The highest urease activities were found at the mouth of the Peace and Shark Rivers (Fig. 5A). Virtually all of the activity was associated with the 0.7–3.0-μm biomass fraction at the three northernmost sites. A different distribution pattern was found for APA (Fig. 5B), with detectable activity only at the Caloosahatchee River and Western Florida Bay stations. The activity in the 0.7–3.0-μm fraction accounted for the majority of activity at these stations. APA was detectable in the <0.2-μm fraction only at the Caloosahatchee River station.

Rates of nitrogen uptake—Specific rates of NO_3^- uptake (as V h^{-1}) were highest off the Caloosahatchee and Shark Rivers, while those of urea uptake were highest off the Shark River (Fig. 6A). However, the absolute rates of N uptake (as μmol N L^{-1} h^{-1}) revealed a consistent gradient of highest rates off the Peace River for both N substrates to lowest rates off the Florida Keys (Fig. 6B). This was a function of the strong gradient in PN, which was almost an order of magnitude higher at the Peace River than at the Florida Keys station (not shown). Rates of NO_3^- and urea uptake were comparable off the Shark River and the Florida Keys, but at the other sites, rates of NO_3^- uptake exceeded those of urea.

Responses to nutrient enrichment in bioassays—Phytoplankton biomass responded positively to N enrichment (organic as well as inorganic forms) at all the stations, with the highest responses in the rivers Peace and Caloosahatchee (Table 2). A higher proportional response to DON was found in western Florida Bay, compared to DIN enrichment. In general, humic acid additions did not greatly enhance phytoplankton biomass. The >3.0-μm phytoplankton fraction contributed most to the observed increase in the total community biomass. Only in the Shark

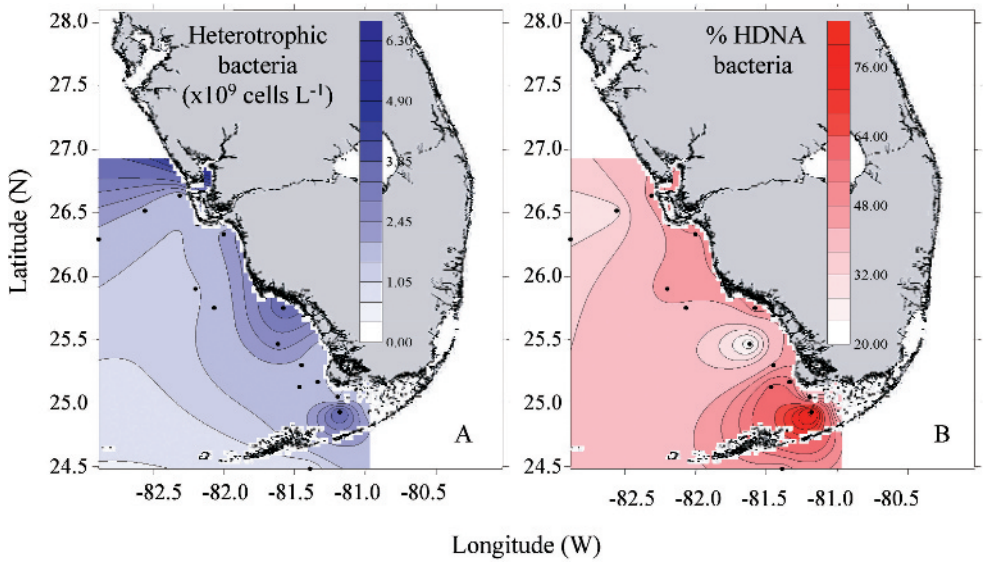

Fig. 4. Contour maps of the bacterial distributions. (A) Data are shown for heterotrophic bacterial abundance and (B) for the percent of bacteria containing high DNA.

River did the smaller size fraction have a significant response to N.

Bacterial response was very low at the mouth of the rivers Peace and Shark (Table 2). At the Caloosahatchee River station bacterial abundance increased ~200% in N treatments and ~50–100% in humic acid and P treatments. In the western Florida Bay also a slight response to humic acid and P additions was observed.

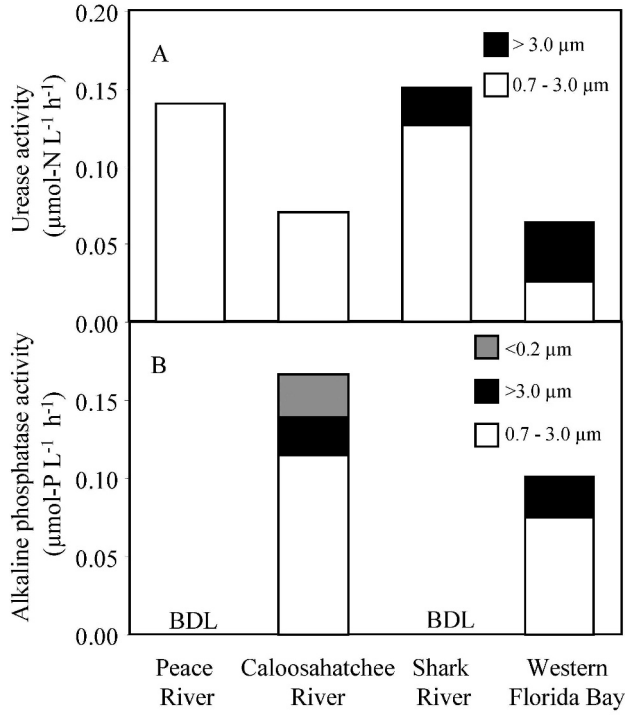

Fig. 5. (A) Activities of the enzymes urease and (B) alkaline phosphatase at each of the station locations indicated. Open bars indicate activity of the 0.7–3.0-μm size fraction, and closed bars indicate the >3.0–μm size fraction. For APA, the gray bar indicates activity in the <0.2-μm size fraction. BDL indicates below detection level.

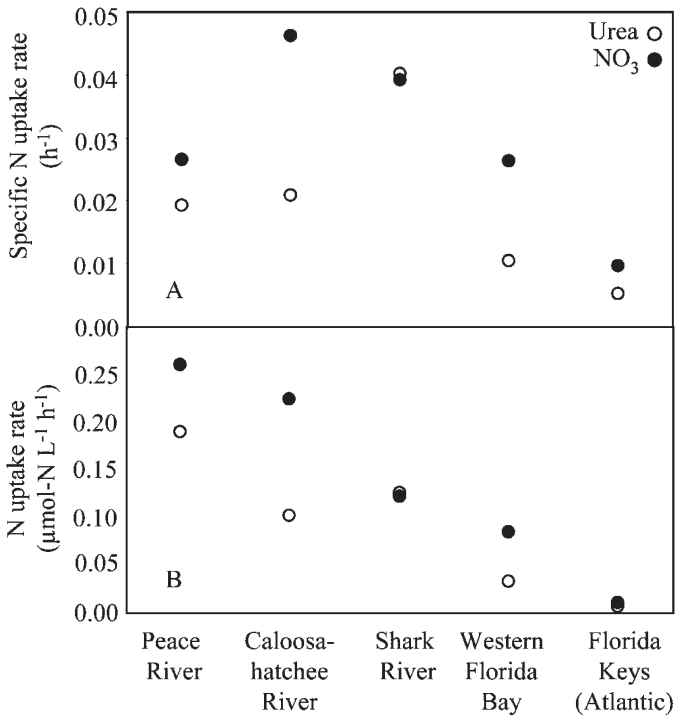

Fig. 6. Rates of uptake of the nitrogenous substrates urea (open circles) and NO_3^- (closed circles) for the station locations indicated. Values are given (A) as specific uptake rates and (B) as total uptake rates.

Table 2. Responses of heterotrophic bacteria and size-fractionated phytoplankton community to nutrient enrichment in bioassay experiments, expressed relative to control after 48 h of incubation (±standard error [SE]). Experiments were conducted with microbial communities from the mouths of the Peace, Caloosahatchee, and Shark Rivers and from western Florida Bay. The nutrient enrichment levels are described in the text.*

River system	Size fraction	Bioassay treatment response (% change from controls)				
		NO_3^-	DON	Humic acid	PO_4^+	DOP
Peace River	Bacterial abundance	23(±3)	10(±6)	23(±10)	8(±11)	14(±15)
	>0.7 μm Chl *a*	572(±15)	267(±32)	−4(±8)	−18(±4)	−16(±0)
	0.7–3.0 μm Chl	−100(±54)	14(±24)	24(±36)	0(±37)	33(±48)
	>3.0 μm Chl *a*	916(±94)	369(±54)	−15(±3)	−25(±9)	−35(±19)
Caloosahatchee River	Bacterial abundance	206(±6)	213(±6)	75(±6)	31(±7)	76(±4)
	>0.7 μm Chl *a*	645(±193)	338(±46)	15(±3)	0(±5)	−14(±4)
	0.7–3.0 μm Chl *a*	99(±140)	88(±11)	9(±13)	−3(±5)	−10(±2)
	>3.0 μm Chl *a*	1,159(±243)	574(±79)	21(±7)	3(±6)	−18(±9)
Shark River	Bacterial abundance	10(±4)	−14(±8)	106(±60)	21(±1)	18(±12)
	>0.7 μm Chl *a*	147(±49)	126(±27)	24(±13)	33(±18)	−1(±6)
	0.7–3.0 μm Chl *a*	114(±53)	70(±29)	34(±11)	54(±31)	6(±12)
	>3.0 μm Chl *a*	217(±42)	245(±23)	2(±17)	−14(±12)	−16(±8)
Western Florida Bay	Bacterial abundance	41(±23)	−12(±7)	47(±6)	18(±1)	68(±7)
	>0.7 μm Chl *a*	58(±42)	167(±25)	41(±7)	−7(±1)	12†
	0.7–3.0 μm Chl *a*	−24(±41)	5(±7)	−19(±27)	−42(±6)	−8†
	>3.0 μm Chl *a*	133(±117)	316(±54)	97(±39)	24(±4)	31†

* Chl *a*, chlorophyll *a*; DON, dissolved organic nitrogen; DOP, dissolved organic phosphorus.
† One of the replicate treatments was lost during incubation.

Discussion

This synoptic study of the southwestern Florida shelf, conducted during a minimal flow period, has revealed significant inshore–offshore gradients as well as significant north–south gradients in nutrients and plankton community composition. Collectively these patterns indicate that terrestrial nutrient inputs are large, even during minimal flow, and that their relative availability leads to both differential nutrient limitation in different regions of the shelf as well as development of a varying plankton community composition. These results also strongly indicate that organic nutrients are an important contributor to the nutrient dynamics of the southwestern Florida shelf.

Nutrient trends—The north–south gradient in the availability of dissolved and particulate nutrients fall into three distinct zones (Fig. 7; Table 3), herein termed zones I, II, and III. Zone I, the mouths of the Peace and Caloosahatchee Rivers, was characterized by a N-limited microbial community, as the stoichiometric proportions of the particulate N and P were consistently <8 and the highest phytoplankton responses were found in bioassays enriched with N, confirming previous bioassay results from this region (McPherson and Miller 1990). This region had the highest abundance of gyroxanthin-diester containing *K. brevis*. Zone II, comprising the Ten Thousand Islands region and the Shark River mouth, was characterized by a particulate stoichiometric N : P ratio that was close to Redfield proportion, 8–24, and an increasing abundance of zeaxanthin-containing cyanobacteria. Although smaller than in the Peace and Caloosahatchee Rivers, the phytoplankton in this region showed modest response in bioassays enriched in N and an even smaller, but still positive, response to enrichments with P. Lastly, Zone III, comprising western Florida Bay and the Florida Keys, had the highest proportion of diatoms, as indicated by the fucoxanthin to Chl *a* ratio. The particulate material of this region was characterized by the highest N : P ratio, >24, and thus was apparently the most P-limited material.

The spatial variability of the dissolved nutrients in these three zones was consistent with the distinct characteristics of their watersheds. The coastal waters influenced by the Peace River (watershed: agricultural, cattle, P mining) had the maximum observed concentrations of NH_4^+, DON, PO_4^{3-}, and DOP (Table 3). At the mouth of the gated Caloosahatchee River (watershed: agricultural, sugar and citrus), inorganic and organic nutrients were significantly lower than those measured at the Peace River mouth. The Ten Thousand Island area and the waters influenced by the Shark River (watershed: Everglades, relatively pristine waters) had similar levels of NH_4^+, DON, and DOP when compared with the Caloosahatchee River estuarine area, but they had lower PO_4^{3-}. These patterns were similar in western Florida Bay (watershed: Everglades). At the Atlantic side of the Florida Keys, DON levels decreased, while an increase in NO_3^- was observed. In contrast, silicate was elevated through the nearshore region. Silicate has been used as a tracer of estuarine outflow within this region (Vargo et al. 2004), and the silicate enrichment observed nearshore and the lack of a significant relationship between fucoxanthin and silicate concentration in the current study indicates that silicate was not a limiting nutrient.

In a previous analysis of N : P nutrient ratios in southwestern Florida coastal waters in the late 1990s,

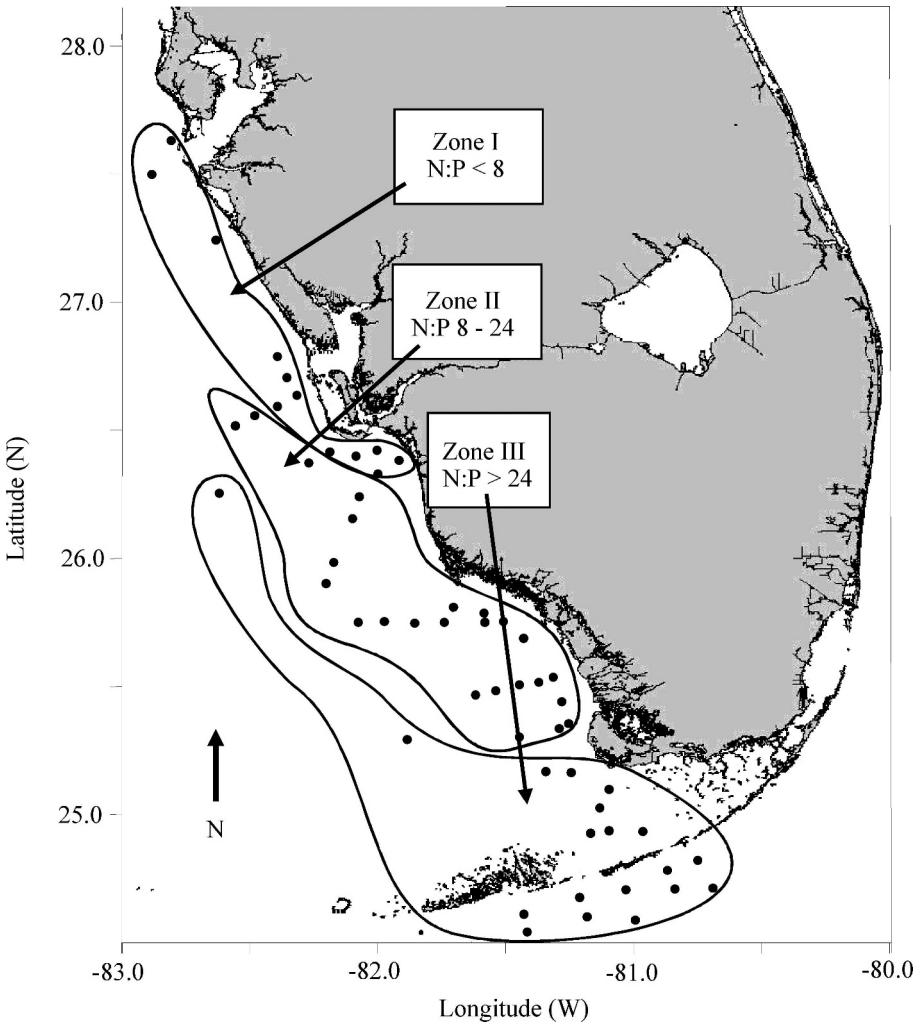

Fig. 7. Map of southwest Florida shelf region indicating the range of N : P ratios in the particulate matter in the three zones characterized in Table 3.

Table 3. Classification summary of the nutrient, phytoplankton, and watershed influences in the different coastal zones in the inner southwest Florida shelf.*

Zone	Watershed influence	Dominant phytoplankton group in receiving waters**	Biomass nutritional status†	Percent of total dissolved phosphorus pool‡	Percent of total dissolved nitrogen pool‡
I	Phosphorus mining, dairy, fruit agriculture	Dinoflagellates	Nitrogen limited	55.6(±6.6) DOP 44.3(±6.6) DIP	94.6(±1.3) DON 0.4(±0.2) NO_3^- 4.8(±1.1) NH_4^+ 0.0(±0.0) Urea
II	Sugar agriculture, Everglades	Cyanobacteria	Balanced	72.0(±4.1) DOP 28.0(±4.1) DIP	88.2(±3.0) DON 1.3(±0.5) NO_3^- 9.9(±2.5) NH_4^+ 1.4(±0.7) Urea
II	Everglades, anthropogenic sewage	Diatoms	Phosphorus limited	88.4(±6.6) DOP 11.6(±6.6) DIP	88.4(±6.7) DON 5.6(±5.0) NO_3^- 5.3(±1.0) NH_4^+ 0.0(±0.0) Urea

* DOP, dissolved organic phosphorus; DIP, dissolved inorganic phosphorus; DON, dissolved organic nitrogen.
** As indicated by pigment composition.
† As indicated by N : P ratio of particulate material.
‡ Values are the average for each zone (±standard error [SE]). NO_2^- was not included as it was at the limits of detection in more than 95% of samples analyzed.

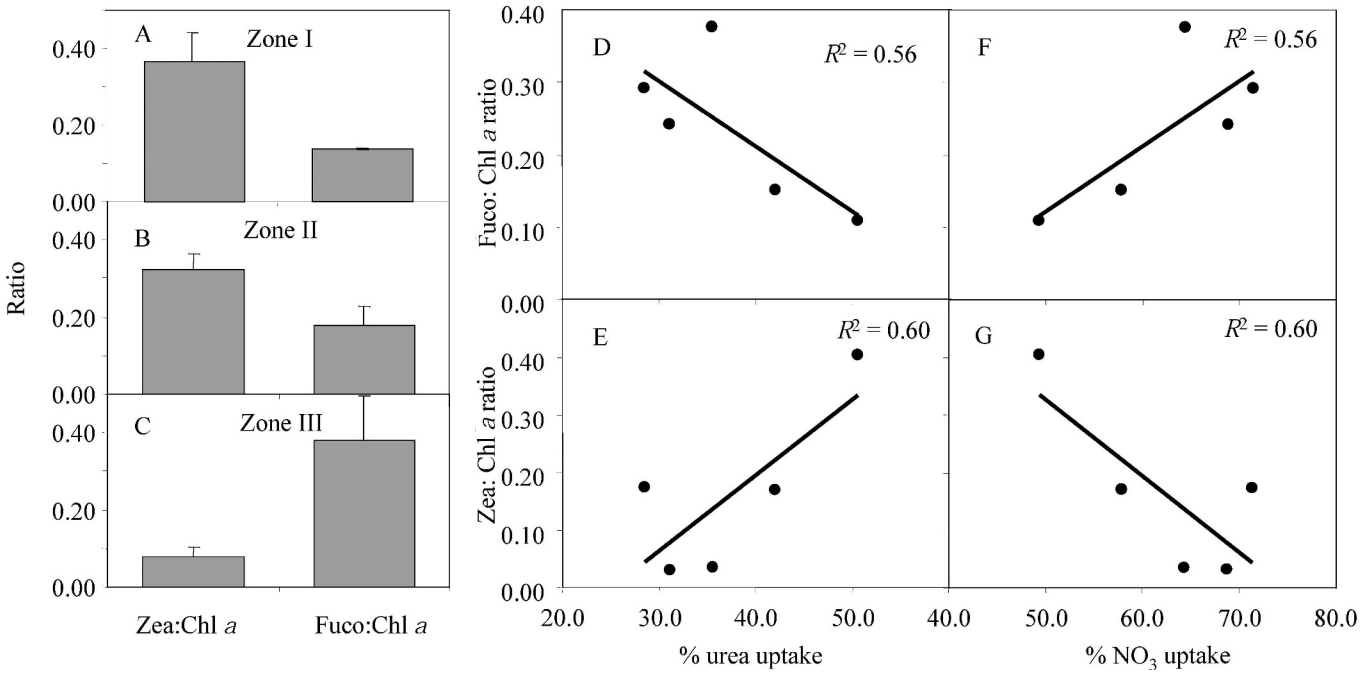

Fig. 8. (A–C) Mean ratios of the pigments zeaxanthin and fucoxanthin relative to Chl *a* for the three zones of the western Florida shelf defined in the text and (D–G) the relationships between the percent of urea and NO_3^- uptake and the pigment ratios fucoxanthin : Chl *a* and zeaxanthin : Chl *a* for all sampled stations.

Brand (2002) also found variations between N and P sources. Our findings and his agree in that DIN was found to be higher in the western Florida Bay region than further north, which is indicative of a sewage or groundwater N source; our findings were also similar in that we both found that high P occurs in the waters receiving drainage from the Peace River. Brand (2002) further suggested that much of the DON in the region of western Florida Bay is unavailable to phytoplankton. However, both direct uptake and bioassay measurements presented here and in previous work in the region (Glibert et al. 2004) indicate that at least a fraction of this DON pool is being readily used.

Phytoplankton composition gradients—The three zones in nutrient composition are also apparent in the phytoplankton groups dominating community composition (Table 3). The southernmost edge of Zone I was dominated by the gyroxanthin-diester–containing *K. brevis*. It has been well recognized in many other areas that dinoflagellates often begin to dominate as the N : P ratio of the dissolved inorganic nutrient pools declines. The high proportion of DON in this zone is also consistent with the increasingly recognized capability of many dinoflagellates to obtain some or all of their nitrogenous nutrition through osmotrophy (Schnepf and Elbrächter 1992; Glibert and Legrande 2006). *K. brevis* has been shown to utilize urea (Shimizu and Wrensford 1993; Bronk et al. 2004) and amino acids (Bronk et al. 2004), and high urease activity was sustained in this region, indicating that urea was being metabolized. Blooms of *K. brevis* occur almost annually in the coastal region between Tampa Bay and Naples (Steidinger et al. 1998). Bloom initiation occurs in an area 18–64 km offshore (Steidinger and Haddad 1981), out of the range of coastal riverine nutrient inputs. Although inorganic nutrient concentrations within and near blooms are generally low (Heil et al. 2001), the proximity of these blooms to coastal sources as well as their common association with temperature and salinity fronts (Vargo et al. 2004) indicate that riverine sources potentially contribute to bloom maintenance. DON release from *Trichodesmium* (Glibert and Bronk 1994) may be another source contributing to bloom maintenance; *Trichodesmium* was found in this study in association with *K. brevis* and has been shown to fix and release sufficient N to support moderately sized *K. brevis* blooms (Mulholland et al. 2006). Although DIN : DIP ratios were lower than Redfield proportions in the region where *K. brevis* was observed (Table 1), DON : DOP ratios were consistently greater, indicating that phytoplankton capable of utilizing DON would be favored over those relying on NO_3^- and NH_4^+ in this region. If the *K. brevis* bloom encountered in this study was indeed being maintained through DON sources, this would indicate that this bloom is not related to eutrophication from DIN. However, the role of anthropogenically derived DON cannot be discounted (Glibert et al. 2006).

In Zone II, a higher proportion of cyanobacteria was found, based on the zeaxanthin : Chl *a* ratio. In this zone, the DIN : DIP ratio was closer to Redfield proportions than in the other zones, and particulate nutrient ratios were also in near-stoichiometric balance. Although DON was a smaller proportion of TDN in this zone, the uptake of one fraction of the DON pool, urea, was strongly related to the relative abundance of zeaxanthin (Fig. 8). Similar positive relationships between the proportion of urea

uptake and the zeaxanthin : Chl *a* ratio have been reported for Florida Bay (Glibert et al. 2004). Collectively these results also support previous work demonstrating that urea can be a significant source of N for cyanobacteria, such as *Synechococcus* sp. (e.g., Berman and Chava 1999; Collier et al. 1999). Size-fractionated urease activity rates indicated that most of the activity was in the 0.7–3.0-μm fraction, and correlation analysis indicated that the enzymatic activity was mainly that of cyanobacteria and heterotrophic bacteria. Urea is a significant anthropogenic form of nitrogen and is now the dominant form of nitrogen used in most agricultural applications, including sugarcane and citrus cultivation (Glibert et al. 2006). If the urea found to be of local importance in this zone is indeed of anthropogenic origin, then this indicates that these cyanobacterial outbreaks may be a symptom of eutrophication from organic loading in this subwatershed.

Lastly, in Zone III, the proportion of fucoxanthin-containing diatoms increased relative to the more northern zones (Fig. 8). This zone had the highest DIN : DIP ratios, resulting from low availability of PO_4^+, and bioassay responses from these samples, although small, showed some response to added P. In this zone, the composition of the DIN pool also shifted, with an increasing proportion of NO_3^- evident. The uptake of NO_3^- was positively related to fucoxanthin and negatively related to zeaxanthin (Fig. 8). These findings are consistent with diatom physiology. Whereas most phytoplankton that utilize inorganic N prefer NH_4^+ over NO_3^- as a nitrogen substrate, many diatoms have the capability for high uptake rates of NO_3^-, and NO_3^- uptake rates are less inhibited by NH_4^+ in many diatoms than in many other phytoplankton groups (Lomas and Glibert 1999, 2000). Although the sources of NO_3^- cannot be identified from this study, it has previously been suggested that sewage- or groundwater-derived NO_3^- from the Florida Keys and the Everglades may be important (Brand 2002). Extrapolating from these results to the diatom-dominated "black water" events that have been noted in this southwest shelf area (Hu et al. 2002; Neely et al. 2004), it is possible that such events were initiated, at least in part, by NO_3^- discharge from this region. The localized peaks in PO_4^{3-} and urea observed in this area, possibly due to localized groundwater discharge or lower Keys inputs, may also contribute to these blooms.

These findings indicate that the dominant nutrient sources of land origin differ from north to south along the southwestern Florida shelf and that these inputs results in a gradient of N-limited to P-limited plankton communities (Table 3). With PO_4^{3-} and DON as the dominant sources in the northern zone, dinoflagellates flourish. In the mid-region, more balanced nutrient conditions exist. Yet this region supports a cyanobacterial community that not only can use inorganic nutrient substrates but that can also exploit DON, especially in the form of urea. Further south, with higher inputs of NO_3^- and lower levels of PO_4^{3-}, diatoms proliferate. These findings indicate that as anthropogenic sources of N, from urea applications in agriculture and NO_3^- in urban and suburban development, increase, the most likely outcomes will be increased blooms of cyanobacteria and diatoms.

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