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Spatial variability in Florida Bay particulate organic matter composition: combining flow cytometry with stable isotope analyses

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Abstract

Long-term management plans for restoration of natural flow conditions through the Everglades increase the importance of understanding potential nutrient impacts of increased freshwater delivery on Florida Bay biogeochemistry. Planktonic communities respond quickly to changes in water quality, thus spatial variability in community composition and relationships to nutrient parameters must be understood in order to evaluate future downstream impacts of modifications to Everglades hydrology. Here we present initial results combining flow cytometry analyses of phytoplankton and bacterial populations (0.1–50 μm size fraction) with measurements of $\delta^{13}C$ and $\delta^{15}N$ composition and dissolved inorganic nutrient concentrations to explore proxies for planktonic species assemblage compositions and nutrient cycling. Particulate organic material in the 0.1–50 μm size fraction was collected from five stations in Northeastern and Western Florida Bay to characterize spatial variability in species assemblage and stable isotopic composition. A dense bloom of the picocyanobacterium, *Synechococcus elongatus*, was observed at Western Florida Bay sites. Smaller *Synechococcus* sp. were present at Northeast sites in much lower abundance. Bacteria and detrital particles were also more abundant at Western Florida Bay stations than in the northeast region. The highest abundance of detritus occurred at Trout Creek, which receives freshwater discharge from the Everglades through Taylor Slough. In terms of nutrient availability and stable isotopic values, the *S. elongatus* population in the Western bay corresponded to low DIN (0.5 μM NH_4^+; 0.2 μM NO_3^-) concentrations and depleted $\delta^{15}N$ signatures ranging from +0.3 to +0.8‰, suggesting that the bloom supported high productivity levels through N_2-fixation. $\delta^{15}N$ values from the Northeast bay were more enriched (+2.0 to +3.0‰), characteristic of N-recycling. $\delta^{13}C$ values were similar for all marine Florida Bay stations, ranging from −17.6 to −14.4‰, however were more depleted at the mangrove ecotone station (−25.5 to −22.3‰). The difference in the isotopic values reflects differences in carbon sources. These findings imply that variations in resource availability and nutrient sources exert significant control over planktonic community composition, which is reflected by stable isotopic signatures.

Introduction

Florida Bay has been influenced by changes in the timing and volume of freshwater flow from the Everglades in the last half century as a result of extensive modification of the natural discharge patterns by water management infrastructure (Davis & Ogden, 1994; Rudnick et al., 1999). This has prompted researchers to explore the extent to which salinity levels in the bay have been affected by these changes (Huvane, 2002). Further, it is hypothesized that human alteration of the

Everglades watershed in the last century facilitating agriculture and urbanization may have increased nutrient loads to downstream Florida Bay leading to unknown impacts on the ecosystem (Fourqurean et al., 1993; Boyer et al., 1999). In the same time frame, biotic and water quality parameters have changed significantly (Lapointe et al., 2002). Historically, Florida Bay was a shallow, optically clear lagoon characterized by low nutrient levels and populated by abundant seagrass communities. However, in recent years significant declines have been observed in water clarity, hypersaline conditions have periodically developed during summer months (Boyer et al., 1999), and widespread die-off events have occurred in the seagrass communities (Zieman et al., 1989; Fourqurean et al., 1993), coincident with phytoplankton blooms (Robblee et al., 1991; Butler et al., 1995; Phlips & Badylack, 1996). Causal mechanisms behind this shift in biotic communities remain the focus of ongoing study; however, the trigger for phytoplankton blooms is not fully understood (Richardson & Zimba, 2002).

Given long term management plans to restore more natural flow conditions through the Everglades, a key concern is the extent to which nutrients passing through or originating from the Everglades impact Florida Bay. Restored flows may alter the forms, amount, and temporal distribution of dissolved nutrients delivered to the bay. Planktonic communities respond quickly due to high population turnover rates and the tight association with water quality. In turn, the structure of these planktonic communities can have significant effects on biogeochemical cycles of nutrients (Lavrentyev et al., 1998). Yet, while much attention in Florida Bay has focused on changes in seagrass abundance and distribution (Zieman et al., 1989; Anderson & Fourqurean, 2003; Fourqurean et al., 2005), aspects of the composition and community dynamics of particulate organic matter (POM) remain poorly understood.

Several studies have taken steps toward characterizing Florida Bay's phytoplankton community applying cell counting and pigment extraction techniques, demonstrating the bay is extremely variable in terms of both species diversity and biomass (Phlips & Badylack, 1996; Lavrentyev et al., 1998; Phlips et al., 1999; Richardson & Zimba, 2002). Cyanobacteria, diatoms, and dinoflagellates have been identified as dominant organisms (Phlips & Badylack, 1996), however relative population abundances of several species in each group vary widely both spatially and temporally (Richardson & Zimba, 2002). Lavrentyev et al. (1998) characterized the microbial plankton community with respect to resource limitation with a short-term bay-wide survey, dividing the bay into three regions based on community structure and elemental stoichiometry. The North-central region of Florida Bay contained the highest cyanobacteria abundance (Lavrentyev et al., 1998). Physical restriction of tidal exchange between this interior zone and the surrounding oceanic environments enhances potential for accumulation of cyanobacterial biomass by increasing water residence times, while euryhaline cyanobacteria species are unaffected by periodic hypersaline conditions (Phlips et al., 1999).

Conspicuously absent in the literature, however, is understanding of the biogeochemical connections between nutrient resources and population dynamics within the phytoplankton and bacterial components of POM. Isotopic-enrichment tracer techniques assessing utilization of organic nitrogen (N) substrates during a cyanobacteria bloom event have shown in one instance that cyanobacterial blooms in Central Florida Bay are associated with high dissolved organic nutrient availability, whereas areas dominated by microflagellate and diatom communities are dependent upon inorganic nutrient resources (Glibert et al., 2004). The utility of natural isotopic abundance measurements to assess biogeochemical coupling between nutrient resources and planktonic communities has not been well explored. It is possible that bulk samples of the 0.1–50 μm POM fraction collected at sites characterized by different water quality parameters and nutrient availability will have distinct isotopic fingerprints based on species composition differences, or may reflect relative nutrient utilization between varying N-sources (nitrate (NO_3^-) versus ammonium (NH_4^+)) and nitrogen versus carbon limitation.

Here we present initial results combining flow cytometry analyses of phytoplankton and bacterial populations with natural abundance carbon and nitrogen isotopic signatures to explore proxies for

biotic assemblage characteristics. Flow cytometry represents an advantage over traditional fluorescence microscopy and HPLC pigment analysis in that it allows efficient and objective analysis of large numbers of particles, with the facility to distinguish organic living and non-living particles (Moreira-Turcq & Martin, 1998). However, limitations in the numbers of pigments analyzed restrict taxonomic resolution as compared to other methods (Veldhuis & Kraay, 2000). Procedures coupling flow cytometry sorters with pyrolysis mass spectrometry for in-line analysis are currently under development (Minor & Nallanthamby, 2004; Pel et al., 2004), however at present sorted sample volumes are insufficient to perform $\delta^{15}N$ analyses, and isotopic values can only be obtained for $\delta^{13}C$.

In our approach, flow cytometry is used to distinguish bacteria, cyanobacteria, eukaryotic phytoplankton, and detritus, allowing a broad view of POM composition at endmember locations in terms of Florida Bay nutrient and salinity gradients. POM composition is then compared with isotopic measurements. Further characterization and exploration of size-fractionated isotopic signatures advances understanding of phytoplankton, microbial, and biogeochemical dynamics in the system.

Methods

Setting

Florida Bay consists of a series of shallow basins (<3 m depth) separated hydrologically by a network of subtidal mudbanks and mangrove islands which restrict water mixing between basins and attenuate both tidal amplitude and current. Freshwater runoff, comparatively rich in dissolved inorganic nitrogen (DIN) and poor in soluble reactive phosphorus (SRP), enters this hydrologically complex system primarily in Northeastern Florida Bay through the Taylor Slough and the C-111 basin. Saline ocean water, comparatively depleted in DIN and enriched in SRP, enters across the western boundary with the Gulf of Mexico and through numerous tidal channels crossing the Florida Keys (Boyer et al., 1999). Interaction between these inflows and limitations on the bay's mixing regime serve to generate gradients in both salinity and nutrient availability across the bay. Salinity increases from the freshwater-affected northeastern region to the south and west to reach marine strength (36‰), with seasonal periods of hypersalinity (40–80‰) in restricted interior basins (Boyer et al., 1999). Nutrient gradients are evident in changing DIN:SRP ratios, which are considered an index of phytoplankton nutrient limitation, from northeast to southwest Florida Bay (Boyer et al., 1999). Long-term monitoring revealed little seasonality in consistently high DIN:SRP ratios in the eastern bay, indicating potentially continuous phosphorus (P) limitation (Fourqurean et al., 1993; Boyer et al., 1999). Western Florida Bay DIN:SRP ratios are over three times lower and are more variable than those in the northeastern region, implying fluctuations between N and P limitation, due not only to lower DIN concentrations in the western bay region than in the east, but also because SRP is periodically supplied through mixing from the Gulf of Mexico (Boyer et al., 1999).

Sampling locations and collection

In November 2003, a "snapshot" of samples was collected at geographic end-member sites in northeastern and western Florida Bay (Fig. 1). Trout Creek (Station 5) represented a freshwater discharge influenced northeastern site (salinity 4.2‰), while Duck Key (Station 4) and South Nest Key (Station 3) were marine northeast Florida Bay sites (salinity 24.5‰). Ninemile Bank (Station 2) and Sprigger Bank (Station 1) were representative of full seawater salinity western Florida Bay locations (salinity 35.5‰). Three sets of water samples were collected at each site after first passing though a 50 μm Nitex screen to exclude larger size classes of zooplankton, phytoplankton, and detritus. Two replicate samples were collected at each station and preserved with formalin (final conc. 1%) for cytometric analysis. Water samples for nutrient analyses (30 ml) were syringe filtered with 0.45 μm GF/F Whatman filters. Finally, 1 l samples were collected for stable isotopic analysis of the 0.1–50 μm POM fraction. All samples were kept in the dark and refrigerated at 5 °C until time of analysis, which occurred within 3 days of sample collection. Salinity was measured using a digital Orion Temp/Salinity Meter.

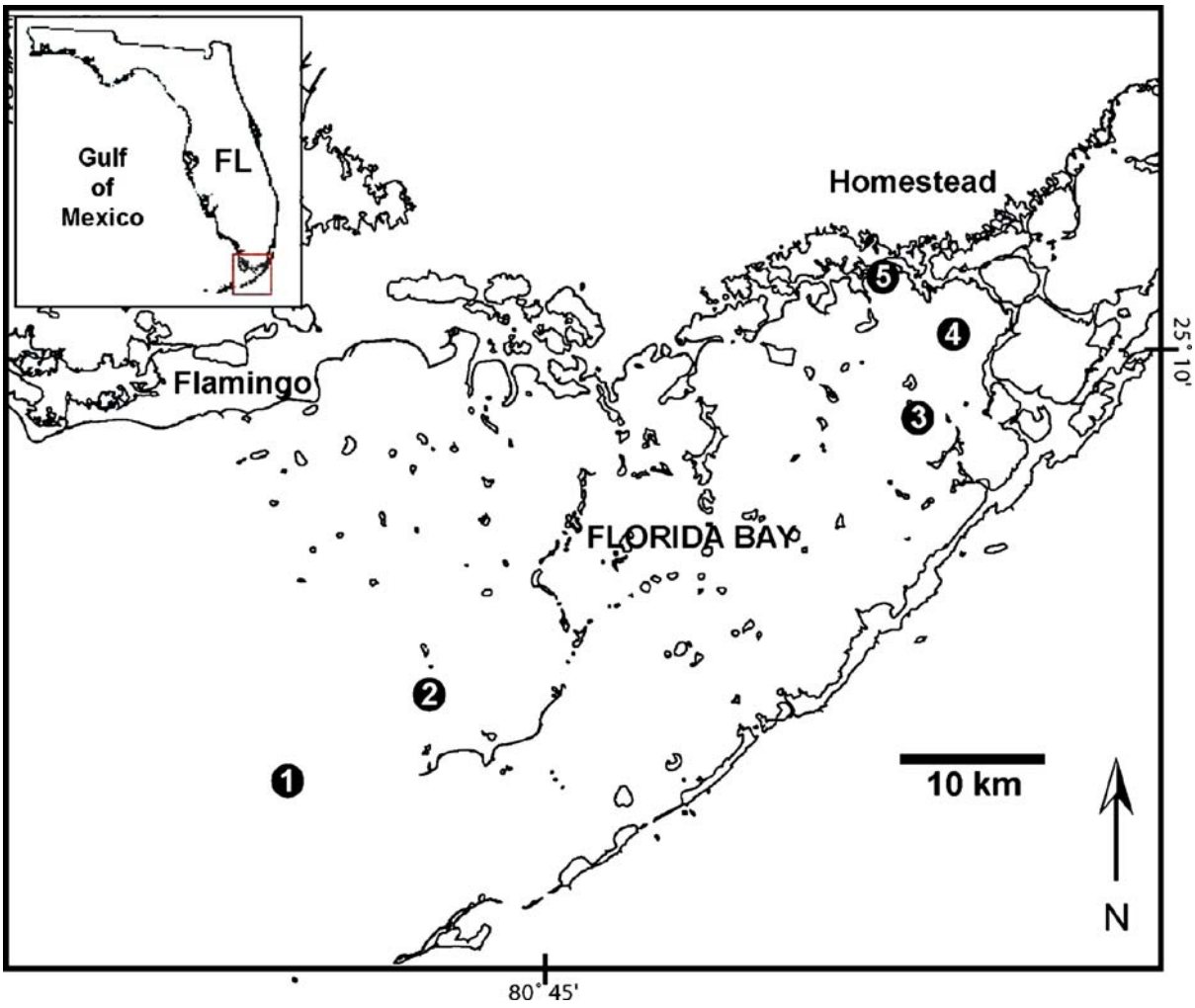

Figure 1. Map of Florida Bay, showing sampling locations in Western Florida Bay at Sprigger Bank (1) and Ninemile Bank (2), in Northeastern Florida Bay at South Nest Key (3) and Duck Key (4), and near the Taylor Slough Everglades outflow at Trout Creek (5).

Flow cytometry analyses

Heterotrophic bacteria and phytoplankton in the 0.1–50 μm POM fraction were counted on a Becton Dickinson FACSort flow cytometer equipped with an argon laser (488 nm). For each particle, two light scatter (side angle light scatter and forward light scatter) and three fluorescence signals were recorded on a 4-decades log scale. All data were analyzed using Win MDI 2.8 (Trotter, 2000) flow cytometric analysis software to define populations by logical gating and generate plots illustrating 50 000 events (particle detections).

A DNA staining procedure was used to enumerate heterotrophic bacteria (Marie et al., 1997). A 500 μl sample aquilot was incubated with 25 μl RNAase (0.1 g l^{-1}, 1:1 mixture of RNAase A & B) for 30 min at 37 °C, followed by the addition of 20 μl of SYBR Green I staining (10^{-5} dilution of commercial stock, Molecular Probes, Eugene, OR) in the presence of 30 mM potassium citrate. SYBR DNA fluorescence was recorded in both the green FL1 (535 ± 15 nm) and red FL3 (>650 nm) fluorescence channels, DNA clusters of bacteria were counted by logical gating from both FL1 vs FL3 and SSC vs FL1 dot plots, and histograms with gate borders set to minima in population fre-

quency distribution. Measured sample volumes for estimates of bacterial abundance (cells ml^{-1}) were calculated from measurement times (30 s) based on weight calibration of flow rates (0.2 $\mu l\ s^{-1}$; Jochem, 2001).

For bulk phytoplankton samples, 1 ml of each replicate sample was analyzed without staining, as phytoplankton pigments exhibit detectable autofluorescence following laser excitation (Hofstraat et al., 1994). Phytoplankton populations were differentiated using chlorophyll autofluorescence, detected in the red FL3 (>650 nm) fluorescence channel, phycoerythrin autofluorescence, detected in the orange FL2 (575 ± 25 nm) fluorescence channel, and side angle light scatter was measured (SSC channel) for proxy analysis of relative cell size. Cell concentrations were calculated based on measurement times (150 s) and weight-calibrated flow rates (1 $\mu l\ s^{-1}$).

Isotopic analyses

Water samples (1 l) were vacuum-filtered through Whatman Anodisc membrane filters (0.1 μm pore size). These filters are aluminum oxide matrix, and thus do not influence carbon and nitrogen stable isotope signatures (Bertoni, 1997). Filters were oven dried (70 °C) and powdered. Powdered filter samples were analyzed following standard Elemental Analyzer Isotope Ratio Mass Spectometry (EA-IRMS) methods on a Finnigan MAT Delta C Isotope Ratio Mass Spectrometer at the Stable Isotope Laboratory of the Southeast Environmental Research Center. $\delta^{13}C$ analyses were conducted after acidifying with 1 N HCl for carbonate removal. $\delta^{15}N$ analyses were conducted on raw filter material. Reproducibility of nitrogen isotope analyses was $\pm 0.2‰$, while carbon reproducibility was $\pm 0.05‰$. All isotopic values are reported using standard delta (δ) notation:

$$\delta = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000 \qquad (1)$$

where R is the isotopic ratio, i.e. $^{15}N/^{14}N$ or $^{13}C/^{12}C$ in either sample or standard.

Nutrient analyses

Surface water filtrates from each site were analyzed for nitrate + nitrite (NO_x^-), nitrite (NO_2^-), ammonium (NH_4^+), and SRP with an Alpkem model RFA 300 water autoanalyzer set to 4 channel simultaneous flow. Nitrate (NO_3^-) concentrations were calculated as $NO_x^- - NO_2^-$. These samples were compared to long-term water quality data sets provided by the SERC-FIU Water Quality Monitoring Network.

Results

Eukaryotic algae (EUK) and four distinct populations of cyanobacteria (numbered 1–4) were differentiated through logical gating of bivariate flow cytometry analysis plots (Fig. 2). Each point on the individual plots represents a single particle detection, thus darker areas indicate higher particle densities. Groups of eukaryotic algae and cyanobacteria are shown within ellipses because they are difficult to distinguish visually in monochromatic plots. Spatial variability in assemblage and population abundance is evident (Table 1). The highest abundances were seen in the low chlorophyll, low phycoerythrin population, *Synechococcus elongatus* (Fig. 2; Cyanobacteria 2), with $801.9 \pm 56.3 \times 10^3$ and $532.9 \pm 25.0 \times 10^3$ cells ml^{-1} present only at the western Florida Bay sites (Ninemile Bank and Sprigger Bank, respectively). *S. elongatus* is known to form blooms in central and western Florida Bay (Lavrentyev et al., 1998; Phlips et al., 1999; Glibert et al., 2004). A second population of *Synechococcus* sp, distinguished by higher phycoerythrin and a smaller size (Fig. 2; Cyanobacteria 3), was present at all stations, but with higher densities at the northeast-

Figure 2. Flow cytometry analyses of Florida Bay phytoplankton collected at five sampling locations in Western (a–d) and Northeastern (e–h) Florida Bay, and proximal to Everglades outflow (i–j). 50 000 events are displayed after acquisition in log mode. The left side plots from each site display phycoerythrin (FL2) against chlorophyll (FL3); the right side plots show Side Angle Light Scatter (relative cell-size proxy) with chlorophyll. All axes are on 4 decade log scale. Eukaryotic algae (EUK) and 4 distinct cyanobacterial populations are shown (circled and numbered 1–4), as determined using logical gating between the plot pairs from each site. Particles not included in definable populations of cells are considered detrital particles.

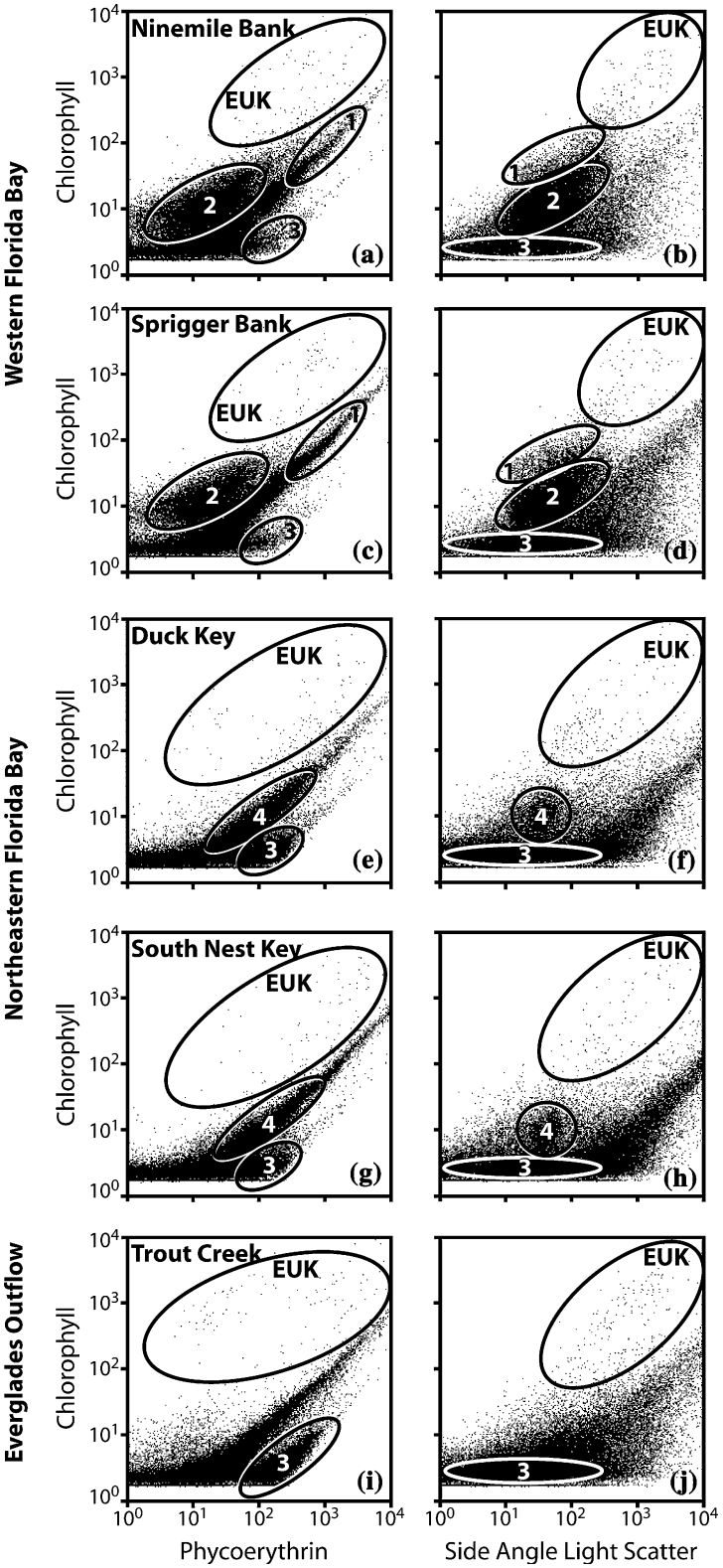
Western Florida Bay
Northeastern Florida Bay
Everglades Outflow
Ninemile Bank
Sprigger Bank
Duck Key
South Nest Key
Trout Creek
EUK
1
2
3
4
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(j)
Chlorophyll
Phycoerythrin
Side Angle Light Scatter
10^0
10^1
10^2
10^3
10^4

Table 1. Mean (± standard deviation) of phytoplankton and bacteria population abundances determined by logical gating (Win MDI; Trotter 1994–2000) for samples collected from Florida Bay during a cyanobacterial bloom, November 2003

Site	Total Phytoplankton	Eukaryotes	Cyanobact. 1	*S. elongatus** Cyanobact. 2	*Syn.* sp.** Cyanobact. 3	Cyanobact. 4	Detritus	Bacteria (cells × 10^6 ml^{-1})
Sprigger Bank	660.3 ± 65.2	1.7 ± 0.2	46.7 ± 1.1	532.9 ± 25.0	81.5 ± 40.3		627.6 ± 47.9	2.058
Ninemile Bank	934.8 ± 87.8	3.5 ± 0.8	79.5 ± 13.9	801.9 ± 56.3	66.8 ± 22.2		655.1 ± 220.0	2.412
South Nest Key	33.7 ± 5.5	0.8 ± 0.1			8.6 ± 3.9	32.1 ± 4.90	261.6 ± 59.6	0.854
Duck Key	166.6 ± 38.9	3.4 ± 0.4			80.0 ± 75.2	37.4 ± 11.3	245.9 ± 71.8	0.623
Trout Creek	520.7 ± 81.6	4.6 ± 1.8			522.0 ± 81.2		1157.0 ± 457.5	1.431

Unless indicated, all values are cells×10^3 ml^{-1}.
**Synechococcus elongatus.*
***Synechococcus* sp.

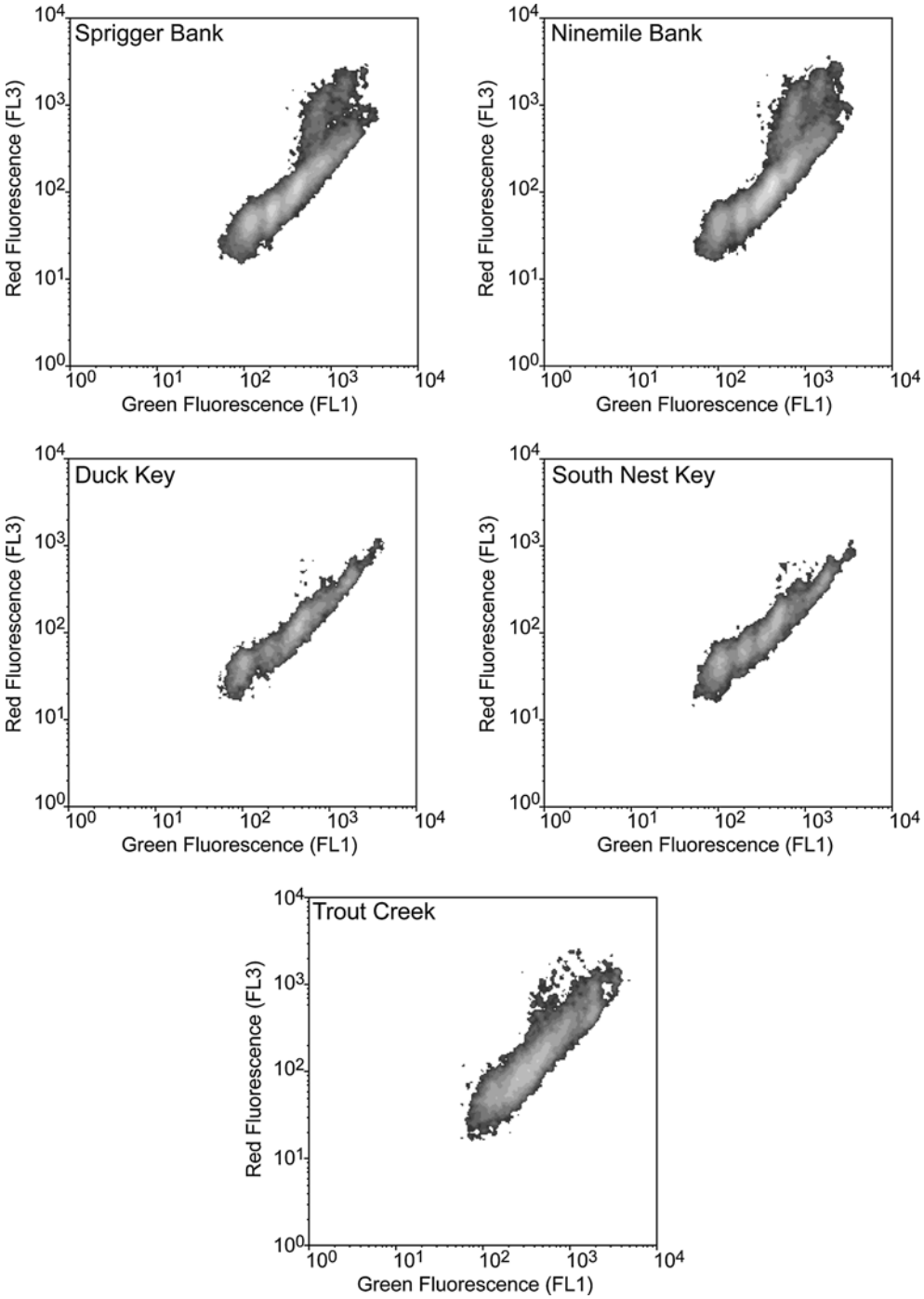

Figure 3. Flow cytometry analyses of November 2003 Florida Bay surface water bacteria samples after DNA staining with SYBR Green. Green Fluorescence (FL1) vs Red Fluorescence (FL3) are shown for all sites on a 4 decade log scale.

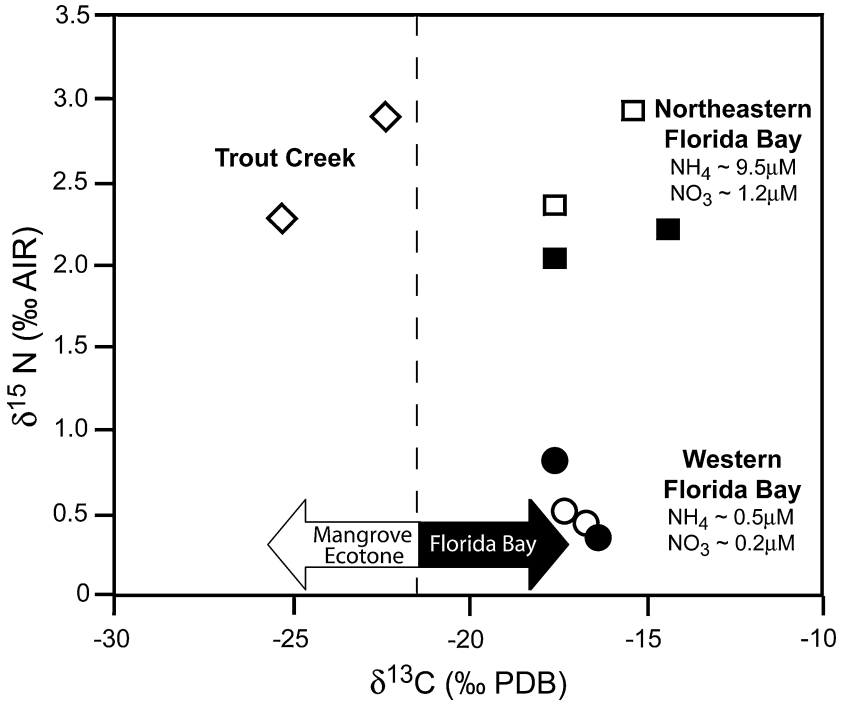

Figure 4. Stable isotope signals in 0.1–50 μm POM collected in November 2003. Everglades Outflow: Trout Creek (◇); Northeast Florida Bay: South Nest Key (□), Duck Key (■); Western Florida Bay: Ninemile Bank (○), and Sprigger Bank (●).

ern sites and with highest abundance ($522.0 \pm 81.2 \times 10^3$ cells ml^{-1}) at Trout Creek. Cyanobacteria 4, similar in size to *S. elongatus*, but with a higher phycoerythrin content, were present at much lower abundance at northeast Florida Bay sites ($32.1 \pm 4.90 \times 10^3$ cells ml^{-1} at South Nest; $37.4 \pm 11.3 \times 10^3$ cells ml^{-1} at Duck Key). Eukaryotic algae were present at all stations in very low abundance, with the largest population at the Trout Creek site ($4.6 \pm 1.8 \times 10^3$ cells ml^{-1}).

High abundances of autofluorescing detrital particles were evident at all stations (Table 1); however, the largest detrital signal occurred in the Everglades freshwater discharge exiting Taylor Slough at Trout Creek. The west Florida Bay stations had threefold higher detritus concentrations than the northeastern sites.

Bacterial communities also differed spatially between western and northeastern Florida Bay (Fig. 3). Up to five populations of bacteria exhibiting different DNA content were evident at all marine Florida Bay stations, but were less distinct at Trout Creek. Two additional populations with higher red fluorescence were abundant at western bay region, present at northeastern sites in much lower abundance; these may represent *Prochlorococcus* sp., or additional bacterial populations. The maximum bacteria concentrations were measured at Ninemile Bank (2.412×10^6 cells ml^{-1}), while much lower bacteria concentrations were found at northeastern sites (0.623×10^6 cells ml^{-1} at Duck Key; 0.854×10^6 cells ml^{-1} at South Nest Key). Trout Creek, in the mangrove ecotone, exhibited higher bacterial abundance than the open water northeast Florida Bay stations (Table 1).

Carbon and nitrogen isotopic signatures demonstrate differences between geographic endmembers of Florida Bay environmental gradients (Figure 4). The Trout Creek location exhibited the most depleted carbon values (−25.3‰ to −22.3‰), whereas the open water locations in both western and northeastern Florida Bay were slightly more enriched, ranging from −17.6‰ to −14.4‰. δ^{15}N, however, differed between west and northeast Florida Bay. At northeastern sites, nitrogen isotopic signatures were 2.0‰ to 3.0‰, while more depleted values were evident at western bay

stations, where a minimum $\delta^{15}N$ value of 0.3‰ was observed. The variation in POM nitrogen isotopic signal is coincident with a large difference in inorganic nitrogen concentrations between endmember locations (Fig. 4). The western sites had over an order of magnitude lower ammonium (NH_4^+) concentrations (9.5 vs 0.5 μM NH_4^+, northeast and west sites, respectively) and nearly an order of magnitude lower nitrate (NO_3^-) levels (1.2 vs 0.2 μM NO_3^-, northeast and west).

Discussion

High spatial heterogeneity in the 0.1–50 μm POM fraction across Florida Bay was reflected by differences in community composition, relative significance of individual sub-populations, total phytoplankton and bacterial abundances, and detritus loads. These differences are not unexpected, as noted in previous studies of the Florida Bay planktonic community (Phlips & Badylack, 1996; Lavrentyev et al., 1998; Phlips et al., 1999; Glibert et al., 2004; Frankovich et al., 2006). Indeed, the physical and chemical variability of the system and the isolated nature of the various basins of Florida Bay certainly lead to the high level heterogeneity in planktonic communities. Populations of *Synechococcus sp*. were present throughout the bay (Fig. 2; Cyanobacteria 3), however in higher abundances in northeastern Florida Bay than in the west. In fact, the highest abundance of *Synechococcus* sp. was found at Trout Creek, the lowest salinity site, proximal to Everglades' freshwater outflow. The dominance of this species in the northeastern, strongly P-limited region of Florida Bay (Fourqurean et al., 1993) supports the conclusions of Stockner (1988) that *Synechococcus* sp. is a superior competitor for phosphorus, coupling high SRP affinity with rapid uptake velocities and the facility for luxury uptake. In addition, this genus is very well adapted to changing environmental conditions, exhibiting a broad range of salinity tolerance, the ability to saturate photosynthesis and growth at very low irradiance, and potential to assimilate dissolved organic nutrients (Stockner, 1988; Phlips et al., 1989; Luo & Mitsui, 1994; Lavrentyev et al., 1998; Glibert et al., 2004). In contrast, Cyanobacteria 4, with higher chlorophyll, and slightly larger-sized cells, were absent from Trout Creek and present in lower abundance in the northeastern bay as compared to the western stations. This may indicate that Cyanobacteria 4 are less tolerant of low salinities and less competitive in nutrient acquisition than typical Florida Bay *Synechococcus* sp.

The most abundant population found throughout Florida Bay in this study was *Synechococcus elongatus*, a larger, phycocyanin-rich strain of the genus exhibiting lower phycoerythrin than the population previously discussed (Phlips et al., 1999). This population represented 80 and 84% of the phytoplankton numerical abundance in western Florida Bay at Sprigger and Ninemile Banks, respectively. These proportions are somewhat lower than those observed by Glibert et al. (2004) during an extensive bloom in November 2002 where *S. elongatus* represented over 99% of the phytoplankton abundance in western and north-central Florida Bay. Further *S. elongatus* represented over 90% of phytoplankton biovolume during fall blooms between 1993 and 1997, though no directly comparable numerical abundance values were given (Phlips et al., 1999). There are several possible explanations for apparent differences between the relative proportions of *S. elongatus* measured in this and prior studies, the most obvious being a smaller bloom in 2003 relative to previous years. Also, values reported here may represent either the early or late period in terms of bloom development, and might not correspond to peak bloom abundance. Furthermore, the maximum abundances noted by both Phlips et al. (1999) and Glibert et al. (2004) were located at sites in the north central region of Florida Bay, rather than the western boundary zone described here; thus it is possible that the high abundances of *S. elongatus* found in western Florida Bay in the present study were coincident with much greater populations that were not sampled at more interior locations. Long-term chlorophyll *a* datasets indicate peak concentrations in Whipray Basin, North-central Florida Bay, exceeded those in the western region and were reached in October 2003. Concentrations in both regions had begun to decline in November (SERC-FIU Water Quality Monitoring Network).

The dominance of *S. elongatus* at the western Florida Bay endmember has interesting implications in terms of both nutrient availability and stable isotopic signatures. Depleted $\delta^{15}N$ isotopic

signatures in the western bay during the *S. elongatus* bloom in November 2003 differed markedly from nitrogen isotopic values of 4.2‰ recorded during a similar bloom in November 2002 (Glibert et al., 2004). Isotopic depletion during a cyanobacterial bloom opposes the expectation that high primary productivity and surface nutrient utilization induces isotopic enrichment, as has been reported in many studies of oceanic and lacustrine POM (Ostrom et al., 1997; Altabet et al., 1999; Teranes & Bernasconi, 2000; Schubert & Calvert, 2001). Here, the 0.1–50 μm POM had δ^{15}N values characteristic of nitrogen fixing bacteria/cyanobacteria, which are habitually near 0‰ in terms of δ^{15}N, reflecting the isotopic signature of atmospheric nitrogen (Gu & Alexander, 1993; Liu et al., 1996; Mahaffey et al., 2003). This ^{15}N-depletion may suggest that nitrogen fixation represents a significant nutrient source for this population, which is consistent with several studies of potential aerobic N-fixation in *Synechococcus* species, including *S. elongatus* (Duerr, 1981; Phlips et al., 1989; Luo & Matsui, 1994; Phlips & Badylack, 1996; Church, 2005). Although nitrogenase activity had been documented in *Synechococcus* sp. cultures in the past (i.e., Duerr, 1981), until recently, unicellular cyanobacteria were not considered important contributors to oceanic nitrogen fixation. However, recent molecular-based techniques identifying nitrogenase gene expression have suggested an active role in nitrogen fixation for these groups (Falcón et al., 2004; Church, 2005). DIN concentrations approximately an order of magnitude lower than in the eastern bay and low DIN:SRP ratios in western Florida Bay, indicative of N-limitation of phytoplankton growth, might provide competitive advantage to nitrogen fixing cyanobacteria in this region.

Other components of the 0.1–50 μm POM that may contribute to the differences in the nitrogen isotopic signal between Florida Bay regions are the heterotrophic bacteria and particulate detritus concentrations observed. Western Florida Bay exhibited much higher abundances in both these particulate categories. While specific populations of the bacterial component were not identified, two clusters displaying high red fluorescence were abundant in the western bay, but were only present at very low abundances at the northeastern open marine sites. It would seem that the very high detritus and bacterial abundance in western Florida Bay are related to the *S. elongatus* bloom; however, since samples amalgamating all components of the 0.1–50 μm POM were analyzed for their isotopic signature, it is impossible to separate the relative importance of each component in terms of δ^{15}N value differences between regions. Yet, it is generally accepted that microbial degradation of autochthonous particulate organic material in marine systems tends to enrich the residual POM by remineralizing isotopically depleted NH_4^+ (Liu et al., 1996); however, the overall POM signal presented here is depleted in the region corresponding to the higher bacterial and detrital abundances.

The northeastern Florida Bay 0.1–50 μm POM fraction exhibited nitrogen isotope signatures more characteristic of nutrient recycling in estuarine waters. However, at 2.0‰ to 3.0‰, these signatures are depleted relative to values reported in many estuaries (and very depleted relative to values measured in highly eutrophic settings; Middleburg & Nieuwenhuize, 2001), which can vary widely, depending on trophic status and anthropogenic nitrogen impacts. Estuarine POM δ^{15}N values range from 4‰ to >20‰, although few, if any, of these estuaries represent the oligotrophic regime characteristic of Florida Bay (Montoya et al., 1991; Rolff & Elmgren, 2000; Middleburg & Nieuwenhuize, 2001). Nitrogen isotopic values from oligotrophic open-ocean settings tend to reflect the isotopic signals of upwelled nitrate (≈5.0‰) altered in some cases by enrichment due to nutrient utilization or denitrification (Miyake & Wada, 1967; Liu & Kaplan, 1989; Schubert & Calvert, 2001). In northeastern Florida Bay, isotopically light POM may reflect the assimilation of nitrogen discharged through the Everglades outflow. Uptake of a relatively depleted nitrogen source delivered by Everglades freshwater outflow is supported by the similarity between the low salinity mangrove ecotone Trout Creek signal and the two northeast bay open water sites. Similar to differences noted at the western stations, the depleted POM signal in northeast Florida Bay is much more depleted than nitrogen isotopic values measured by Glibert et al. (2004) in Little Madeira Bay, November 2004 (8.63‰). This station is located within 5 km of all northeastern loca-

tions sampled in the work presented here. The differences between these studies (November 2002 versus November 2003) reflect the variable nature of the system in terms of water quality, nutrient sources, and storm induced perturbations. The potential that the comparatively depleted estuarine isotopic signal reported here indicates uptake of an isotopically light nitrogen source may be supported by the fact that ammonium concentrations were nearly tenfold higher than nitrate concentrations at the northeastern sites in November 2003. $\delta^{15}N$ signatures of phytoplankton incorporating remineralized ammonium are more depleted (Checkley & Entzeroth, 1985), and ^{15}N-tracer experiments confirmed that NH_4^+ is generally assimilated preferentially over NO_3^- in most phytoplankton (Montoya et al., 1991; Velinsky & Fogel, 1999; Maguer et al., 2000). High NH_4^+ concentrations in northeastern Florida Bay suggest NH_4^+ as the predominant nitrogen source for primary production, causing observed $\delta^{15}N$ signatures in this region. Nitrification of an isotopically light ammonium source might also produce an isotopically depleted nitrate pool, which could be assimilated by phytoplankton (Cifuentes et al., 1989). Because inorganic phosphorus concentrations are persistently limiting in this region, it is unlikely that primary productivity increases generate high enough nitrogen uptake rates to deplete available pools, and thus no isotopic enrichment of photosynthetic autotrophs is evident.

Carbon isotopic signatures did not differ between northeastern and western Florida Bay, suggesting similar carbon acquisition mechanisms and/or relative carbon limitation between plankton communities in different regions of the bay. $\delta^{13}C$ values of $-17.6‰$ to $-14.4‰$ fell within the published range of values from estuarine phytoplankton (Gearing et al., 1984; Velinsky & Fogel, 1999; Rau et al., 2001). Although the more enriched values may indicate some degree of carbon limitation or a shift toward a carbon concentration mechanism (CCM) whereby bicarbonate (HCO_3^-) is used as a carbon source. HCO_3^- becomes the dominant dissolved inorganic carbon (DIC) species available under conditions of increasing pH related to high photosynthetic uptake of dissolved CO_2. A number of studies have demonstrated that many microalgal genera, including *Synechococcus*, are capable of switching to a CCM that facilitates assimilation of HCO_3^- in response to increasing HCO_3^- concentrations and dissolved CO_2 limitation (Badger & Andrews, 1982; Mayo et al., 1986; Fogel et al., 1992; Popp et al., 1998; Matsuda et al., 2001; Rau et al., 2001). It is reasonable then, that the cyanobacterial populations at both northeastern and western Florida Bay stations employ this mechanism under carbon stress. Yet, high photosynthetic rates assumed inherent to cyanobacterial blooms are not reflected by POM $\delta^{13}C$ enrichment due to carbon limitation and decreases in isotopic discrimination. Open connectivity between western Florida Bay and the Gulf of Mexico, and diurnal tidal exchange may serve to maintain DIC availability at a similar level to that in northeastern Florida Bay even though the northeast exhibits much lower productivity levels. An alternative explanation for the relatively enriched $\delta^{13}C$ values of marine Florida Bay stations is the fact that seagrass detritus may represent either a significant component of the particulate detritus, or may represent a carbon source used by the microbial community. Seagrass $\delta^{13}C$ signatures are more enriched than those typical for C_3 plants, varying seasonally between $-13.5‰$ and $-5.2‰$, likely attributable to an active CCM and HCO_3^- uptake (Fourqurean et al., 2005; Anderson & Fourqurean, 2003). Seagrass associated carbon, either in the form of microbially released dissolved CO_2 or as a particulate detritus component of the 0.1–50 μm POM, would serve to generate the more enriched carbon isotopic values evident at marine stations in Florida Bay.

The $\delta^{13}C$ signature of the Trout Creek 0.1–50 μm POM fraction was more isotopically depleted then those at open water marine sites. This depletion may reflect differing carbon sources between the brackish mangrove ecotone and open marine systems, perhaps indicating that the Trout Creek outflow is influenced by dissolved or particulate organic carbon associated with terrestrial sources (Kendall & McDonnell, 1998). This contention is supported not only by the station's proximity to the mangrove ecotone of the southern Everglades, but also by a more than fourfold higher concentration of detritus at the Trout Creek site, compared to the open marine sites in northeastern Florida Bay. The carbon isotopic signa-

tures evident at the station imply that a large component of the detritus is likely of mangrove origin with some autochthonous inputs from the water column community.

Conclusion

The isotopic composition of POM in near-shore and estuarine settings can provide important information about ambient environmental conditions, biotic community structure and biogeochemical cycling within the system. This information may be used to infer changes in primary productivity and water quality parameters in increasingly environmentally sensitive coastal settings. Combining flow cytometry with stable isotopic analyses provides unique insight into ecological controls on carbon and nitrogen isotopic signatures of POM, improving interpretations. In many marine settings, carbon and nitrogen isotopic signatures have been interpreted in terms of surface nutrient utilization and productivity levels, yet here we present data from a cyanobacterial bloom showing nitrogen isotopic depletion, rather than enrichment. This isotopic depletion has implications regarding the importance of biologically fixed nitrogen as a nutrient source during bloom events, particularly in nitrogen-limited marine environments. Further work examining other indicators of N-fixation, such as nitrogenase enzymatic assays or mesocosm experimentation measuring uptake of isotopically labeled N_2 gas, is needed to elucidate the role fixed nitrogen plays in South Florida coastal environments.

The spatial variability evident in Florida Bay planktonic species assemblages, detritus concentrations, and corresponding isotopic compositions suggests that bulk POM isotopic values combining all size fractions may not be easily attributed to external nutrient forcings without closer examination. More extensive size-fractionated POM sampling, distributed geographically and over longer-term time-series, is necessary to more fully understand carbon and nitrogen cycling within the South Florida coastal zone. Further work examining temporal variability in planktonic assemblages and corresponding isotopic compositions, taken in conjunction with water quality variables and the isotopic composition of dissolved inorganic nutrient compounds will greatly enhance understanding of nitrogen and carbon cycling in Florida Bay and adjacent marine systems.

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