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FLORIDA BAY

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SPECIAL ISSUE

FLORIDA BAY

Guest Editors

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FLORIDA BAY: A SUBTROPICAL SYSTEM INCREASINGLY INFLUENCED BY MULTIPLE STRESSORS

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INTRODUCTION TO THE SPECIAL ISSUE

Subtropical coastal ecosystems are increasingly experiencing a range of natural and anthropogenic stressors, ranging from climate change to eutrophication. Algal blooms, loss of benthic habitat, altered biogeochemical fluxes and food web changes are occurring in many subtropical systems throughout the world. As with their temperate counterparts, these stressors and their effects are synergistic and difficult to reverse. Considerably less is known about how these subtropical systems function, however, which makes management of these systems especially challenging and their further study critical.

Florida Bay is one such system which has experienced a history of both natural and anthropogenic stressors. Since the onset of industrialization in the 1880's, the health of the Florida Bay ecosystem has been negatively impacted by changes in land use, changes in water management practices, alteration of freshwater flow patterns within the Everglades and coastal eutrophication (Fourqurean and Robblee 1999). Chronic negative impacts on primary producers have been well documented (e.g., declines in seagrass distribution and abundance (Robblee et al. 1991, Zieman et al. 1988; Fourqurean et al. 1993)), as have increases in pelagic algal blooms (Phlips and Badylak 1996, Butler et al., 1995, Phlips et al. 1995, Glibert et al. 2004), and decreases in coral health (Chiappone and Sullivan 1994, Szmant and Forrester 1994), and economically important nursery shrimp ground (Nance 1994, Costello and Allen 1996), sport fisheries (Tilmant 1989) and predators such as manatees (McIvor et al. 1994) within the Bay. The large, unprecedented picocyanobacterial bloom in the eastern region of the Bay that began in 2005 and continued through 2008 is just the most recent manifestation of these impacts. This bloom served to highlight the potential impacts of a series of new stressors on Bay health: the passages of hurricanes Katrina, Rita and Wilma and the initiation of road construction to widen the causeway connecting the Florida Keys and the mainland. Associated with the hurricanes were altered water management flows and canal releases to Florida Bay and road construction was associated with clear-cutting and mulching of mangroves. Collectively these stressors altered nutrient and organic matter delivery to the Bay (Glibert et al. 2009a).

Herein is presented a collection of papers that touch on a range of issues in Florida Bay. The first paper is a description of the water quality status and trends with a discussion of the factors that con-

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tributed to the 2005-2008 algal bloom (Glibert et al. 2009a). Several additional papers follow on the topic of algal growth and the factors that contribute to cyanobacterial dominance. Richardson (2009) describes the comparative competitive strategies of cyanobacteria and diatoms, while Heil et al. (2009) compare the alkaline phosphatase activity of cyanobacteria and bacteria, and McCarthy et al. (2009) examine water column nitrogen cycling and the role of sediment nitrogen regeneration in sustaining water column primary production. Algal growth rates are presented from a langragrian experiment in a paper by Vargo et al. (2009). A comparison of nutrient availability and phytoplankton responses from Florida Bay and Moreton Bay, Australia, is given by Glibert et al. (2009b).

The net ecosystem production of the *Thallasia testudinum*-dominated seagrass community in Florida Bay is described by Nagel et al. (2009), and lastly, the mangrove community and their stressors characterized by carbon and nitrogen isotopic discrimination is given by Mancera- Pineda et al. (2009).

The recent unprecedented algal blooms in Florida Bay are evidence that this unique subtropical ecosystem responds to natural and anthropogenic stressors in complex ways that impact most or all biotic components of the entire ecosystem. Despite this ecological complexity, some impacts, trends and relationships associated with altered nutrient inputs and cycling resulting from these stressors are becoming evident. These relationships, some of which may typify lagoonal estuaries in general (Glibert et al. 2010), suggest that management decisions regarding the nutrient sources to these estuaries are critical to their continued ecological functioning.

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FLORIDA BAY: WATER QUALITY STATUS AND TRENDS, HISTORIC AND EMERGING ALGAL BLOOM PROBLEMS

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ABSTRACT

Florida Bay is a unique subtropical estuary that while historically oligotrophic, has been subjected to both natural and anthropogenic stressors, including hurricanes, coastal eutrophication and other impacts. These stressors have resulted in degradation of water quality in the past several decades, most evidenced by reoccurring blooms of the picocyanobacterium *Synechococcus* spp. Major nutrient inputs consist of freshwater flows to the eastern region from runoff and regulated canal releases, inputs from the Everglades to the central region via Taylor Slough, exchanges with the Gulf of Mexico, which include intermittent Shark River inputs to the western region, stormwater and wastewater from the Florida Keys, and atmospheric deposition. These nutrient inputs have resulted in a transition from strong phosphorus (P) limitation of phytoplankton in the eastern bay to nitrogen (N) limitation in the western bay. Large blooms of *Synechococcus* were most pronounced in the central bay region, in the area of transition between P and N limitation, in the mid-1990s. Although non-toxic, these blooms, which have continued intermittently through the early 2000s, resulted in significant seagrass and benthic organism mortalities. A new suite of stressors in 2005, including the passages of Hurricanes Katrina, Rita, and Wilma, additional canal releases, and the initiation of road construction to widen the main roadway leading to the Keys, were correlated with a large *Synechococcus* bloom in the previously clear, strongly P- limited, northeastern region of the bay. Sustained for 3 years, this bloom was accompanied by a shift from P limitation to N limitation during its course. Nutrient bioassay experiments suggest that this bloom persisted due to the ability of *Synechococcus* to access organic N and P sources, microbial and geochemical cycling of organic and inorganic nutrients in the water column and between the water column and sediments (both suspended particles and benthos), and decreased grazing by benthic fauna due to their die-off.

Keywords: Synechococcus blooms, hurricane impacts, nitrogen, phosphorus, organic nutrients

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INTRODUCTION

Florida Bay is a shallow, sub-tropical lagoon estuary located at the southern end of the Florida peninsula between the Everglades and the Florida Keys (Figure 1). Although historically oligotrophic, significant natural and human perturbations in the past several decades in both its watershed and in Florida Bay itself have led to episodic as well as, in some cases, prolonged picocyanobacterial blooms that now threaten the ecological health of this system (Phlips and Babylak 1996, Hitchcock et al. 1998, Phlips et al. 1999, Glibert et al. 2004). Climate change and anticipated sea level rise will further affect this system in ways that are not yet well understood. Long-term management plans for the southern Florida area call for restoration of the natural flow conditions within the Everglades, which will have additional effects on the system. This paper aims to provide a short synopsis of the long term trends in chemical and biological characteristics in Florida Bay, with a focus on nutrients and water quality, and to describe initial observations of an unusual algal bloom that occurred in the eastern bay from 2005-2008.

FLORIDA BAY

Florida Bay has an open boundary with the Gulf of Mexico on its western border, but exchange along this boundary is limited by broad carbonate mud banks. Exchange with the Atlantic Ocean on the eastern and southern boundaries is limited to narrow tidal passes in the Florida Keys. Riverine input is minimal, and the major freshwater input is via slowly flowing surface water from the Everglades watershed after traversing freshwater wetlands and a mangrove forest ecotone, then discharging to northern Florida Bay. Flushing within the bay is minimal and residence times are long (Nuttle et al. 2003), thus the hydrology of the system dominates the ecological and geochemical dynamics. During the rainy season, Florida Bay receives considerable freshwater flow from the Everglades, and the system functions more like an estuary. During the dry season, the bay becomes a marine lagoon.

Florida Bay's unique geomorphology includes a vast interconnected network of banks and shoals that create barriers to hydrologic circulation. The banks form about 40 distinct quasi-isolated basins

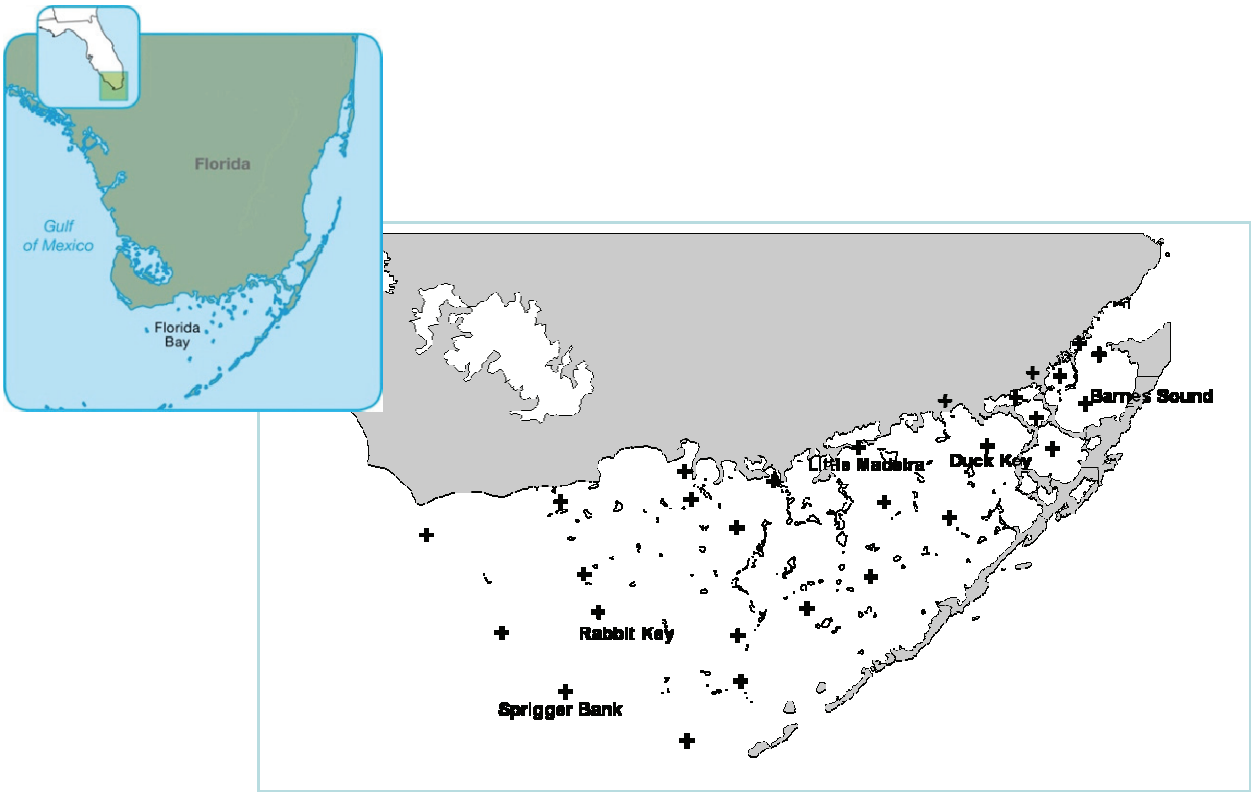

Figure 1. Map of Florida Bay showing its general location and highlighted stations.

(Nuttle et al. 2003) where water transport is often via cross-bank flow or through narrow inter-basin channels (Lee et al. 2006). Despite its interposition between the Atlantic Ocean and the Gulf of Mexico, wind-driven exchange dominates almost all of the tide and hydrologic circulation in eastern and central Florida Bay. The net effect of restricted circulation in the estuary is that high evaporation and long residence times can concentrate salts, particularly in the central bay, which periodically (often annually) experiences hypersaline conditions, with salinities as high as 70 (Boyer et al. 1997, Brewster-Wingard et al. 1999, Smith and Pitts 2001).

Major nutrient sources to Florida Bay derive from Everglades surface discharge, atmospheric deposition, groundwater inputs, the Florida Keys, and exchange with marine end members. Freshwater flows from the Everglades into the eastern bay region are comprised of runoff and regulated releases from the C-111 Canal. Canal releases are impacted by the extensively developed southeastern Florida region and incumbent requirements for flood protection. Central Florida Bay receives freshwater flow that traverses the Everglades wetlands and mangroves via Taylor Slough. In western Florida Bay, exchange with the Gulf of Mexico water impacts phosphorus (P) availability as marine concentrations are higher than concentrations in Everglades discharges. Nitrogen (N) availability is also impacted in the western bay, as outflows from Shark River Slough seasonally affect this western edge. In addition, the increasing population and development of the Florida Keys has resulted in increased nutrient inputs from septic and sewage that locally impacts the waters, including the canals, along the southern boundary of the bay (US EPA 1999). Nutrient and freshwater flows are thus complex, resulting from the regulated channelized flow to the east, sheet flow and runoff from the Everglades, exchange with the Gulf of Mexico and Atlantic Ocean, and rainwater and groundwater inputs (Boyer et al. 2007). Weather patterns also influence nutrient loading to the bay; nutrient inputs typically increase with the summer wet season and decrease significantly during the winter dry season (Rudnick et al. 1999, Madden 2009).

The patterns of freshwater input and circulation establish four major water quality zones within the bay, each exhibiting coherent patterns of salinity and water quality (Boyer et al. 1997). Discharge from the Everglades and ocean exchange through tidal passes most influences the eastern bay. The central bay is hydrologically isolated, receives low freshwater input, and has a high water residence time. The western bay is characterized by marine inflows from the Gulf of Mexico, and is affected by freshwater discharge from Shark River Slough onto the western Florida Shelf which is periodically directed into the bay by prevailing currents. A fourth water quality sector, the northern bay, includes the mangrove transition zone at the Everglades-Florida Bay interface which is typified by seasonal freshwater inundation, extensive mangrove wetlands and interspersed with shallow ponds and small channels (Boyer 2007). The combination of different and spatially distinct nutrient sources and the dominance of carbonate sediment have historically led to a system that is P limited in the eastern region, yet tending toward N limitation in the west (Tomas 1999; Brand 2002, Glibert et al. 2004, Boyer 2007).

Throughout the 20th century, the hydrology, water budget and flow regime of Florida Bay have been significantly altered by the filling of Atlantic tidal passes between several Keys, and by engineered channelization of water flow away from historic flow paths across the bay's northern boundary (Madden 2009). Population centers in Miami and Homestead/Florida City to the north and the Keys to the south have changed patterns of land use, water consumption and nutrient regimes in the cross-boundary flows to Florida Bay.

Due to the shallow nature of the bay and historic high water clarity, submerged aquatic vegetation (SAV, seagrass) has dominated primary production, playing a key role in sediment stabilization, benthic nutrient exchange and habitat provision. Their sediment-binding capacity serves to reduce resuspension and bottom scouring, thereby promoting water clarity and enhanced benthic primary production (Zieman 1982). SAV covers an estimated 5,500 km^2 of the greater Florida Bay and Keys area in meadows dominated by turtle grass (*Thalassia testudinum*), often mixed with shoal grass (*Halodule wrightii*). Manatee grass (*Syringodium filiforme)* is found in generally deeper waters nearer the Gulf of Mexico and widgeon grass *(Ruppia maritima)* occurs in the fringes of the mangrove transition zone near fresher water

(Zieman 1982). SAV is the keystone community of the Florida Bay ecosystem, playing roles in many important physico-chemical (Yarbro and Carlson 2008), autotrophic (Fourqurean et al. 2002) and higher trophic (Ley and McIvor 2002, Lorenz et al. 2002) functions of the bay's ecology.

LONG-TERM WATER QUALITY STATUS AND TRENDS

Prior to the 1990s, records of water quality were largely anecdotal, or reported on a sporadic basis. Water quality including chlorophyll *a* (Chl *a*), inorganic and total nutrients, turbidity and hydrographic parameters, has been monitored monthly at up to 28 stations in Florida Bay beginning in 1989 (Boyer and Briceño 2008, Table 1). Everglades discharge and nutrient loading rates to Florida Bay have been measured at several creek sites since 1995 (Hittle et al. 2001, Woods and Zucker 2008).

Inputs to the eastern bay from the Everglades Panhandle are characteristically very low in P, as the calcium carbonate substrate of the Everglades marl and Florida Bay sediments effectively scavenges P from the water column, binding and sequestering it in a variety of forms (Nielson et al. 2006). Freshwater inputs tend also to be relatively higher in N compounds, particularly dissolved organic nitrogen (DON). The P transported to the bay is rapidly removed from the water column by pelagic and benthic biota and retained within the bay's carbonate sediments. Most of the P in the eastern bay is found in the sediments either in the solid phase as loosely bound oxy-hydroxides or apatite, while P available to benthic autotrophs is limited to the aqueous phase in interstitial porewaters in the sediments (Koch et al. 2001). Water column productivity is thus generally very low in the east, while benthic plants have access to this limiting nutrient in the sediments (Fourqurean et al. 2002, Nielson et al. 2006). In contrast to P, much of the N transported to the bay remains in the water column, with DON subject to decomposition.

The central bay is a region with a long water residence time, with low tidal influence and little direct freshwater inflow. This region is influenced by its connectivity to both eastern and western bay waters, with the former being an N source and the latter being a P source (Brand 2002). This convergence of nutrients and long water residence time often leads to the highest Chl *a* concentrations and water column productivity in the system (Madden 2009). SAV and microphytobenthos are also much more productive in this region than in the eastern bay (Fourqurean and Robblee 1999). N, mostly in the form of NH_4^+, is generally readily available to benthic plants in sediment pools (Jackson and Burd 2002).

The central bay transitions from P limitation (eastward) to N limitation (westward) and autotrophs in the central bay are generally more nutrient-sufficient than in adjacent areas (Glibert et al. 2004). It is of note, however, that while phytoplankton biomass is generally P limited in the east and N limited in the west, heterotrophic bacteria appear to be most responsive to (i.e., limited by) P in the west and N in the east (Glibert et al. 2004). Differential P metabolism of the heterotophic bacteria compared to the phytoplankton may ultimately be very important for the availability of different nutrients for the autotrophs (Van Mooey et al. 2009).

In the western area of the bay, which is more distant from major Everglades inputs, available N is in somewhat lower supply, while P forms are in greatest supply from flux across the Gulf boundary. Some phytoplankton blooms occur in the west, especially when favorable currents bring Everglades discharge and associated N into the bay from the Florida Shelf, and extant blooms from the southwest Florida Shelf waters may be physically transported to this region (Steidinger et al 1998; Neely et al 2004; Heil et al 2007). The P load of the Gulf waters is enhanced by the discharge of terrestrial P into western Florida coastal waters.

Nutrients from the Everglades flow through the mangrove transition zone at the bay-wetland interface, and inputs are related to seasonal patterns of freshwater discharge (Sutula et al. 2003, Davis et al. 2004). Nutrient loading and nutrient concentration increase with increasing water discharge during the wet season, although not linearly. Loading of P from the Everglades to Florida Bay occurs during the wet season mostly as dissolved organic P (DOP), in very low concentrations. During the dry season, P is typically imported into the mangrove ecotone from Florida Bay. The output of N to the bay from the Everglades is mostly as DON, in high concentrations, resulting in a significant N

Table 1. Parameters sampled in the Florida Bay monitoring program and long-term medians for the entire bay (all) and for the central, eastern and western regions of the Bay.

Variable	Zone	Median	Min	Max	n
Chlorophyll a ($\mu g\ L^{-1}$)	All	0.84	<0.03	35.61	3612
	Central	1.79	0.11	35.61	542
	East	0.55	<0.03	11.35	2284
	West	1.55	0.14	22.08	786
DO - surface ($mg\ L^{-1}$)	All	6.6	0.4	12.3	3633
	Central	6.4	2.8	12.3	545
	East	6.7	0.4	11.7	2289
	West	6.3	3.0	11.5	799
DO - bottom ($mg\ L^{-1}$)	All	6.5	1.4	13.4	3414
	Central	6.3	1.5	12.2	514
	East	6.7	1.4	13.4	2174
	West	6.2	3.0	11.1	726
Salinity-surface	All	31.9	0.2	63.0	3691
	Central	34.0	8.7	63.0	554
	East	28.9	0.2	54.3	2324
	West	35.0	16.5	52.0	813
Salinity-bottom	All	31.3	0.2	63.0	3376
	Central	33.2	11.9	63.0	510
	East	28.4	0.2	54.3	2140
	West	34.7	16.6	51.0	726
NO_3^- (μM-N)	All	0.36	<0.03	11.0	3580
	Central	0.21	<0.03	5.71	537
	East	0.64	<0.03	11.0	2268
	West	0.14	<0.03	7.21	775
NH_4^+ (μM-N)	All	2.28	<0.03	120	3592
	Central	3.64	<0.03	120	535
	East	2.78	<0.03	82.1	2277
	West	0.78	<0.03	24.4	780
PO_4^{3-} (μM-P)	All	0.03	<0.03	0.8	3570
	Central	0.03	<0.03	0.8	537
	East	0.03	<0.03	0.5	2260
	West	0.03	<0.03	0.3	773

loading and very high molar TN:TP ratios, near 200, in the export.

Compared to other estuaries, Florida Bay has low Chl *a* in general (i.e., non-bloom periods) and, in particularly in the eastern bay (Phlips et al. 1999, Glibert et al. 2004). Median Chl *a* in the eastern bay was 0.85 $\mu g\ L^{-1}$ from 1989 to 2004 (Boyer et al. 1999). In the central bay, where N and P are more balanced due to the convergence of Gulf and Everglades nutrient inputs, Chl *a* concentrations are generally highest, with a median of 2.34 $\mu g\ L^{-1}$, regularly exceeding 10 $\mu g\ L^{-1}$ and occasionally exceeding 20 $\mu g\ L^{-1}$ during blooms (Phlips et al. 1999). Median concentrations of Chl *a* in the western bay are more moderate, 1.93 $\mu g\ L^{-1}$. Phytoplankton in this area respond to additions of N and Si, indicating that both nutrients limit production (Tomas et al. 1999).

Phytoplankton community composition within Florida Bay varies within the major regions of the bay. In the north- central region, diatom blooms (eg. *Thalassiosira* spp.) often occur in basins adjacent to the mangrove fringe. Phytoplankton abundance

and composition in the western bay exhibit a seasonal variation, peaking in late summer to winter and typically dominated by centric (*Rhizosolenia* spp.) and pennate diatoms (*Cocconeis*, *Navicula*, and *Surirella* sp.; Phlips and Badylak 1996). High-biomass blooms of both the toxic dinoflagellate *Karenia brevis* and the N_2 -fixing cyanobacteria *Trichodesmium* are occasionally transported into western Florida Bay from the Gulf of Mexico. In contrast, phytoplankton biomass in the eastern and central bays tend to be highest in late summer and fall (Phlips et al. 1999), dominated by picocyanobacteria (*Synechococcus* spp.). Prior to 2005, the northeastern bay phytoplankton community was a diverse mixture of diatoms, cyanobacteria, microflagellates and dinoflagellates, including ciguatera- associated species and *Pyrodinium bahamense* (Hitchcock et al. 2007).

After decades of highly transparent waters, areas of Florida Bay became increasingly turbid in the early 1990s. Turbidity, measured as NTU, increased between 1989 and 1992 by factors of 2, 4, and 20 in the eastern, western and central bays, respectively (Boyer et al. 1999). From a comparative study, Stumpf et al. (1999) reported the water column to be relatively clear in 1987, with a bay-wide mean downwelling light attenuation (Kd) of 0.51 m^{-1} while in 1995 the mean attenuation was 2.82 m^{-1}. Much of this increased turbidity was due to increased resuspension of carbonate bottom sediments (Boyer et al. 1999). The abrupt changes are likely associated with a significant loss of SAV in the late 1980s, leading to reduced sediment binding, increased sediment resuspension and increased nutrient availability and release from the benthos, fueling phytoplankton blooms (Hunt and Nuttle 2007). Turbidity has somewhat declined through the late 1990s and into the 2000s, although it seems to have stabilized at a higher level than in previous decades, likely a consequence of continuing picocyanobacterial blooms and more resuspended sediments.

The decline in SAV in fall 1987 destroyed 4,000 ha of *Thalassia*, and thinned an additional 23,000 ha (Robblee et al. 1991), resulting in the loss of 30% of the community (Hall et al. 1999, Durako et al 2002). Maximum loss of *Thalassia* occurred in the highest density beds, especially in the north central bay, and loss of this keystone species caused a cascade of ecological effects. The nursery function of Florida Bay was affected, exhibited by landings of spiny lobster (Butler et al. 1995) and pink shrimp at Tortugas Banks that plunged in 1988 to their lowest levels in decades (Robblee et al. 1991). Game fish landings also declined as SAV community composition shifted (Matheson et al. 1999).

Beginning in 1991, phytoplankton blooms began to appear in the central and western bay regions (Boyer et al. 1999, Stumpf et al. 1999). A 100% mortality of sponges near the Florida Keys in these areas ensued and several genera of sponges permanently disappeared from the bay (Fourqurean and Robblee 1999). These blooms continued through the 1990s and into the mid-2000s (Richardson and Zimba 2002, Glibert et al. 2004), with a recent expansion of bloom activity to the eastern and southern bays. Low-level SAV die-offs continued through 2008 as *Thalassia* ebbed and increased in the central bay region.

THE 2005-2008 ALGAL BLOOM

In September 2005 an unprecedented phytoplankton bloom appeared in the historically clear waters of northeastern Florida Bay (Rudnick et al. 2007). This eastern bay phytoplankton bloom was first observed as a regional phenomenon, extending from Duck Key in eastern Florida Bay to Card Sound in southern Biscayne Bay (Rudnick et al. 2007). The bloom persisted for 3 years, 2005-2008, although it varied in intensity and in specific location. Peak Chl *a* concentrations, > 20 μg L^{-1}, were observed in Blackwater Sound, Barnes Sound, and Lake Surprise, and extensive underway mapping suggests that the bloom was spatially centered around U.S Highway 1 in this region during its first year. As is typical of blooms that historically occur in central Florida Bay, this bloom was dominated by *Synechococcus* spp. A metagenomic profile of the 2005 bloom showed that the plankton community was dominated by 3 organisms from the same clade of *Synechococcus*: *Synechococcus* sp. WH8101 (Woods Hole), *Synechococcus* sp. CB 0201 (Chesapeake Bay) and *Synechococcus* sp. RS 9708 (Gulf of Aqaba), and that the plankton community was distinctly different from that of the benthic community (Boyer , unpub. data).

The initiation of this bloom was roughly coincident with two major system perturbations. First,

in 2005, Florida Bay was impacted by the passage of a series of intense hurricanes, Katrina (August), Rita (September) and Wilma (October), which resulted in significant increases in flows of freshwater and nutrients to the eastern part of the bay. Also, in April of 2005, construction began on the expansion of an 18-mile causeway connecting the mainland and the Florida Keys, resulting in clear-cutting and mulching of acres of adjacent mangroves and extensive mangrove soil excavation and tilling. These factors, individually or synergistically, appear to have contributed to nutrient and/or organic matter availability and potentially initiated this bloom.

Katrina was notable in south Florida for its associated high rainfall and runoff, which resulted in a rapid decrease in salinity in south Florida estuaries, particularly close to the coast (Rudnick et al. 2007). Opening of structure S-197 in the lower C-111 canal discharged significant amounts of freshwater and associated nutrients into Manatee Bay and Barnes Sound in southern Biscayne Bay. This resulted in a major input of total P to Manatee Bay, followed by locally elevated Chl a concentrations. Hurricane Rita also resulted in high rainfall in late September 2005, although much of this was over eastern Florida Bay and southern Biscayne Bay and discharges from the C-111 were far less than during Hurricane Katrina. In early October, monthly water quality sampling documented that highly elevated TP concentrations existed through the region (from Duck Key to Barnes Sound; Figure 2). Hurricane Wilma in late October was notable for its strong winds and associated storm surge which exceeded that of any other hurricane of the very active hurricane season of 2005 (Rudnick et al. 2007).

A relationship between total P inputs from Katrina-related canal discharges and the subsequent *Synechococcus* bloom appears likely in Manatee Bay. One day after Hurricane Katrina, and the resultant discharge of C-111 water through S-197, total P concentrations in Manatee Bay increased to 84 ppb (= 2.6 μM-P; Rudnick et al. 2007). Within 2 weeks, Chl *a* increased to >6 μg L^{-1} in both Manatee Bay and adjacent Barnes

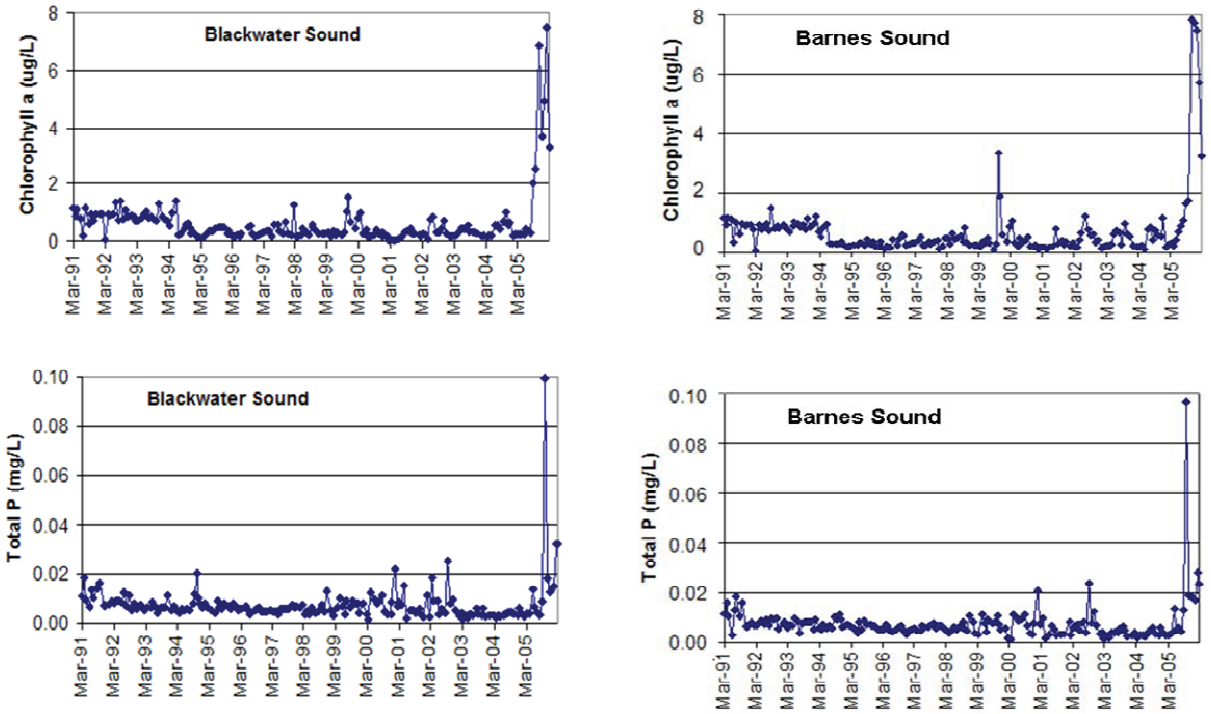

Figure 2. Long-term records (March 1991 to March 2006) of Chlorophyll *a* (μg L^{-1}) (top panels) and total phosphorus (bottom panels) for Blackwater Sound in eastern Florida Bay and Barnes Sound in southern Biscayne Bay. Data are from the coastal water quality monitoring program maintained by Florida International University and the South Florida Water Management District. The peak in biomass in 2006-07 can be seen, followed by its progressive decay to 2008.

Sound, far above previously recorded values (Boyer and Briceño 2008). However, high TP concentrations were found regionally in early October 2005. Concentrations as high as 60 ppb (=1.9 μM-P) were observed at Duck Key (Rudnick et al. 2007, data not shown). Highest concentrations during this period were found to spatially bracket US Highway 1 and were higher near Key Largo than the Florida mainland.

Throughout the first years of this bloom, both the central region of the bloom and elevated total P levels occurred in proximity to the highway construction (Rudnick et al. 2007; Figure 3). Based on these spatial patterns and the chronology of bloom events, it appears that the proximate cause of the bloom was elevated total P. The mechanism by which highway construction may have contributed to P release is unclear, but may have been related to the extensive cutting and mulching of mangrove trees along the highway. The debris was not fully contained or isolated from the water. This organic matter may have competitively interacted with carbonates for binding sites for the P, in turn mobilizing P for biological uptake (Glibert et al. 2007). The sufficiency of P for ambient phytoplankton assemblages was apparent in bioassay experiments. During the first year of the 2005-2008 bloom, algal biomass increased in response to N, either inorganic or organic, but not to P (Figure 4).

Once initiated, the eastern phytoplankton bloom appeared to be sustained via a number of interactive mechanisms common to prior blooms in Florida Bay. Extensive mortality of *Thalassia testudinum* and benthic macroalgae, particularly in deeper portions of Blackwater and Barnes Sounds (1.5-2 m), occurred following the cyanobacterial bloom initiation (Rudnick et al. 2008). These die-backs and biogeochemical interactions likely provided additional nutrient sources to sustain the blooms, yielding a sustained period of low light for SAV and further mortality. Moreover, a massive sponge mortality event also occurred in Blackwater and Barnes Sounds and in Manatee Bay between July and October 2005 (Rudnick et al. 2008), decreasing grazing pressure by these benthic organisms on the phytoplankton. The sponge mortality event, however, may have been initiated in advance of Hurricane Katrina. Finally, the long residence times in the affected basins is likely to have promoted retention of the nutrient material and created a tightly recycled system of biologically and abiotically sequestered nutrients that sustained the

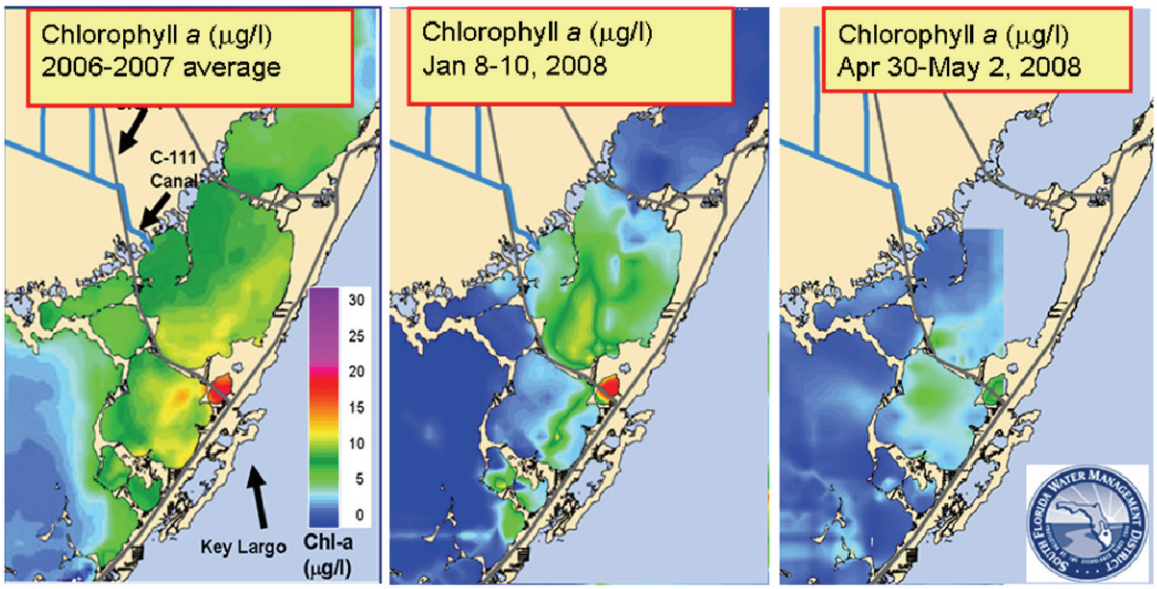

Figure 3. Chlorophyll a (Chl *a*, μg L^{-1}) concentrations in eastern Florida Bay and southern Biscayne Bay, measured as in vivo fluorescence approximately bi-monthly by the Dataflow surface mapping system (Madden and Day 1992). Left panel is an average of six surveys. Other panels show specific surveys during the decline of the bloom.

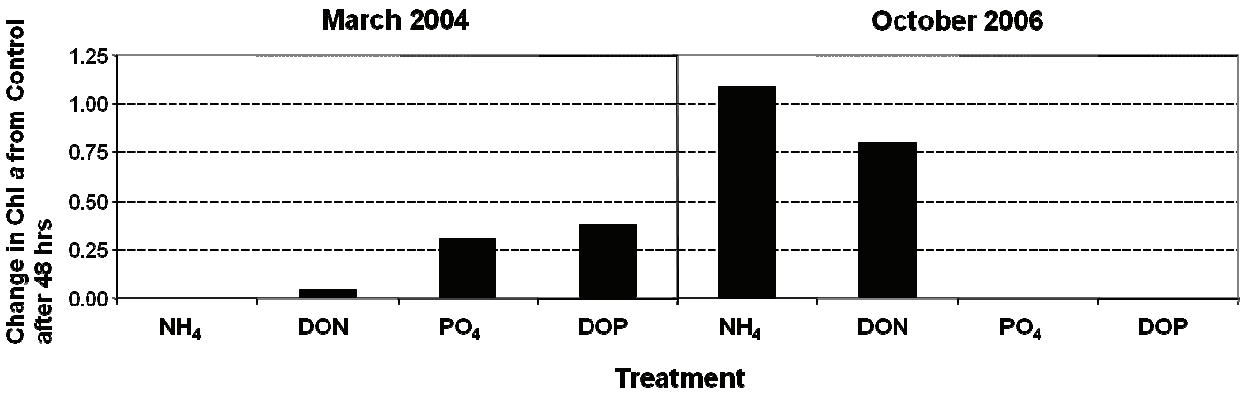

Figure 4. Biomass response expressed as changes in Chlorophyll *a* from control (no addition) after 48 hours in nutrient addition bioassays with water collected from Sunset Cove Station incubated under natural temperature and 50% of ambient irradiance in 2004, before the eastern bay bloom, and 2006, during the eastern bay bloom. Additions were composed of 10 μM NH_4^+, 10 μM –N L^{-1} DON (prepared as 5 μM–N L^{-1} urea, 2.5 μM–N arginine and 2.5 μM–N glutamine), 2.5 μM PO_4^{3-} or 2.5 μM DOP (as glycerophosphate).

bloom. Thus, there were multiple biological, physical and human alterations to the eastern region of Florida Bay in late summer of 2005, all of which appeared to play a role in initiating or sustaining the phytoplankton bloom that ultimately lasted about 3 years.

Interestingly, as the algal bloom became prolonged, its composition changed from being nearly exclusively composed of *Synechococcus* to one of a mixed community with proportionately more flagellates, some of which were heterotrophic. The common microbial grazers of *Synechococcus* are heterotrophic protozoa and ciliates (e.g., Campbell and Carpenter 1986, Caron et al. 1991, Strom 1991), but some dinoflagellates may also consume *Synechococcus* (Legrand et al. 1998, Jeong et al. 2005, Glibert et al. 2009). Grazing experiments conducted in the eastern bay in 2006, in which size fractionated Chl *a* responses were determined following exposure to bloom water containing a vari-

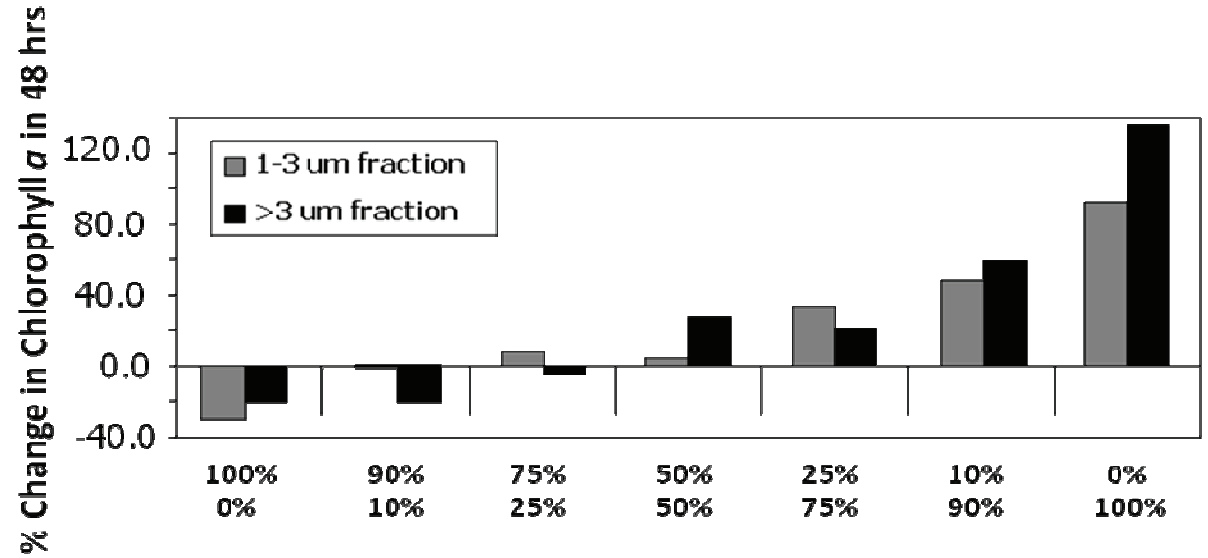

Figure 5. Representative result for one modified dilution experiment in which sample water from Blackwater Sound (eastern Florida Bay) was mixed with water from Barnes Sound (southern Biscayne Bay) in the proportions indicated. The top line on the x axis indicates the percentage of water from Blackwater Sound, dominated by flagellates, and the second line indicates the percentage of water from Barnes Sound, dominated by *Synechococcus* spp. The change in size-fractionated chlorophyll *a* was determined after 48 hrs of incubation. Reprinted from Glibert et al. (2010) with permission of the publisher.

able proportion of flagellates showed that, indeed, these microbial grazers were controlling the picocyanobacterial community (Figure 5; Glibert et al. 2010). Thus, the evolution of the bloom, the initiation of which was correlated with an injection of P and organic matter from hurricanes and construction activities, resulted in ecosystem impacts, some of which led to benthic regenerative processes which continued to fuel the bloom. This scenario is supported by simulation modeling of the episodic loading of P into a basin region with the same mean flushing rate calculated by transport model for Barnes Sound by the FATHOM model (Nuttle et al. 2003, Madden and McDonald, unpub. data). The mean Chl *a* concentrations attained (20 μg L^{-1}) and duration of the bloom (3 years) in the model result approximate the empirical data from Florida Bay (Madden and McDonald unpub. data).

As the bloom aged, and as additional microbial species became established, micrograzing became an important controlling factor and the bloom eventually either was reduced to a new stable stage of reduced cell concentrations, declined completely in some sub-basins, or may have been flushed from the system by water turnover. Seemingly unrelated, in summer of 2007, another geographically distinct phytoplankton bloom, also dominated by *Synechococcus* spp., was observed in southern Florida Bay, centered between Twin Key Basin and Islamorada (Rudnick et al. 2008). From Chl *a* distributions in satellite observations it seems likely that this bloom was initiated *de novo* and is not the result of transport and seeding from the eastern bay bloom. This southern bay bloom also was accompanied by a large scale sponge mortality event (Butler and Behringer 2008).

SUMMARY

This synopsis has highlighted the temporal and spatial trends in water quality and biota in Florida Bay and has demonstrated that plankton blooms are recurring phenomena, although the most recent events appear to be unusual in strength, duration and regional location within Florida Bay. While external nutrient loading remains an important concern, particularly with regard to the downstream consequences of Everglades restoration or anticipated changes in flow due to regional warming and sea level rise, the low flushing and long residence times of eastern and central Florida Bay amplify the importance of internal nutrient cycling and biotic interactions. Benthic nutrient fluxes from surface sediment and potentially from groundwater exchange greatly exceed watershed inputs. SAV community dynamics, rapid cycling of organic and inorganic nutrients in the benthos, in the water column and between the benthos and water column, and the sensitivity of the biota to disturbance appear to be the key determinants in the nutrient cycles of the bay. The bay's sensitive trophic status may be poised between alternative stable states of benthic and pelagic dominance; when algal blooms occur a new set-point becomes established around a pelagic-based system at the expense of critical benthic habitats. Although the unusual and prolonged bloom event did eventually subside, indicating a degree of ecosystem resilience, Florida Bay appears to be an ecosystem that is particularly sensitive to multiple stressors that result in large, long-lasting changes in the state of the ecosystem.

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PHYSIOLOGICAL CHARACTERISTICS AND COMPETITIVE STRATEGIES OF BLOOM-FORMING CYANOBACTERIA AND DIATOMS OF FLORIDA BAY

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ABSTRACT

The atypical appearance of expansive, persistent, and recurring phytoplankton blooms frequently dominated by cyanobacteria (*Synechococcus* spp.) in Florida Bay, FL, beginning in the early 1990s, prompted public concern and interest in the cause of these blooms. The physiological attributes of the dominant cyanobacteria species and selected bloom-forming and non-bloom diatom species from Florida Bay were examined in the laboratory to determine how interspecific differences may influence a species' dominance in a bloom in the bay. Attributes included light and salinity growth responses, phosphate growth kinetics, and nitrogen (N)- and phosphorus (P)-competitiveness. The broad salinity tolerances and low-salinity optima of the cyanobacteria *Synechococcus* cf. *elongatus* and *Synechocystis* sp. may contribute to their dominance in hypersaline and oligohaline regions of the bay. In moderate salinities, the greater light-use growth efficiencies and maximum growth rates of the diatoms *Chaetoceros* cf. *salsugineus* and *Thalassiosira* cf. *oceanica* favor their dominance during periods of light saturation or light limitation. Phosphate half-saturation constants for growth, minimum equilibrium phosphate requirements, and P-limited competitive outcomes established *S.* cf. *elongatus* as the superior phosphate-competitor between salinities of 15 and 50. Although *S.* cf. *elongatus* dominance was decidedly enhanced under P-limitation, N-limitation promoted codominance of cyanobacteria and diatoms. Frequent bloom dominance by *S.* cf. *elongatus*, the superior phosphate-competitor – coupled with the conspicuous scarcity of *Skeletonema costatum*, the inferior phosphate-competitor, in a bay that experiences chronically low levels of available phosphorus – supports the hypothesis that P-limitation may play an influential role in species composition and dominance in Florida Bay blooms.

Keywords: Florida Bay, cyanobacteria, *Synechococcus*, nutrient competition

INTRODUCTION

Florida Bay is a large, shallow-water lagoon located at the southern tip of Florida, between the Florida Keys and the Everglades. The climate is subtropical, with a mean annual water temperature of 26 °C (Boyer et al. 1999). This approximately 2200-square kilometer area has historically had a clear water column supporting low phytoplankton biomass and large seagrass meadows with fringing mangrove communities (Zieman et al. 1989). Florida Bay waters are influenced by exchange with Gulf of Mexico waters to the west, Atlantic Ocean waters to the southeast, and freshwater inputs along its northern boundary.

The bay has been divided into three broad geographic and ecological zones – the eastern, central, and western zones – defined by water-quality parameters (Boyer et al. 1999) and phytoplankton nutrient bioassay results (Tomas et al. 1999). Phytoplankton distribution patterns also suggest a subdivision of the central region into a north-

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central and a south-central region (Steidinger and Phlips 1996). Each zone is effectively divided by mudbanks into a network of sub-basins, restricting water exchange between the basins and allowing some sub-basins to develop substantial differences in water quality, water-residence time, and phytoplankton biomass and composition.

High rates of evaporation, coupled with extended periods of drought and long water-residence times have periodically created large hypersaline areas, particularly within the central region of the bay (Robblee et al. 2001). Seagrass mass-mortalities, beginning in 1987 (Robblee et al. 1991), resulted in areas of increased sediment-derived turbidity, particularly in the central and western regions of the bay. The spatial patterns of water-column concentrations of total phosphorus (P) and total nitrogen (N) within the bay are approximately the inverse of each other. P is lowest in the east and increases from east to northwest across the bay, whereas N is generally lowest in the western region of the bay and increases eastward, being highest along the northeastern boundary of the bay. The spatial gradients of P and N have been hypothesized to reflect their west Florida shelf and freshwater runoff source waters, respectively (Fourqurean et al. 1993, Brand 2002). The ability of carbonate sediments to bind inorganic phosphate (McGlathery et al. 1994) contributes to the chronic low levels of phosphate found in Florida Bay, particularly in the eastern region (Fourqurean et al. 1992).

The phytoplankton community is both taxonomically diverse and species rich, estimated collectively at more than 250 species. Phytoplankton blooms consist largely of a mix of centric and pennate diatoms, cyanobacteria, dinoflagellates, and flagellates (Steidinger et al. 2001). For the most part, blooms are both spatially and temporally distinct and characteristically dominated by different taxa. The predominant phytoplankton group in the western zone blooms is diatoms, although cyanobacteria have at times dominated samples, particularly where the western and central zones converge. The western-zone blooms occur in fall and winter, and are typically dominated by openwater shelf species (e.g., in Rhizosoleniaceae). Smaller blooms in the summer are commonly composed of *Chaetoceros* (Steidinger et al. 1995). The central-zone blooms are composed of diatoms (e.g., *Cyclotella* and *Chaetoceros*) and cyanobacteria (e.g., *Synechococcus* and *Synechocystis*) and are commonly dominated, numerically and in biovolume, by *Synechococcus* (Steidinger et al. 1998, Phlips et al. 1999). Central-zone blooms tend to originate in the north-central region in the summer, spread principally into the south-central region as the bloom grows during the fall, and dissipate during the winter to spring period by shrinking to a small bloom located in the north-central zone (Steidinger et al. 1998, Phlips et al. 1999). Large, recurring seasonal blooms of cyanobacteria and/or diatoms have been documented since approximately 1991 in the western and central zones. In contrast, the eastern zone has been infrequently influenced by phytoplankton blooms (Steidinger and Phlips 1996), at least until the large cyanobacterial bloom beginning in 2005 (Rudnick 2007).

Phytoplankton relative abundance may be explained by interspecific differences in each species' population growth and loss factors, with dominance by a single or several species occurring when the dominant species maximize their net growth to a greater degree than any of the other co-occurring phytoplankton species. Consequently, the dominance of cyanobacteria in Florida Bay may result from ecophysiological characteristics that give the cyanobacteria a competitive advantage over other competing eukaryotic phytoplankton. In general, characteristics considered to contribute to cyanobacteria's frequent dominance of the phytoplankton community include N_2-fixing ability, high nutrient affinities, enhanced uptake and storage capabilities, ability to assimilate diverse organic compounds, low grazing losses, resistance to viral infection, and an ability to flourish under physical and chemical environmental conditions that would be suboptimal for other algae (e.g., high pH, low light, low dissolved oxygen, high sulfides, and extremes in salinity) (Oliver and Ganf 2000, Paerl 2000, Stockner 1988, Lavrentyev et al. 1998). In contrast, the higher specific growth rates of most diatoms (Furnas 1990), is one of the principal reasons given for their frequent dominance of the phytoplankton community.

The recurring growth and development of blooms typically dominated by cyanobacteria, particularly in the north-central region of Florida Bay – an area with a history of hypersaline conditions (Robblee et al. 2001), high turbidity and reduced

irradiance that may periodically limit light (Phlips et al. 1995), and alternating P- and N-limitation (Tomas et al. 1999) – led to hypotheses that salinity, light, and nutrient availability may influence phytoplankton species abundances in Florida Bay. To that end, this study focuses on how the ecophysiological characteristics of selected species of bloom-forming (*Synechococcus* cf. *elongatus*, *Synechocystis* sp., and *Chaetoceros* cf. *salsugineus*) and nonblooming species (*Thalassiosira* cf. *oceanica* and *Skeletonema costatum*) from Florida Bay may contribute to their relative dominance. Species responses to salinity, light, N- and P-availability, and interspecific N- and P-competitive interactions are investigated.

METHODS

Phytoplankton

Growth responses to light and salinity variations, P-growth kinetics, and competitiveness for N and P were determined using nonaxenic clonal laboratory cultures of cyanobacteria and diatom species isolated from Florida Bay. Isolates were maintained in the Florida Fish & Wildlife Conservation Commission Culture Collection (CCFWC). The dominant bloom-forming species were *Synechococcus* cf. *elongatus* (Naegeli) (CCFWC 402), *Synechocystis* sp. (CCFWC 401), and *Chaetoceros* cf. *salsugineus* (Takano), all of which are observed commonly throughout the bay and regularly as dominant components of algal blooms in the north-central region (Steidinger et al. 2001). The less abundant species *Thalassiosira* cf. *oceanica* Hasle (synonymous with *Cyclotella nana* Hustedt Guillard clone 13-1) and *Skeletonema costatum* (CCFWC 400) are also found in the bay but do not commonly form large blooms or dominate the phytoplankton community (Steidinger et al. 1995). *S. costatum* was isolated from western Florida Bay during the latter part of this study and was therefore unavailable for many of the experimental analyses. Hereafter, the species will be referred to by their genus.

Stock cultures were maintained in natural seawater collected from the Gulf of Mexico approximately 40 miles offshore, diluted to a salinity of 25 with deionized water, autoclaved, and enriched to "f/2" (Guillard and Ryther 1962) nutrient levels. Aseptic techniques were used throughout. Stock unialgal cultures were maintained in exponential growth by semicontinuous dilution. Experiments were conducted in growth chambers at 25 ^{o}C, a 12/12 h (L/D) photoperiod, and 150 μmol photons m^{-2} s^{-1} provided by 60-watt cool-white fluorescent lights, unless noted otherwise.

Analytical Methods

Salinity was determined by a temperature-corrected hand-held refractometer. Irradiance was determined by a Biospherical QSL-100 PAR irradiance meter with a 4π sensor or a LiCor PAR meter with a cosine-corrected 2π sensor when the light source was strictly unidirectional. Cyanobacteria cells were counted using epifluorescence techniques (Booth 1993, MacIssac and Stockner 1993); diatom species were preserved in Lugol's, sedimented, and counted according to the procedures outlined by Venrick (1978). Cell biovolumes were determined by measuring a minimum of ten cells at 400-1000X and calculated using standard geometric shapes. Specific growth rates (d^{-1}) in each flask were calculated by a linear least-squares regression of the natural log of either the *in vivo* fluorescence values (Turner Designs 10 AU fluorometer) or sample cell counts vs. time during log-phase growth. Soluble reactive phosphorus (SRP) concentrations > 0.4 μM were determined with a 1 cm Pyrex cell by the Strickland and Parsons method (1972). SRP concentrations < 0.4 μM were determined using a 10 cm cell and the MAGIC method (Karl and Tien 1992), which allows for accurate determinations to approximately 0.01 μM.

Growth Response to Salinity

The influence of salinity on each species growth rate was examined using "f/2"enriched artificial seawater (ASW) (Parsons et al. 1984). Populations were fully acclimated to the experimental salinities of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 for three weeks before measuring growth-rates. Species were established at each of the experimental salinities from stock cultures maintained at a salinity of 25 by gradually increasing or decreasing the salinity in increments of 3 by the addition of ASW or deionized water. Dilute suspensions of acclimated cells were inoculated into replicate test tubes (25 x 125-mm Pyrex) containing 30 ml of growth medium. Tubes were inverted twice daily. Growth rates were determined from daily measurements of *in vivo* fluorescence using a

Turner Designs 10 AU fluorometer (Brand et al. 1981).

Growth Response to Irradiance

The influence of light on each species growth rate was determined using experimental growth conditions and methodologies similar to those used for the salinity growth-rate experiments, except instead of test tubes, flasks (250-ml Pyrex) containing 50 ml of growth medium were used. Populations were acclimated to experimental light conditions ranging from 11 to 350 μmol photons m^{-2} s^{-1} for at least two weeks before taking any growth-rate measurements. A semicontinuous-batch-culture technique was used to maintain experimental flasks in steady-state exponential growth rates at all experimental irradiances (Brand et al. 1981). Each species' irradiance growth-response curve (U -E curve) was determined at a salinity of 25 by fitting the growth vs. irradiance data to the hyperbolic tangent function (Yoder et al. 1979)

$$U = U_m \, \mathrm{Tanh} \, (\alpha_g \, E \, U_m^{-1})$$

where U is the growth rate (d^{-1}); U_m is the maximum growth rate (d^{-1}); E is the irradiance (μmol photons m^{-2} s^{-1}); and α_g is the light use growth efficiency or slope of the initial linear portion of the curve (growth div d^{-1} / μmol photons m^{-2} s^{-1}). The function parameters U_m and α_g for each species were derived from the species U-E curves. The irradiance approximating the onset of light-saturated growth, E_s, was estimated by dividing U_m by α_g. The compensation growth-irradiance level (E_c) for each species (i.e., E at U = 0) was estimated by linear, least-squares regression of growth rate vs. irradiance made during balanced growth in the light-limited regions of the growth curves (E < 40 μmol photons m^{-2} s^{-1}) following Geider et al. (1985).

Phosphorus Growth Kinetics and Equilibrium Resource Competition (ERC) Theory Minimum Phosphorus Requirement

The relationship between the growth rate and the external phosphate concentration was determined for each species by using single-species, short-term batch cultures (Kilham 1978, Tilman 1981, Sommer 1986, and Grover 1989). Prior to inoculating the experimental flasks, algae were depleted of P by batch growth in P-limited media ("f/2", except phosphate = 0.3 μM). The cells were judged to be starved of their internal P pools when the population reproduction rate as determined by *in vivo* fluorescence and cell counts was zero. Stationary-phase, P-depleted cells were inoculated into duplicate 1-liter Pyrex flasks containing artificial seawater enriched to "f/2" media except for phosphate, which was added at concentrations ranging from 0.03-5.0 μM. Initial experimental cell densities ranged from 5-74 cells ml^{-1} and experiments were 4-5 days in length. Phosphate growth-kinetic parameters were estimated by linear and nonlinear methods. Nonlinear regression was used to fit the substrate-dependent growth of each species to the Monod model as described by the equation

$$U = U_m \, (S / K_u + S)$$

where U is the growth rate, U_m is the maximal growth rate, S is the limiting-nutrient concentration, and K_u is the half-saturation constant for growth. Linear transformations of the substrate-dependent growth of each species (Dowd and Riggs 1965) were also used to estimate each species nutrient kinetic parameters, U_m and K_u. The linear transformation used was

$$S / U = (K_u / U_m) + (1 / U_m) \, S$$

where S is the phosphate concentration, U is the species' growth rate, K_u is the half-saturation constant for growth, and U_m is the maximal growth rate. The index of competitive ability for a limiting resource, R*, as described in the theory of equilibrium resource competition (ERC theory) (Tilman, 1982), was calculated using the equation

$$R^* = U \, K_u / (U_m - U)$$

where R* is the amount of the limiting resource that a species needs for a reproductive replacement rate equal to its loss rate, and K_u, U_m, and U are as previously defined. A growth-mortality rate of 0.2 d^{-1} (equivalent to an experimental steady-state dilution rate of 0.2 d^{-1}) was used with species' U_m and K_u values to calculate phosphate-related R* values. R* values were then used to construct zero-net-growth isocline (ZNGI) plots for each species according to Tilman (1982). Phosphate growth-kinetic parameters and R* values were determined at salinities of 15, 25, and 50 in order to examine

the influence of salinity on species' K_u and R*. This salinity range included both optimal and suboptimal salinities for growth of each species. The potential influence of bacterial biomass was minimized by drawing inocula from stock cultures that were transferred frequently to new glassware and maintained at high relative growth rates.

Competition Experiments

Mixed-species and paired-species competition experiments were conducted to examine the influence of P-limitation, N-limitation, and type of limiting N or P, upon interspecific competition. In the mixed-species competition experiments cyanobacteria and diatom species, with the exception of *Skeletonema*, were placed together in experimental flasks. In the paired-species competition experiments, the diatom species (*Thalassiosira, Chaetoceros*, and *Skeletonema*) were paired with each other in flasks. All competition experiments were carried out using semicontinuous dilution, which consisted of a single daily manual dilution. This approach has been found to approximate the steady-state conditions required by competition experiments for testing predictions of ERC theory (Kilham 1978). Competitive outcomes were determined by calculating changes in species' total biovolumes over time. Flasks (250 ml Pyrex Erlenmeyer) containing 100 ml of the appropriate culture medium were diluted each day by removing 20 ml of culture, followed by adding 20 ml of fresh media of the appropriate salinity and nutrient composition; this resulted in a dilution rate of 0.2 d^{-1}.

At the initiation of the experiments, duplicate flasks of each treatment were inoculated with species from exponentially growing stock cultures. The initial inoculum of each species in each flask was such that species' initial biomasses had similar total biovolumes. The 20 ml of culture removed daily was used for cell counts. Control flasks containing only a single species of each competitor were included to verify that each species was able to grow and survive under the experimental conditions. Media formulations consisted of artificial seawater (Parsons et al. 1984) enriched to "f/2" concentrations, except for N and P, which were added to flasks at desired concentrations. The forms of N and P used were nitrate, ammonium, phosphate, and glycerophosphate. The concentrations of nitrate and phosphate used in the P-limited experiments were 50 μM nitrate and 0.2 μM phosphate in the mixed-species flasks and 880 μM nitrate and 1.0 μM phosphate in the paired-diatom species flasks. N-limited experiments were 3 μM nitrate and 1.5 μM phosphate in the mixed-species flasks and 72 μM nitrate and 36 μM phosphate in the paired-diatom species flasks. The concentrations used when the limiting P form was glycerophosphate were 50 μM nitrate and 0.2 μM glycerophosphate, and when the limiting N form was ammonium were 6.0 μM ammonium and 3.0 μM phosphate. Paired-species competition experiments were used to simplify individual competitive differences for interpretation of effects of limiting N and P. *Skeletonema costatum*, isolated from western Florida Bay, was unavailable at the onset of the study and was therefore not a part of the mixed-species competition experiments. Additional competition experiments examining the separate and combined influences of P-limitation, N-limitation, salinity, and frequency of delivery of the limiting nutrient upon interspecific competition are not presented but can be seen in Richardson (2004).

Statistical Analyses

Table and figure data are presented as mean and standard error of the mean (SEM). Each species' irradiance growth-response curve was determined by fitting the hyperbolic tangent function (Yoder et al. 1979) to calculate U_m and α_g. A linear transformation of the Monod model (Dowd and Riggs 1965) was constructed (SAS v 9.2, Cary, NC) to calculate U_m and K_u (as well as their standard errors) for the substrate-dependent growth curves. Model parameters were compared between species at each salinity and within each species for all salinities using multiple t-tests with a Bonferonni adjustment to maintain an overall error rate of 0.05 for the irradiance growth-response and substrate-dependent models. E_s was derived from the irradiance growth-response parameters U_m and α_g, and R^* was calculated from the substrate-dependent parameters U_m and K_u (Tilman, 1982). A linear model was constructed from a subset of the data (less than 40 μmol photons m^{-2} s^{-1}) in order to calculate E_c. Error terms for E_s, R^*, and E_c were estimated using indeterminate error propagation rules for sums, products, and quotients and are presented as absolute errors (Swartz and Miner 1977).

RESULTS

Growth Response to Salinity

Growth responses to variations in salinity followed taxonomic lines (Fig. 1). The two cyanobacteria had broader salinity-tolerance ranges and had growth rates that were at least 60% of maximal growth rates (U_m) between salinities of 5 and 60. In contrast, the two diatoms had narrower salinity tolerances, with *Thalassiosira* unable to grow at salinities below 10 or above 55; *Chaetoceros* grew at a salinity of 5 but barely grew at 60. Although all four species had relatively broad salinity-optima ranges, the cyanobacteria salinity optima (10-20) were lower than those of the diatoms *Thalassiosira* (25-40) and *Chaetoceros* (15-30). The U_m in divisions d^{-1} for each species at its salinity optimum was 1.06 for *Synechococcus*, 0.97 for *Synechocystis*, 2.11 for *Chaetoceros,* and 2.46 for *Thalassiosira*. Between salinities of 15 and 50, the diatom species' U_m values exceeded those of the cyanobacteria species, but at salinities of 5 and 60, the U_m values of the cyanobacteria exceeded those of the diatoms.

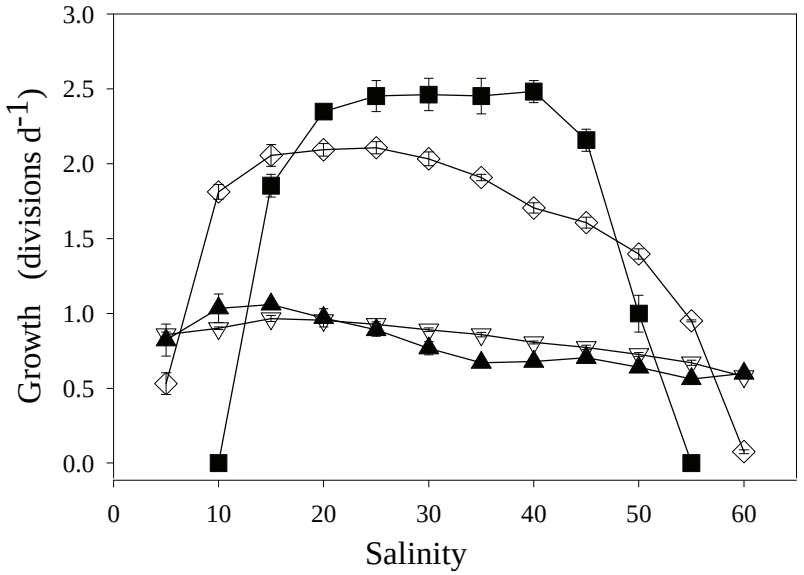

Figure 1. Salinity-response growth curves, plotted as mean growth (± SE) vs. salinity of two cyanobacteria species (*Synechococcus* cf. *elongatus* = closed triangle, *Synechocystis* sp. = open triangle) and two diatom species (*Chaetoceros* cf. *salsugineus* = open diamond, and *Thalassiosira* cf. *oceanica* = solid square).

Growth Response to Irradiance

The hyperbolic tangent function effectively described the growth vs. irradiance (U-E) responses of cyanobacteria and diatom species. The species responses once again followed taxonomic lines (Fig. 2), with the U_m, α_g, and E_s values of the diatoms being greater than those of the cyanobacteria (Table 1). T-tests found all species' U_m values at a salinity of 25 to significantly differ from each other ($p > 0.05$), except between the diatoms. Comparisons

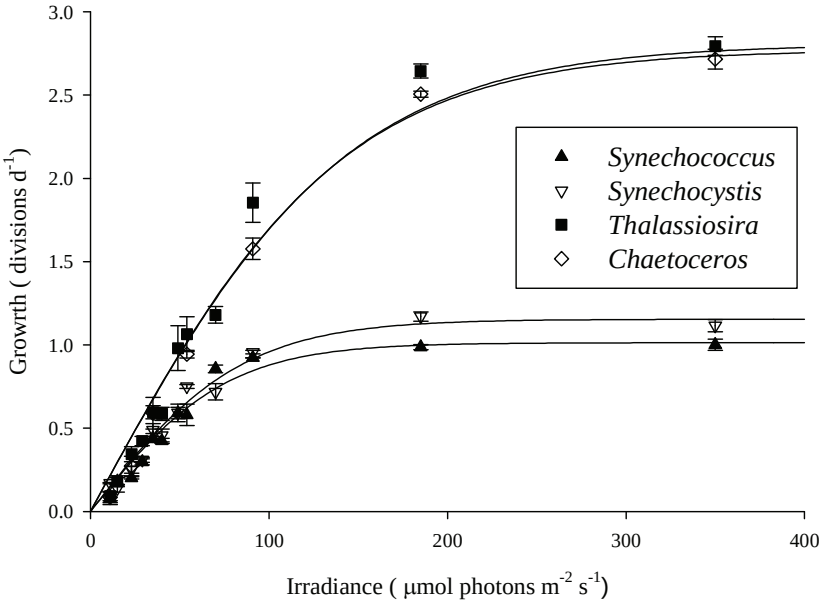

Figure 2. Irradiance-response growth curves of two cyanobacteria species (*Synechococcus* cf. *elongatus*, *Synechocystis* sp.) and two diatom species (*Chaetoceros* cf. *salsugineus*, and *Thalassiosira* cf. *oceanica*). All curves are nonlinear least-squares regressions fit to the Jassby-Platt hyperbolic tangent function. Values are means ± SE.

Table 1. Growth-curve parameters for each species' response to variations in irradiance. U_m (divisions d^{-1}) and α_g (divisions d^{-1} / μmol photons m^{-2} s^{-1}) with standard errors in parentheses were determined using nonlinear least-squares regressions fit to the Jassby-Platt hyperbolic tangent function. E_s (μmol photons m^{-2} s^{-1}) and E_c (μmol photons m^{-2} s^{-1}) with absolute-error estimates [±] were calculated using U_m and α_g values, and linear regression slope and y-intercept values, respectively, as described in text.

Species	**U_m**	**α_g**	**E_s**	**E_c**
Synechococcus cf. *elongatus*	1.01 (0.02)	0.013 (0.0005)	75 [±5]	5.0 [±4]
Synechocystis sp.	1.15 (0.02)	0.014 (0.0005)	84 [±5]	2.8 [±5]
Thalassiosira cf. *oceanica*	2.90 (0.06)	0.021 (0.0007)	139 [±7]	6.5 [±3]
Chaetoceros cf. *salsugineus*	2.77 (0.05)	0.020 (0.0008)	140 [±8]	8.1 [±8]

between species' α_g values all showed significant differences ($p > 0.05$) except between cyanobacteria species and between diatom species. E_s values indicated lower light-saturation intensities are needed by the cyanobacteria than by the diatoms. E_c values suggest that all species have similarly low minimum-light requirements. No photoinhibitory response was observed in any of the species' growth rates up to an irradiance of 350 μmol photons m^{-2} s^{-1} (Fig. 2).

Phosphorus Growth Kinetics and ERC Theory

Minimum P Requirements

Growth responses, as a function of external phosphate concentrations at a salinity of 25, are shown in Figure 3 (see Richardson 2004, Figs. 3-1, 3-2, 3-3, and 3-4 for salinities of 15 and 50). U_m and K_u values derived from a linear transformation of the Monod model and R* values calculated using those parameters are presented in Table 2. Species' U_m values at each salinity were significantly different from each other ($p > 0.05$), except between the cyanobacteria species at salinities of 15, 25, and 50 and between the diatom species at 15 and 25. Comparisons of species' K_u values at each salinity (excluding the negative K_u values for *Synechococcus* at a salinity of 15 and *Synechocystis* at salinities of 15 and 50) revealed no significant interspecific differences ($p > 0.05$). Calculated R* values had large absolute errors. No statistical comparisons were performed on R* values. Qualitative trends in species' K_u and R* values at a salinity of 25 suggest interspecific differences in K_u and R* values, from smallest to largest being *Synechococcus* < *Synechocystis* ≈ *Chaetoceros* < *Thalassiosira*. The qualitative pattern predicts that, under steady-state P-limiting conditions, *Synechococcus* is the superior phosphate-affinity competitor, *Thalassiosira* the most inferior competitor, and *Synechocystis* and *Chaetoceros* intermediate competitors.

Statistical comparisons revealed that salinity influenced species U_m but not K_u. U_m values for each species were significantly different at salinities of 15, 25, and 50 ($p > 0.05$), except for *Synechococcus* at 15 and 25. K_u values for each species were not significantly different at salinities of 15, 25, and 50 ($p > 0.05$). The diatoms' R* values were elevated at the suboptimal salinity of 50 (Table 2). ZNGI plots for *Synechocystis* and *Synechococcus* (Fig. 4) reflect the inability to calculate R* values when K_u values were negative. Instead, the maximum R* values for *Synechocystis* at a salinity of 15 and *Synechococcus* at salinities of 15 and 50 were estimated using each species' respective U_m, and the lowest K_u determined at that salinity (i.e., 0.003 μM phosphate at a salinity of 15 and 0.07 μM phosphate at 50). Because K_u values

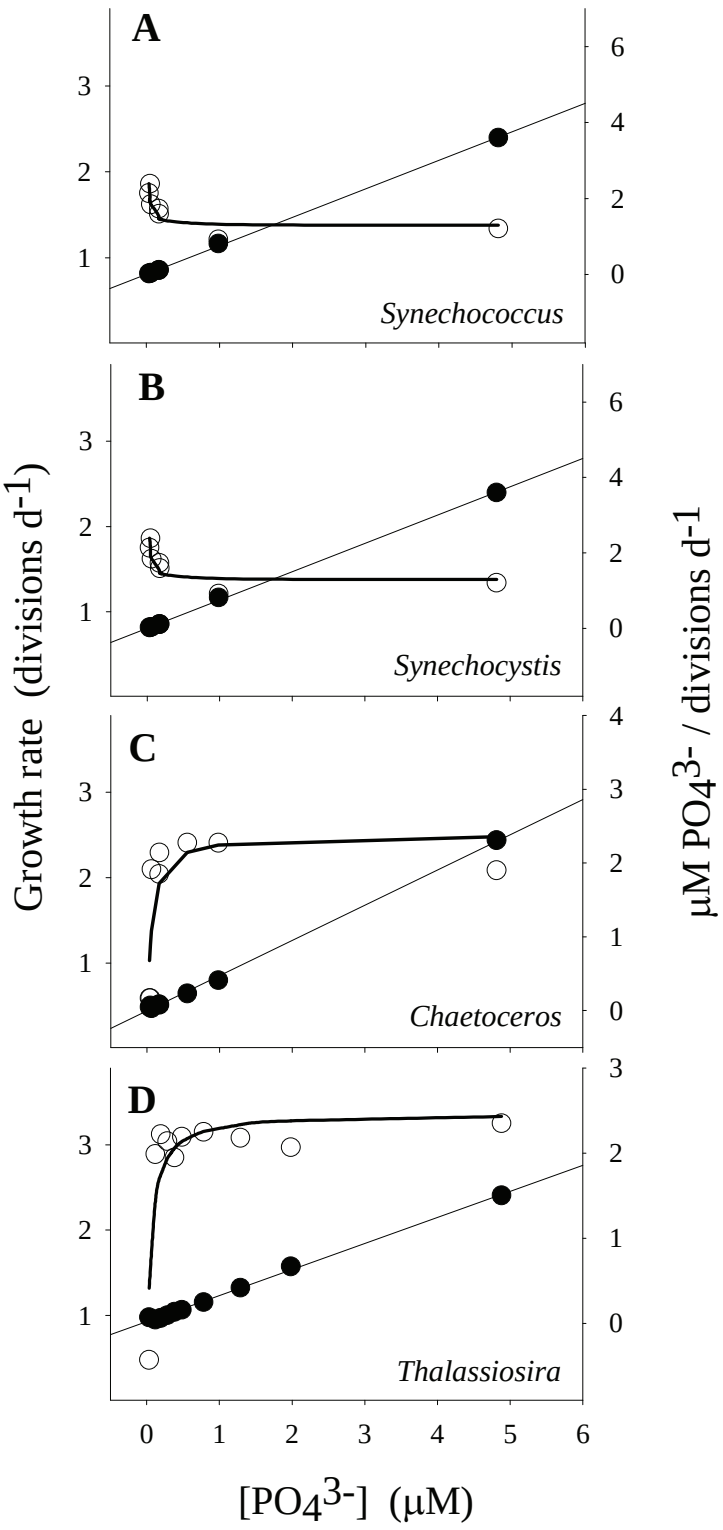

Figure 3. Phosphate-dependent algal growth kinetics at a salinity of 25. (A) *Synechococcus* cf. *elongatus*, (B) *Synechocystis* sp., (C) *Chaetoceros* cf. *salsugineus*, and (D) *Thalassiosira* cf. *oceanica*. Nonlinear Monod model – (Growth rate vs. [PO_4^{3-}]) and linear transformation – (([PO_4^{3-}] / divisions d^{-1}) vs.

for *Synechococcus* and *Synechocystis* were negative, they likely were lower than the lowest determined K_u. As a result, the actual R* values for *Synechococcus* and *Synechocystis* at salinities of 15 and 50 are expected to lie somewhere below the estimated maximum values (Fig. 4).

The ZNGI lines combining phosphate concentration and salinity, above which a species will experience a net increase in population size and below which it will experience a net decrease, predict that *Thalassiosira*, with the highest R* values, was the most inferior phosphate competitor between the salinities of 15 and 50. At a salinity of 25, *Synechococcus* was predicted to be the superior phosphate-competitor, with *Synechocystis* and *Chaetoceros* being intermediate competitors.

Competition Experiments

Mixed-species competition outcomes under P-limitation (phosphate and glycerophosphate) at a salinity of 25 reveals that *Synechococcus* dominated by biovolume, *Synechocystis* was the next most abundant species by biovolume, and both *Thalassiosira* and *Chaetoceros* were competitively excluded (Figs. 5A and 7A). At salinities of 15 and 50, competitive outcomes were largely comparable to those at 25 except at 50 *Chaetoceros* was not excluded (see Richardson 2004, Figs. 3-8A and 3-10A). In paired-diatom species competition experiments under P-limitation (phosphate only) at a salinity of 25 (which included *Skeletonema*), *Chaetoceros* excluded both *Thalassiosira* and *Skeletonema*, and *Thalassiosira* excluded *Skeletonema* (Fig. 5B-D). An overall relative ranking of the species' competitiveness for phosphate at salinities of 15, 25, and 50, from

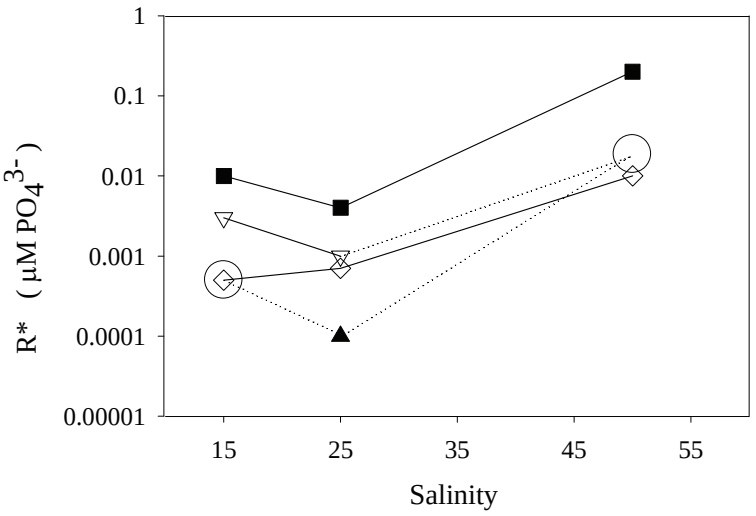

Figure 4. Zero-net-growth isoclines (ZNGI). R* calculated using linear-derived U_m and K_u values. *Synechococcus* cf. *elongatus* = ▲, *Synechocystis* sp. = Ñ, *Chaetoceros* cf. *salsugineus* = ◊, *Thalassiosira* cf. *oceanica* = ■. Estimated maximum R* for *Synechocystis* sp. at a salinity of 50 and *S.* cf. *elongatus* at salinities of 15 and 50 = O. Estimated ZNGI's for *S.* cf. *elongatus* and *Synechocystis* sp. = ···. Maximum R* values for *S.* cf. *elongatus* and *Synechocystis* sp. at salinities of 15 and 50 estimated using each species U_m and the lowest K_u determined at that salinity.

strongest to weakest is *Synechococcus* > *Synechocystis* ≥ *Chaetoceros* > *Thalassiosira* (> *Skeletonema*, at a salinity of 25). The relative ranking of the species' competitiveness for glycerophosphate at a salinity of 25, from strongest to weakest is *Synechococcus* > *Synechocystis* > *Thalassiosira* and *Chaetoceros*.

In mixed-species competition experiments under N-limitation (nitrate and ammonium) at a salinity of 25, *Synechococcus* dominated by biovolume, followed by *Synechocystis* and *Thalassiosira*, with *Chaetoceros* being competitively excluded under nitrate-limitation and nearly excluded under ammonium-limitation (Figs. 6A and 7B). Similar competitive outcomes occurred at salinities of 15 and 50; *Chaetoceros* was competitively excluded, and *Synechococcus, Synechocystis, and Thalas-*

Table 2. Phosphorus-dependent growth kinetic parameters at salinities of 15, 25, and 50 for two species of cyanobacteria (*Synechococcus* cf. *elongatus* and *Synechocystis* sp.) and two diatom species (*Chaetoceros* cf. *salsugineus* and *Thalassiosira* cf. *oceanica*). U_m = maximum growth rate (divisions d^{-1}), K_u = half saturation constant for growth (μM phosphate), and R* = the index of competitive ability for the limiting resource (μM phosphate). Negative values are represented by ---. Standard errors are in parentheses, and absolute errors are in brackets.

	Um	**Ku**	**R***
Salinity of 15			
Synechococcus	1.30 (0.08)	---	---
Synechocystis	1.96 (0.02)	0.003 (0.02)	0.0003 [" 0.002]
Chaetoceros	1.63 (0.02)	0.004 (0.2)	0.0005 [" 0.03]
Thalassiosira	1.45 (0.05)	0.07 (0.07)	0.01 [" 0.01]
Salinity of 25			
Synechococcus	1.33 (0.01)	0.0007 (0.02)	0.0001 [" 0.004]
Synechocystis	1.39 (0.02)	0.007 (0.02)	0.001 [" 0.004]
Chaetoceros	2.10 (0.04)	0.007 (0.03)	0.0007 [" 0.004]
Thalassiosira	3.27 (0.06)	0.07 (0.04)	0.004 [" 0.002]
Salinity of 50			
Synechococcus	0.95 (0.003)	---	---
Synechocystis	0.99 (0.02)	---	---
Chaetoceros	1.38 (0.03)	0.07 (0.05)	0.2 [" 0.008]
Thalassiosira	0.33 (0.02)	0.1 (0.07)	0.3 [" 0.1]

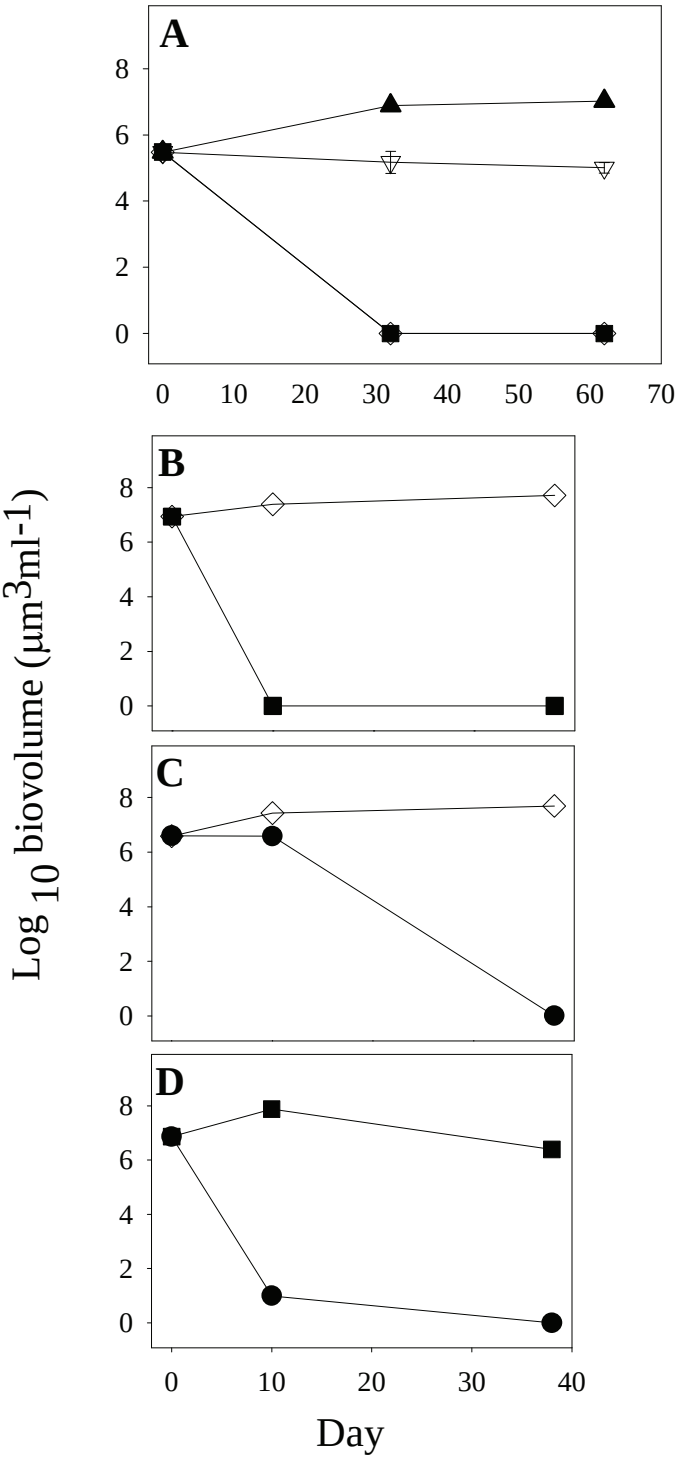

Figure 5. P-limited competition experiments at a salinity of 25. (A) mixed-species (50 μM nitrate, 0.2 μM phosphate), (B-D) paired-diatom species (880 μM nitrate, 1.0 μM phosphate). *Synechococcus* cf. *elongatus* = filled triangles, *Synechocystis* sp. = inverted tiangles, *Thalassiosira* cf. *oceanica* = filled squares, *Chaetoceros* cf. *salsugineus* = open diamonds, S*keletonema costatum* = filled circles

siosira were all dominant species, albeit with slight shifts in their relative biovolumes, except for the exclusion of *Thalassiosira* at a salinity of 50 (Figs. 3-12A and 3-14A in Richardson 2004).

The relative ranking of the species' competitiveness for nitrate at salinities of 15, 25, and 50, from strongest to weakest is *Synechococcus* ≥ *Synechocystis* > *Thalassiosira* > *Chaetoceros*. In paired-diatom species competition experiments under N-limitation (nitrate only) at a salinity of 25 (which included *Skeletonema*), *Skeletonema* excluded both *Thalassiosira* and *Chaetoceros*, and *Thalassiosira* dominated *Chaetoceros* (Fig. 6B-D). The rapid exclusion of *Thalassiosira* by *Skeletonema* (Fig. 6B) and the slight dominance of *Synechococcus* and *Synechocystis* over *Thalassiosira* (Fig. 6A) together suggest that the relative ranking of species competitiveness for nitrate at a salinity of 25, from strongest to weakest is *Synechococcus* ≥ *Synechocystis* ≥ *Skeletonema* > *Thalassiosira* > *Chaetoceros*.

DISCUSSION

The growth of the study species in response to salinity and light agree with growth characteristics reported previously for these taxa (Guillard and Ryther 1962, Erickson and Farrow 1965, Glover et al. 1987, Kana and Glibert 1987, Stockner 1988, Orlova and Selina 1993, Phlips and Badylak 1996). Differences between the salinity-related growth curves of the study species indicate that salinity may influence their relative abundance and dominance of a bloom in Florida Bay. Oligohaline and hypersaline conditions (salinities < 10 or > 50), in the presence of sufficient nutrients and light, would favor the more euryhaline cyanobacteria (*Synechococcus* and *Synechocystis*) over the diatoms (*Chaetoceros* and *Thalassiosira*). Hypersaline conditions occur regularly in the central and eastern zones of Florida Bay. Since 1955, the median

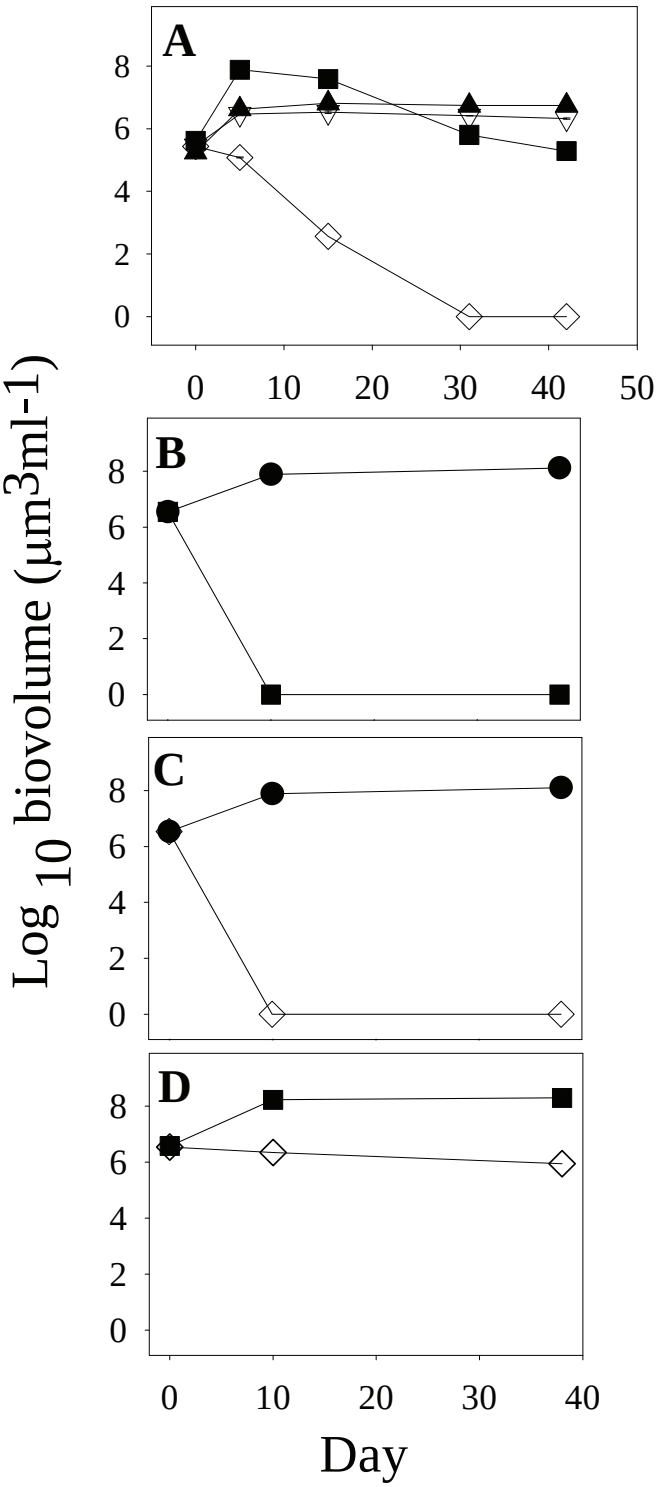

Figure 6. N-limited competition experiments at a salinity of 25. (A) mixed-species (3 μM nitrate, 1.5 μM phosphate), (B-D) paired-diatom species (72 μM nitrate, 36 μM phosphate). *Synechococcus* cf. *elongatus* = filled triangles, *Synechocystis* sp. = inverted triangles, *Thalassiosira* cf. *oceanica* = filled squares, *Chaetoceros* cf. *salsugineus* = open diamonds, *Skeletonema costatum* = filled circles

monthly salinities ranged from 29–40 in the western zone, 21–57 in the central zone, and 13–51 in the eastern zone (Roblee et al. 2001). In general, hypersalinity appears first and is most persistent in the central-zone of Florida Bay, where salinities exceeded 40 for 60% of the months since 1955 (Robblee et al. 2001). Consequently, hypersalinity exceeding a salinity of 50 may contribute to dominance by the more euryhaline *Synechococcus* and *Synechocystis* in the central zone as well as the eastern zone of Florida Bay. Oligohaline conditions (salinity <10), which would favor the cyanobacteria over the diatoms, are principally restricted to the northern and northeastern boundary regions of Florida Bay during periods of above-average rainfall (Roblee et al. 2001), but blooms dominated by cyanobacteria have not been documented to either originate or persist in these low-salinity regions of the bay (Phlips et al. 1999). Consequently, unlike hypersalinity, oligohaline conditions are not likely to be an important factor contributing to the development of large, expansive, and recurring cyanobacteria-dominated blooms in the north-central region or the northeastern region of the bay.

Despite their extremes, baywide salinities are for the most part moderate. Since 1955, the mean monthly salinities of the western, central, and eastern zones of the bay were approximately 36, 42, and 33, respectively (Roblee et al. 2001). Under nutrient- and light-sufficient conditions and moderate salinities (between 15 and 45), development of diatom-dominated blooms (*Chaetoceros* and *Thalassiosira*) would be favored, because their maximal growth rates are substantially higher than those of the cyanobacteria species (*Synechococcus* and *Synechocystis*) throughout this salinity range. As a result, the prevailing moderate salinities found throughout the bay are more favorable for the development of diatom-dominated blooms.

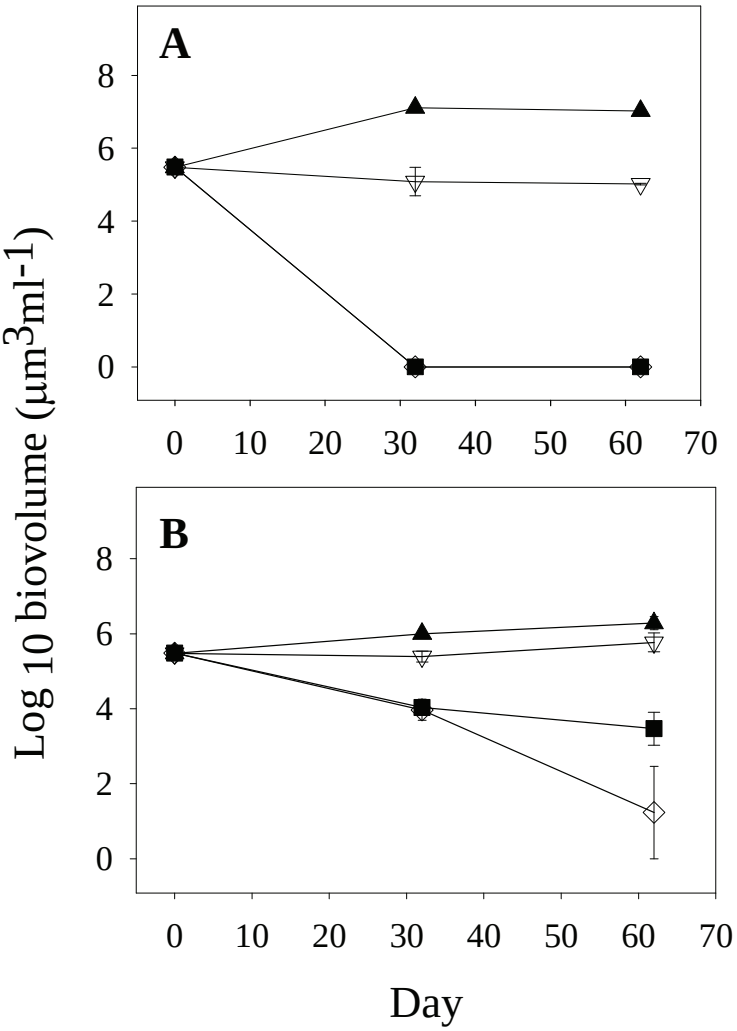

Figure 7. Mixed-species competition experiment at a salinity of 25. (A) P-limited (50 μM nitrate, 0.2 μM glycerophosphate), (B) N-limited (6.0 μM ammonium, 3.0 μM phosphate). *Synechococcus* cf. *elongatus* = filled triangles, *Synechocystis* sp. = inverted triangles, *Thalassiosira* cf. *oceanica* = filled squares, *Chaetoceros* cf. *salsugineus* = open diamonds

Light availability and algal physiological adaptations to light have been found to influence the composition of phytoplankton communities (Oliver and Ganf 2000). Physiological adaptations affording a competitive growth advantage at low irradiance include a lower minimum irradiance (E_c), lower irradiance saturation (E_s), and higher light-use efficiency (α_g). Large temporal and spatial differences in water-column irradiances are found in Florida Bay (Boyer et al. 1999). Particularly during the winter months, the north-central and northwestern regions experience reduced irradiances that are at or near the threshold level for the onset of phytoplankton light limitation (2-5 E m^{-2} d^{-1} PAR) (Phlips et al. 1995). The higher light-use growth efficiencies (α_g) of the diatoms, compared with those of the cyanobacteria, indicate that under light-limiting conditions the diatoms grow faster per unit increase in photosynthetically active radiation (PAR) than do the cyanobacteria (Fig 2, Table 1). This would give the diatoms a competitive advantage that may contribute to diatom bloom dominance during periods of reduced irradiance. This competitive advantage may contribute to the large diatom-dominated blooms that occur in winter in the northwestern region (Phlips et al. 1999) and to the less frequent diatom-dominated blooms in the north-central region (Steidinger et al. 1995). Neither *Synechococcus* nor *Synechocystis* demonstrated an ability to regulate their buoyancy—a physiological adaptation shared by numerous cyanobacteria that that can provide them with a competitive advantage when water-column irradiances become growth limiting (Oliver and Ganf 2000).

The cyanobacteria are, in one respect, better adapted to low irradiances than are the diatoms. The lower E_s values of the cyanobacteria allow them to attain their maximal growth rates at lower irradiances than the diatoms. Nevertheless, because of the cyanobacteria's lower α_g and U_m values, this adaptation does not give the cyanobacteria a competitive advantage over the diatoms that could contribute to cyanobacteria bloom dominance. Although light levels in the bay may occasionally limit the growth of phytoplankton in the northwestern and north-central regions, phytoplankton light-limiting conditions are infrequent in Florida Bay, because mean midday PAR values near the sediment surface are mostly above 250 μmol photons m^{-2} s^{-1} (Carlson et al. 2001). These irradiances far exceed

the similarly low minimum-irradiance requirements (E_c) determined for the study species, and, therefore, differences in E_c values would not be expected to influence their relative abundances in the bay.

Although light-limited environments may influence a species' relative dominance through interspecific differences in E_s, E_c, and α_g, light-saturated environments may affect community structure through interspecific differences in U_m and the species' susceptibility to photoinhibition. Species differences in U_m and photoinhibition may play an important role in determining bloom dominance in Florida Bay because water-column irradiances are predominantly nonlimiting. Under growth-saturating irradiances, when the diatoms' higher α_g values do not give them a competitive advantage over the cyanobacteria, the diatoms still have an advantage because of their much higher light-saturated U_m values. The common occurrence of diatom-dominated blooms throughout much of the bay may be due in part to their much higher maximum growth rates (U_m) at light saturation, particularly during the summer when irradiances are high and sediment-derived turbidity is low. This may contribute to the formation of small diatom blooms usually being dominated by *Chaetoceros* spp. in the western zone during summer (Steidinger et al. 1995).

The study species' U-E curves illustrate the superior competitiveness of the diatoms under both limiting and saturating irradiances, exceeding the cyanobacterial growth rates by ~ 0.7 divisions d^{-1} at 75 μmol photons m^{-2} s^{-1} (light-saturated growth rate for the cyanobacteria) and ~ 1.4 divisions d^{-1} at 175 μmol photons m^{-2} s^{-1} (light-saturated growth rate for the diatoms). The diatoms' greater U_m values would be expected to be more influential in determining their dominance over the cyanobacteria than would their greater α_g values, because on average, water-column irradiances baywide exceed the diatoms' saturating irradiance of 175 μmol photons m^{-2} s^{-1}. The magnitude of the competitive advantage that the diatoms have over the cyanobacteria is proportionately reduced as irradiance decreases. It is only at irradiances of ~ 50 μmol photons m^{-2} s^{-1} and below, when the difference in growth rate is ~ 0.3 divisions d^{-1} or less, that the cyanobacteria may be competitive with the diatoms. Consequently, development of cyanobacteria-dominated blooms in Florida Bay must be promoted by some factor(s) other than light availability. Although the study species' potential was not determined for photoinhibition above an irradiance of 350 μmol photons m^{-2} s^{-1}, a large photoinhibitory response by natural populations of cyanobacteria in Florida Bay is unlikely given the typical development and growth of large cyanobacteria-dominated blooms during periods of high ambient irradiance, which occur during the summer and early fall.

The recurrence of large and persistent cyanobacteria-dominated blooms in regions of Florida Bay that are often hypersaline and N- or P-limited (Tomas et al. 1999) suggest that interspecific differences in the dominant bloom species' nutrient competitiveness (N and P K_u, and R* values) and the influence of salinity on that competitiveness (U_m, K_u, and R* values) may be influential in determining their relative dominance. Interspecific differences in K_u, and R* values have the potential to influence phytoplankton community structure (Tilman 1982, Grover 1989, Sommer 1989), and suboptimal environmental conditions may lower species' competitiveness by elevating K_u and R* values (Paasche 1975, Tilman et al. 1981). The absence of interspecific differences in K_u or R* values for the study species, as well as the lack of any salinity effect on K_u or R* values, may be due to the large errors associated with those values. Wide variances in K_u values are commonly encountered because species' K_u values are often so low that growth occurs at nutrient concentrations below those that can be accurately prepared and measured (Grover 1989).

The similarly low phosphate K_u values of *Synechococcus*, *Synechocystis*, and *Chaetoceros* suggest that the species are well adapted to the low-P environment of Florida Bay, and may contribute to their baywide distribution and their high abundance during blooms. The K_u values obtained for the Florida Bay isolates (0.0007–0.1 μM phosphate), are well within the range of K_u values reported for microalgal species (Healy 1985, Grover 1989, Sommer 1989). The presence of bloom species with very low P K_u values in the phytoplankton community of Florida Bay should not be unexpected, because nutrient bioassays have provided direct evidence that a large portion of the phytoplankton community in Florida Bay consistently experiences P-limitation (Tomas et al. 1999). The

lowest K_u value of 0.0007 µM phosphate obtained for *Synechococcus* would be expected for a P-specialist well adapted to a P-limited environment.

The P-limited competitive outcomes at salinities of 15, 25, and 50 were in agreement with the ERC theory prediction that, under phosphate-limiting equilibrium conditions, *Synechococcus* would competitively displace, and given sufficient time exclude, the three other species at a salinity of 25, beginning with the most inferior competitor *Thalassiosira*. The outcomes (rates and magnitudes of competitive displacement and exclusion) verified that the general qualitative trends of interspecific differences in K_u and R* between the cyanobacteria and diatom species were real. Small differences in competitive ability (R*) were revealed as small differences in species' final biovolumes due to minor competitive displacements. The large differences in final biovolumes, visible as exclusion, indicated large differences in competitive abilities. The rapid rates of exclusion of the poor phosphate-competitors *Skeletonema* and *Thalassiosira*, under P-limitation, were indicative of large competitive differences (in R* values) between them and the superior phosphate-competitor *Synechococcus.* The displacement but not exclusion of *Synechocystis* by *Synechococcus* under P-limitation reflected a smaller difference in phosphate-related competitive abilities. Although a qualitative trend was found that suggested the diatoms' competitive abilities were reduced via elevated K_u and R* values at the suboptimal salinity of 50, differences in the relative magnitude and rate of displacement in P-limited competitive outcomes at salinities of 15, 25, and 50 did not support the existence of any real quantitative differences.

Dissolved organic phosphorus (DOP) may be an important source of P for the phytoplankton of Florida Bay, as it has been found to be the dominant form of P in the water column, being on average approximately an order of magnitude greater than the dissolved inorganic phosphorus (DIP) concentration (Lavrentyev et al. 1998). DIP concentrations in the bay have been found to range from undetectable to 0.33 µM, while organic phosphorus can range from 0.04 to 2.03 µM (Fourqurean et al. 1993). Although the most readily used form of P by both diatoms and cyanobacteria is generally phosphate (Healy 1982), other organic forms of P are utilized as well. High potential alkaline phosphatase activity found throughout Florida Bay, coupled with low concentrations of phosphomonoesters, suggest that organic phosphomonoesters are rapidly hydrolyzed and used by microbial assemblages in the bay (Heil et al., this volume, Koch et al. 2009). Interspecific differences in the study species' abilities to utilize DOP were considered because differences in the ability to utilize DOP have been documented for diatoms and cyanobacteria (*Synechococcus* sp.) (Kuenzler 1970) and differences may provide species with a competitive advantage. The similar competitive outcomes (cyanobacteria dominance and diatom exclusion) in the mixed-species competition at a salinity of 25, when either phosphate or glycerophosphate was the limiting form of P, confirmed the superior competitive ability of the cyanobacteria for both phosphate and a simple organic phosphate ester. The ability of the cyanobacteria to effectively outcompete the diatoms for dissolved organic forms of P in Florida Bay may provide them with a competitive advantage when phosphate levels become extremely low and could contribute to their relative dominance under P-limited conditions.

Phytoplankton species commonly demonstrate nutrient-competitive tradeoffs in which species that are superior competitors for one major nutrient are more often than not inferior competitors for another major nutrient (Tilman and Kiesling 1984). Competitive outcomes at a salinity of 25 were examined for N- and P-specialization tradeoffs, because this salinity was not suboptimal for any of the species. The relative competitive ranking from strongest to weakest competitor for phosphate was *Synechococcus* > *Synechocystis* > *Chaetoceros* > *Thalassiosira* > *Skeletonema*, whereas that for nitrate was *Synechococcus* ≥ *Synechocystis* ≥ *Skeletonema* > *Thalassiosira* > *Chaetoceros*. The relative competitive rankings highlight the pronounced N- and P-specialization tradeoffs of the diatom species. *Skeletonema* exhibited the most pronounced tradeoff, being the most inferior phosphate-competitor and a strong nitrate competitor. *Thalassiosira* was similarly a better competitor for nitrate than for phosphate, while *Chaetoceros* showed the opposite tradeoff, being a poor nitrate-competitor but good phosphate-competitor. Because the cyanobacteria (particularly *Synechococcus*) were such superior phosphate-competitors, a tradeoff in N- and P-specialization comparable to that seen for

Skeletonema was expected, producing poor nitrate-competitors. Yet, no comparable tradeoff was seen in the cyanobacteria species. Competitive outcomes found the cyanobacteria to be both superior phosphate-competitors and strong nitrate-competitors.

The potential influence of ammonium on the development of cyanobacteria-dominated blooms, particularly in the north-central region of Florida Bay, was considered because ammonium can be the dominant form of N, reaching concentrations as high as ~26 µM (Lavrentyev et al. 1998), and some field and experimental evidence suggests that cyanobacteria growth is favored by ammonium while algal growth is favored by nitrate (Blomqvist et al. 1994). The similar competitive outcomes, when either ammonium or nitrate were the limiting forms of N, confirmed the strong competitive ability of the cyanobacteria for both nitrate and ammonium. The strong competitive ability of the cyanobacteria for both nitrate and ammonium may be particularly advantageous in Florida Bay where mineralized N may at times be the dominant form of available N. Although the competitive abilities of the cyanobacteria and diatom species for nitrate and ammonium may be important, *in situ* differences in the use of various N substrates by cyanobacteria and diatoms in Florida Bay, suggest that the availability of different forms of N may also influence the formation of cyanobacteria- and diatom-dominated blooms. Glibert et al. (2004), using pigment ratios, found the relative cyanobacteria biomass (ratio of cyanobacteria biomass to phytoplankton biomass) to be positively correlated with the rate of uptake of urea, and negatively correlated with the rate of uptake of DIN (nitrate, nitrite, and ammonium), while the opposite pattern was observed for the relative diatom biomass.

The small tradeoff in N- and P-competitiveness makes *Synechococcus*, relative to the other species, better equipped to increase in biomass during periods of P-limitation and experience little or no loss in biomass during periods of N-limitation. The strong N- and superior P-competitiveness of *Synechococcus* may not be solely the result of interspecific differences in phosphate or nitrate affinities (K_u) but rather be the result of combined influences of nutrient uptake and assimilation. For example, in this study, the cyanobacteria's nitrate and phosphate affinities (low K_u values), velocities (high V_m / K_s ratios), and luxury-storage attributes appear suited to the temporal variation of limiting P and N introduced by the daily single manual removal and addition of 20% of the culture medium.

Skeletonema costatum, a common bloom species in Florida's estuarine and coastal waters, is surprisingly uncommon in Florida Bay and is conspicuously absent from the central and eastern regions of the bay (Steidinger et al. 1995, Steidinger et al. 2001). Although it has been described as a good N-competitor (Rijstenbil et al. 1989), capable of overwhelmingly dominating the phytoplankton community when N is apparently the only limiting nutrient (Kilham and Hecky 1988), the same cannot be said for its P-competitiveness. The rapid exclusion of *Skeletonema* by *Thalassiosira* and the exclusion of *Thalassiosira* by the other species in the P-limited competition experiments indicate a large disparity in P-competitiveness between *Skeletonema* and the dominant bloom species of the bay.

The finding in this study that *Skeletonema* was the most inferior phosphate-competitor may help explain its conspicuous absence from the predominantly P-limited eastern zone of Florida Bay. Its absence from the eastern zone may also result from P deficiency, as its P requirement may be relatively high based on its high ATP requirements (Lavrentyev et al. 1998). In contrast, the finding in this study that *Skeletonema* was a strong nitrate-competitor seems at odds with its infrequent occurrence in the western zone of Florida Bay, which is predominantly N-limited, as well as its absence from the central zone, which alternates between P- and N-limitation. This anomaly may be explained in part by *Skeletonema*'s large P- and N-specialization tradeoff, which makes it a strong nitrate competitor and the most inferior phosphate competitor. Its absence from the central zone and rarity in the western zone may be largely the consequence of its inferior phosphate-competitiveness, such that any cumulative gains in *Skeletonema* biomass made during N-limiting conditions are overwhelmed by large and rapid biomass losses during P-limitation.

The large and persistent cyanobacteria-dominated blooms in the north-central region and the northeastern region resemble conditions found in the competition experiment's flasks in having relatively constant but low water-turnover rates,

similar and fairly constant rates of algal biomass loss and gain, an algal biomass that remains at or near a determined carrying capacity for extended periods of time, and an algal biomass that may be determined for extended periods of time by a single (N or P) limiting nutrient. Nutrient bioassays have indicated that the standing algal biomass in the north-central bloom region may be determined by a single nutrient and be at or near its carrying capacity (Tomas et al 1999). The north-central and northeastern regions also have low rates of water turnover (on the order of months; Nuttle 2008). The long water-residence times would also be expected to promote phytoplankton growth supported mainly by nutrient recycling (i.e., phosphate, DOP, nitrate, and ammonium) in the water column and the benthos—conditions that may facilitate nutrient limitation and competition. Although ERC theory is not considered to be applicable to dynamic environments such as estuaries (Roelke et al. 1999), the approximate steady-state characteristics shown by cyanobacteria-dominated blooms in the north-central and northeastern regions suggest a system where nutrient-based (e.g., N and P) ERC theory interspecific competitive interactions may influence bloom dynamics. As such, the N- and P-limited competitive outcomes in the competition experiments may reflect competitive processes occurring in Florida Bay.

The competitive advantage that *Synechococcus* has over the diatom species during P-limitation at all salinities and N-limitation at low and high salinities may contribute to its bloom dominance in the eastern region where P-limitation prevails, as well as the north-central region where alternating P - and N-limitation and hypersaline conditions occur. The influence that N- and P-availability and salinity may have on microalgal bloom dominance and thus the type and quantity of phytoplankton carbon available for higher trophic levels in Florida Bay may have potential management implications. Given sufficient N and P to support large algal blooms in the bay, water-management practices that promote P-limitation, irrespective of salinity, have the potential to enhance cyanobacteria-dominated blooms, whereas practices that promote N-limitation have the potential to either enhance mixed cyanobacteria-diatom blooms at moderate salinities or cyanobacteria-dominated blooms at extreme salinities. Management practices that promote long water-residence times in Florida Bay would be expected to enhance cyanobacteria-dominated blooms over diatom-dominated blooms by creating conditions that favor species with superior nutrient competitive abilities over species with superior maximum growth rates. Although the results of this study indicate that P-limitation has the potential to play an important role in the widespread occurrence of expansive and persistent *Synechococcus*-dominated blooms, it is only one of many factors (see introduction) that may contribute to cyanobacteria dominance of the phytoplankton community in Florida Bay.

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SIZE-FRACTIONATED ALKALINE PHOSPHATASE ACTIVITY ALONG A GRADIENT OF NITROGEN TO PHOSPHORUS LIMITATION IN A CARBONATE DOMINATED SUBTROPICAL ESTUARY

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ABSTRACT

Phytoplankton communities in Florida Bay, a shallow, subtropical, carbonate dominated estuary, are characterized by an east-west gradient of nitrogen (N) to phosphorus (P) limitation which would be expected to influence the dynamics of dissolved organic phosphorus (DOP) utilization by phytoplankton within the Bay. We examined the bioavailability of DOP utilization from 2002 to 2005 at six stations across this nutrient gradient by measuring size fractionated alkaline phosphatase activity (APA) and its regulation by PO_4^{3-} using direct measurements and 48 hr nutrient addition bioassays. APA was present year round throughout the Bay under both N and P limiting conditions and ranged from 0.00 to 1.58 μM P L^{-1} h^{-1} and 0.00 to 9.71 μmol P μg Chlorophyll *a* (Chl *a*)$^{-1}$ h^{-1} when normalized to Chl *a* biomass. A significant amount of total APA, 42.6 (±24.7%) on average for the entire study, was present in the dissolved (<0.2 μm) fraction. The amount of dissolved and particulate APA present showed no relationship with location, season, Chl *a* or PO_4^{3-} and DOP concentrations. APA in the particulate fraction was inversely related to PO_4^{-3} uptake rate of the entire microbial assemblage (>0.2 μm fraction), however, suggesting some physiological regulation of APA was present. Variable amounts of APA were present in the bacterial (0.2 – 1.0 μm) and larger (1.0 – 3.0 μm and >3 μm) plankton fractions throughout the year, and its presence in both N, P or grazer limited populations (as suggested by biomass response to nutrient additions after 48 hours) suggests that APA is used by diverse microbial assemblages throughout Florida Bay when PO_4^{3-} and DOP concentrations are low (i.e. less than 0.17 μM and 0.47 μM respectively) as a P acquisition strategy. Enzymatic activity was repressed with 2.0 μM PO_4^{3-} additions in all samples except bloom populations with Chl *a* > 1.0 μg L^{-1}, further suggesting that microbial communities in Florida Bay, despite maintaining APA under both N and P limitation, have the ability to switch from organic P to inorganic P when presented with PO_4^{3-} sources.

*Keywords:*Florida Bay, alkaline phosphatase

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activity, phytoplankton, bacteria, size-fractionated, phosphorus uptake, DOP, PO_4^{3-}

INTRODUCTION

Phytoplankton nutrient dynamics and nutrient cycling in subtropical estuaries are not as well studied as that of phytoplankton communities in temperate systems, especially those subtropical estuaries in which phosphorus (P) limitation occurs (Hu et al. 1990, Fourqurean et al. 1993, Glibert et al. 2004, 2006, Xu et al. 2008, 2009, Koch et al. 2009). Florida Bay is a semi-enclosed, shallow, P limited sub-tropical estuary subject to extreme physical, chemical and geological gradients, all of which influence pelagic nutrient dynamics within the Bay. Salinities can range from 0 to >50 depending upon hydrological and climatological conditions, the extent of mixing with the Gulf of Mexico, and large changes in freshwater inputs related to water management and precipitation patterns of a subtropical climate. High temperatures drive surface evaporation such that evaporation often exceeds precipitation and tidal flushing, resulting in hypersaline conditions (Lee and Johns 2007). Nutrient inputs and conditions can also vary greatly with flow regimes. Elevated dissolved organic nitrogen (DON) inputs occur in the northwestern, northern and northeastern regions of the Bay from the Everglades, while elevated anthropogenically-derived dissolved inorganic nitrogen (DIN) and DON inputs occur to the northeastern Bay and elevated P inputs occur to the western Bay from the Gulf of Mexico. Additionally, the geology of the region influences nutrients dynamics of phytoplankton via geochemical interactions between shallow carbonate sediments and P, which can result in significant P limitation in the eastern Bay. The combined impact of these spatial and temporally disparate nutrient sources to the Bay is that dissolved inorganic P concentrations are generally low, <0.2 μM (Boyer et al., 2006; Boyer et al., 1999) throughout the Bay, as are dissolved organic phosphorus (DOP) concentrations (Glibert et al. 2004).

These diverse hydrological, chemical and geological influences result in a Bay characterized by a strong east to west gradient in water quality (Boyer et al. 1997, 1999, Hitchcock and Brand 2007) and a system in which bacteria (Cotner et al. 2000), phytoplankton (Fourqurean et al. 1993, Phlips and Babyck 1996, Lavrentyev et al. 1998; Boyer et al. 2006, Armitage et al. 2005), seagrasses (Fourqurean et al. 1992, Powell et al. 1989, Armitage et al. 2005) and macroalgae (Lapointe 1989, Armitage et al. 2005) are generally P limited. Seagrass, phytoplankton and bacteria have also been shown to exhibit different levels of P limitation within this gradient, however (Fourqurean and Zieman 1992, Glibert et al. 2004). This is not unexpected given seagrasses reliance on sedimentary nutrient sources and differences in the ability of some cyanobacteria such as *Synechococcus* spp., the recent cause of large blooms in Florida Bay (Glibert et al. 2009), to substitute non-phosphorus containing lipids for P-containing lipids to alleviate P limitation (Van Mooey et al. 2009). Some evidence of N limitation of phytoplankton has been reported from the western Bay, which is significantly influenced by P inputs from Gulf of Mexico waters (Boyer et al. 1997, 1999, Glibert et al. 2004) to its western regions. The central Bay, an area of large picocyanobacterial blooms of *Synechococcus* spp. in the 1990s (Phlips et al. 1999, Phlips and Babychak 1999) has been characterized by variable limitation of phytoplankton populations, generally either N or Si limited. While concentrations of both inorganic N and P are low throughout the Bay (Boyer et al. 2006), concentrations of dissolved organic N and P differ greatly, with DON present at up to 65 μM concentrations, while DOP is generally <0.5 μM (Boyer et al. 1997, 1999, Glibert et al. 2004). The low concentrations of both PO_4^{3-} and DOP throughout the Bay suggest that DOP cycling and bioavailability may play a role equivalent to that of PO_4^{-3} in regulating primary producers as well as heterotrophs in the Bay.

Uptake and assimilation of DOP by microorganisms requires alkaline phosphatase (AP), a cell surface enzyme that facilitates hydrolysis of DOP compounds to an organic carbon moiety and PO_4^{3-} which is subsequently available for uptake. Measurable amounts of AP have been reported associated with seagrasses (Koch et al. 2009), macroalgae (Lapointe 1989) and phytoplankton populations (Glibert et al. 2004, Koch et al. 2009) in Florida Bay. The biological and nutrient factors regulating AP activity (APA), especially along this east to west gradient of water quality and P to N nutrient limitation of pelagic primary producers in Flor-

ida Bay, is not well understood however. We examined seasonal and spatial patterns of APA in size fractionated phytoplankton populations across Florida Bay over a 4 year period, in conjunction with nutrient bioassay experiments, to determine how this enzyme regulates P supply across a water quality and nutrient limitation gradient within Florida Bay.

METHODS

Station sampling

Sampling efforts took place over 7 to 9 day periods in November 2002, March 2003, July 2003, March 2004, August 2004 and June 2005. Seven regularly sampled stations were sampled across a gradient in the Bay (Fig. 1) which encompassed previously documented regions in the transitional Gulf (#1, Sprigger Bank), west-central (#2, Rabbit Key), central (#3, Barnes Key, #4, Rankin Bight) and eastern Florida Bay (#5, Little Madiera Bay, #6, Sunset Cove, and #7, Duck Key). During each field effort a single station was sampled each morning between 0800 and 1000 hr. Approximately 100 L of water was collected from 0.1 m below the surface in rinsed (10% HCl, repeated DIW rinses) 20 L carboys, stored in the shade and returned to the laboratory for processing within1 hr of sampling. In the laboratory, station water was subdivided for station measurements and for nutrient addition bioassays. APA of the station water was measured immediately after subsampling according to Perry (1972). Approximately 300 ml of <3.0 μm, <1.0 μm and <0.2 μm fractions of the station water were obtained using Nuclepore filters with gentle (< 5 mm p.s.i.) pressure. Unfiltered station water and each fraction was divided into two clean (10% HCl, repeated DIW rinses) beakers: one beaker served for ambient measurements while the 2nd beaker was amended to a final concentration of 2 μM PO_4^{3-} immediately prior to the first fluorescence measurement via the addition of an appropriate volume from a $K_2H_3PO_4$ stock solution. Fluorescence of replicate samples of each fraction was determined after the addition of methyl fluorescein phosphate reagent and at 15 minute intervals thereafter for 1 hr using a Turner Design fluorometer. Optimal measurement time was determined during the November 2002 sampling from measurements conducted at 15 min intervals for 4 hrs to determine the linearity in fluorescence response in all station APA measurements. No attempts were made to examine the fluorescence kinetics of the Perry (1972) method for Florida Bay samples.

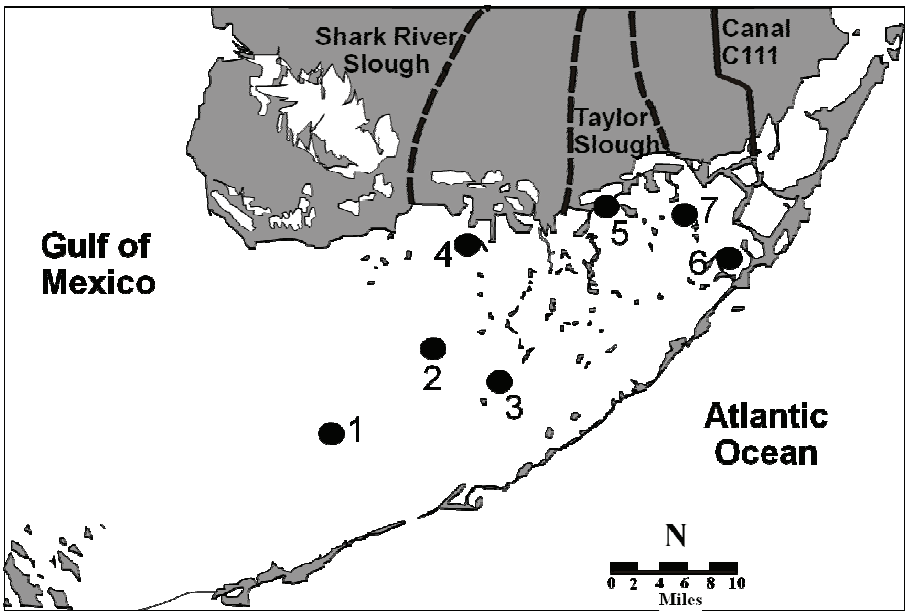

Figure 1. Map of Florida Bay with the locations of stations sampled from 2002 to 2006. Station numbers refer to the following named locations: #1) Sprigger Bank; #2) Rabbit Key; #3) Barnes Key; #4) Rankin Bight; #5) Little Madeira; #6) Duck Key and #7) Sunset Cove.

Station water was also analyzed for size fractionated (total, >3.0 μm, >1.0 μm) chlorophyll *a* (Chl *a*). Water was filtered through 0.2 μm, 1.0 μm and 3.0 μm Millipore Nucleopore filters, the filters were immediately frozen and Chl *a* was analyzed within 2 weeks of sample collection according to the method of Holm-Hansen et al. (1967). Fluorescence filter blanks were run for both sizes of Nucleopore filters during analyses; blank values were less than 0.01% of sample fluorescence. Size fraction ranges of Chl *a* for normalization of APA values were calculated by subtraction. Rep-

licates of the first aliquot of sample were filtered through precombusted (450°C for 4 h) GF/F filters to yield subsamples for analysis of total dissolved (TDP) and particulate P (PP) according to Solorzano and Sharp (1980). Filtrates from TDP sampling were frozen for subsequent analysis of dissolved nutrients. Concentrations of dissolved inorganic nutrients (NO_3^-, NH_4^+, PO_4^{3-}) were determined in triplicate using autoanalysis techniques (Atlas et al. 1971; Gordon et al. 1994).

Phosphorus Uptake Measurements

Phosphorus uptake was determined for each of the size fractions for which APA activity was determined by PO_4^{3-} disappearance from both natural and nutrient amended (final concentration, 2 μM PO_4^{3-}) samples. An additional 600 ml of station water was size fractionated coincident with APA measurements. Replicate samples of each size fraction were either left unamended, or amended with a PO_4^{3-} addition to a final concentration of 2 μM PO_4^{3-} and incubated immediately after amendment at 60% of ambient irradiance (achieved through neutral density screening) in floating corrals moored to a dock in Florida Bay located adjacent to the laboratory on Key Largo). After 30-60 minutes, bottle contents were filtered through 0.2 mM Nucleopore filters and the filtrate retained for PO_4^{3-} analysis as described above. Uptake was determined by disappearance of PO_4^{3-} after subtraction of appropriate blanks.

RESULTS

APA was present at all stations and in all size fractions throughout the study, although a large range of both volumetric- and biomass-normalized activities were found (Table 1). Volumetric-based APA ranged from 0.00 to 1.58 μM P L^{-1} h^{-1}, with the maximum value observed at Duck Key in the eastern Bay and minimum values consistently observed at Sprigger Bank in the western Bay. Dissolved APA ranged from 0.00 to 1.09 μM P L^{-1} h^{-1}, and constituted, on average, 42.6% (±24.7) of total activity at stations. Dissolved APA activity was generally highest during the summer sampling months in the eastern Bay region (Fig. 2) and lowest in the western Bay and displayed no relationship with either PO_4^{3-} or DOP concentrations (Fig. 3). Dissolved APA also displayed no relationship with the nutrient status of ambient microbial assemblages (as evident by biomass response in 48 hr nutrient addition bioassays; data not shown, see Glibert et al. 2004, 2009). Greatest percentages of dissolved APA were present when populations were both N and P limited (e.g. Rankin Bight and Little Madiera Bay stations in July 2003). Although no statistically significant relationship was observed between dissolved APA and Chl *a* (Fig. 4), the highest APA value observed in the dissolved fraction occurred at the Rankin Bight station in July 2003 during a bloom of the diatom *Cyclotella choctawatcheeana* in which a Chl *a* concentrations of 11.92 μg L^{-1} was measured.

Volumetric-based APA rates in the total particulate fraction (Fig. 4) were highest at lower Chl *a* concentrations, ≤0.50 μg L^{-1}, (except for the aforementioned diatom bloom in Rankin Bight and a *Synechococcus* spp. bloom in Barnes Basin in November 2002; data not shown), which exhibited the highest particulate activity observed). Volumetric-based APA also displayed no relationship with either PO_4^{3-} or DOP concentration (Fig. 3). Average station biomass-based APA ranged from 0.00 to 1.88 μM P μg Chl a^{-1} h^{-1}, and was highest in the 0.2 – 1.0 μm fraction for all stations (Table 1, Fig. 2) followed by the >3.0 μm fraction in the western Bay and the 1.0-3.0 μm fraction in the eastern Bay. No other consistent patterns were observed in either the seasonal or spatial allocation of activity between the different size fractions (Fig. 2), although the relative percentage contribution of each fraction tended to be similar across the Bay during each sampling period except in areas of high bloom biomass (e.g. Rankin Bight in July 2003).

Uptake rates of PO_4^{3-} for the total microbial assemblage (>0.2 μm fraction) ranged from 0.00 to 1.39 μM P L^{-1} h^{-1} and were generally highest at stations with the lowest volumetric based APA activity and lowest at stations with the highest APA activities, < 1.25 μM PO_4^{3-} L^{-1} h^{-1} (Fig. 5), but variable in the range between the two extremes.

A suppressive effect of additions of 2.0 μM of PO_4^{3-} to the APA of ambient assemblages was evident in all samples except those in which Chl *a* concentrations exceeded 1.0 μg L^{-1}. This response was typical of responses to PO_4^{3-} additions for all sampling periods, so only March 2004 data is shown (Fig. 6C, D). Suppression of APA activity

Table 1. Summary of ambient size fractionated Chlorophyll *a* and APA data from all stations during all sampling periods along a west to east transect in Florida Bay. Dissolved APA is defined as the APA present in the <0.2 μm size fraction.

Station	Size Fraction	Chlorophyll *a* (μg L^{-1}) Mean (±S.D)	Range	n	Volumetric-based APA (μM P L^{-1} h^{-1}) Mean (±S.D)	Range	n	Biomass-normalized APA (μM P mg Chl a^{-1} h^{-1}) Mean (±S.D)	Range	n
Sprigger Bank	>0.3 μm	0.50 (±0.35)	0.23 – 1.06	5	0.03 (±0.08)	0.00 – 0.17	5	0.29 (±0.64)	0.00 – 0.22	5
	1.0-3.0 μm	0.15 (±0.09)	0.00 – 0.24	5	0.05 (±0.06)	0.00 – 0.14	5	0.25 (±0.32)	0.00 – 0.77	5
	0.2 – 1.0 μm	0.22 (±0.23)	0.00 – 0.48	5	0.06 (±0.08)	0.00 – 0.20	5	0.39 (±0.51)	0.00 – 1.09	4
	>0.2 μm	0.84 (±0.35)	0.49 – 1.29	6	0.12(±0.12)	0.01 – 0.35	6	0.12 (±0.10)	0.01 – 0.22	5
	<0.2 μm				0.03 (±0.03)	0.00 – 0.07	5			
Rabbit Key	>0.3 μm	0.28 (±0.23)	0.07 – 0.60	5	0.04 (±0.04)	0.00 – 0.10	5	0.52 (±0.40)	0.19 – 1.17	5
	1.0-3.0 μm	0.15 (±0.20)	0.00 – 0.50	5	0.04 (±0.05)	0.00 – 0.12	5	0.12 (±0.20)	0.00 – 0.47	5
	0.2 – 1.0 μm	0.12 (±0.16)	0.00 – 0.41	5	0.10 (±0.06)	0.00 – 0.15	5	1.44 (±1.41)	0.00 – 3.38	5
	>0.2 μm	0.91 (±1.23)	0.17 - 3.39	6	0.39 (±0.17)	0.24 – 0.69	6	0.62 (±0.31)	0.27 – 1.00	5
	<0.2 μm				0.18 (±0.19)	0.06 – 0.51	5			
Barnes Basin	>0.3 μm	0.23 (±0.10)	0.10 – 0.34	4	0.06 (±0.05)	0.04 – 0.13	4	0.33 (±0.24)	0.00 – 0.59	5
	1.0-3.0 μm	0.06 (±0.07)	0.00 – 0.16	4	0.04 (±0.04)	0.00 – 0.09	4	0.54 (±0.52)	0.00 – 1.24	4
	0.2 – 1.0 μm	0.19 (±0.07)	0.13 – 0.26	4	0.19 (±0.11)	0.04 – 0.29	4	1.74 (±1.79)	0.29 – 4.74	5
	>0.2 μm	2.10 (±3.66)	0.29 – 8.64	5	0.77 (±0.35)	0.39 – 1.17	5	0.69 (±0.43)	0.26 – 1.22	4
	<0.2 μm				0.42 (±0.40)	0.16 – 1.01	4			
Rankin Bight	>0.3 μm	1.11 (±0.82)	0.27 – 2.20	5	0.12 (±0.18)	0.00 – 0.44	5	0.29 (±0.27)	0.00 – 0.62	5
	1.0-3.0 μm	2.07 (±4.50)	0.00 – 10.11	5	0.13 (±0.16)	0.00 – 0.38	5	0.00 (±0.00)	0.00 – 0.00	5
	0.2 – 1.0 μm	0.37 (±0.32)	0.09 – 0.87	5	0.17 (±0.11)	0.04 – 0.33	5	0.81 (±0.83)	0.17 – 2.24	5
	>0.2 μm	2.97 (±4.41)	0.73 – 11.92	6	0.55 (±0.38)	0.29 – 1.25	6	0.37 (±0.43)	0.00 – 1.10	5
	<0.2 μm				0.31 (±0.44)	0.10 – 1.09	5			
Little Madiera Bay	>0.3 μm	0.15 (±0.32)	0.20 – 0.54	4	0.14 (±0.19)	0.00 – 0.41	4	0.54 (±0.59)	0.00 – 1.36	4
	1.0-3.0 μm	0.11 (±0.07)	0.00 – 0.43	4	0.14 (±0.13)	0.00 – 0.29	4	0.68 (±0.59)	0.28 – 1.36	3
	0.2 – 1.0 μm	0.19 (±0.27)	0.05 – 0.43	4	0.21 (±0.18)	0.04 – 0.41	4	1.09 (±1.08)	0.09 – 2.24	3
	>0.2 μm	0.29 (±0.63)	0.29 – 0.93	5	0.73 (±0.40)	0.37 – 1.26	5	0.89 (±0.44)	0.44 – 1.49	4
	<0.2 μm				0.37 (±0.27)	0.14 – 0.60	4			

Table 1. Continued.

Sunset Cove	>0.3 μm	0.10 (±0.19)	0.10 – 0.32	4	0.04 (±0.05)	0.00 – 0.10	4	0.23 (±0.28)	0.00 – 0.60	4
	1.0-3.0 μm	0.16 (±0.17)	0.02 – 0.37	4	0.03 (±0.02)	0.00 – 0.05	4	0.65 (±1.22)	0.00 – 2.48	4
	0.2 – 1.0 μm	0.68 (±0.36)	0.00 – 1.37	4	0.22 (±0.16)	0.03 – 0.40	4	1.88 (±3.01)	0.00 – 5.35	3
	>0.2 μm	0.59 (±0.69)	0.16 – 1.53	4	0.56 (±0.34)	0.18 – 0.89	5	0.75 (±0.98)	0.23 – 2.21	4
	<0.2 μm				0.39 (±0.27)	0.09 – 0.75	4			
Duck Key	>0.3 μm	0.10 (±0.22)	0.08 – 0.32	5	0.03 (±0.03)	0.00 – 0.08	5	0.26 (±0.19)	0.00 – 0.47	5
	1.0-3.0 μm	0.04 (±0.03)	0.00 – 0.08	5	0.05 (±0.07)	0.00 – 0.16	5	0.60 (±0.67)	0.00 – 1.70	5
	0.2 – 1.0 μm	0.14 (±0.15)	0.02 – 0.37	5	0.17 (±0.06)	0.12 – 0.26	5	0.87 (±0.15)	0.71 – 1.00	3
	>0.2 μm	0.13 (±0.32)	0.12 – 0.47	5	0.71 (±0.51)	0.29 – 1.58	5	1.46 (±1.57)	0.39 – 4.15	5
	<0.2 μm				0.32 (±0.22)	0.06 – 0.55	5			

Figure 2. Average (as % of total) contribution of dissolved (<0.2 μm) and particulate (0.2 – 1.0 μm, 1.0 – 3.0 μm, and >3.0 μm) fractions of volumetric-based alkaline phosphatase activity to total activity measured along a west to east transect of stations during each sampling periods A) March 2003, B) July 2003, C) March 2004, D) August 2004 and E) June 2005. Stars (*) indicate that a station was not sampling.

Figure 3. Dissolved (<0.2 μm) and particulate (>0.2 μm) volumetric-based alkaline phosphatase activity versus ambient PO_4^{3-} and DOP concentrations for all stations during all sampling periods.

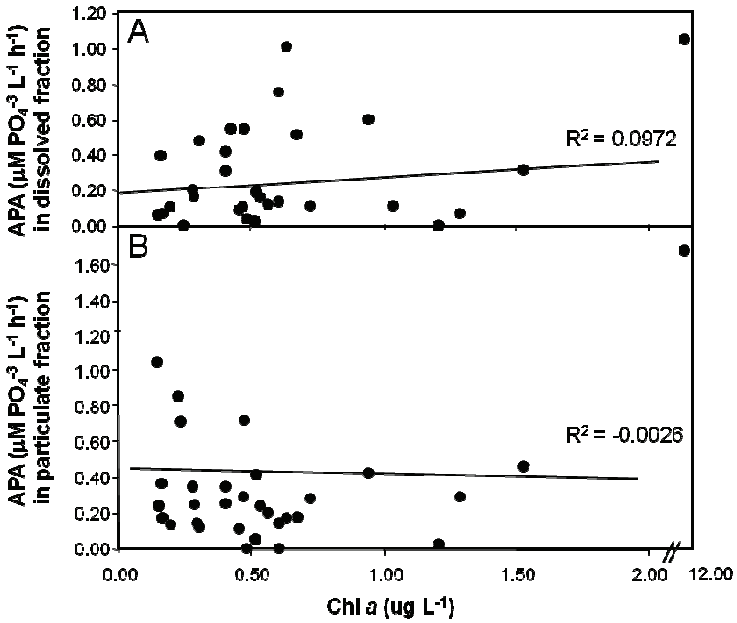

Figure 4. Volumetric-based alkaline phosphatase activity in the A) dissolved (<0.2 µm) and B) particulate (>0.2 µm) fractions versus Chl *a* concentrations for all stations over the entire sampling period.

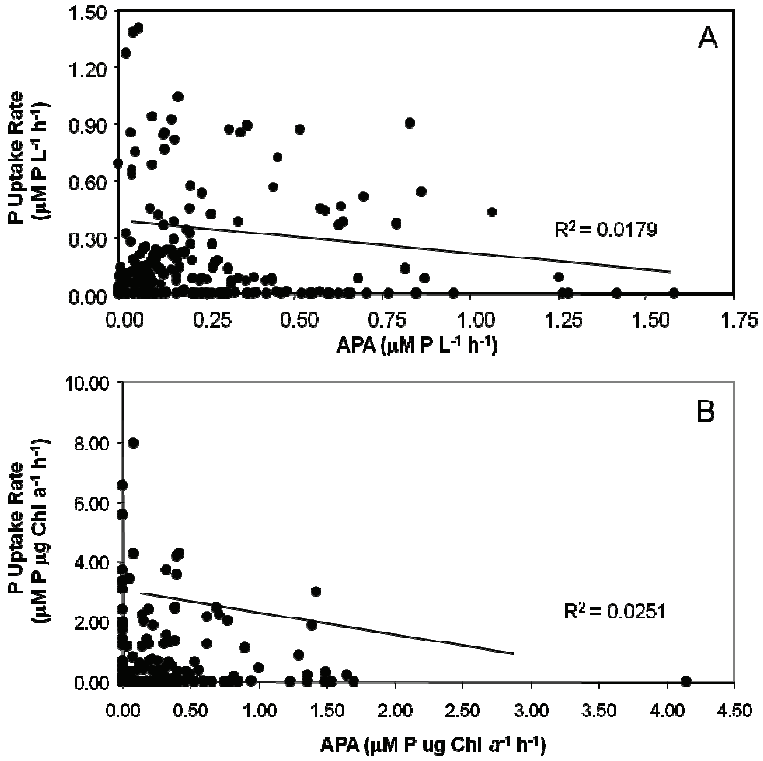

Figure 5. PO_4^{3-} uptake rates of the entire microbial assemblage versus A) volumetric-based alkaline phosphatase activity and B) biomass normalized alkaline phosphatase activity for all stations during all sampling periods at ambient PO_4^{3-} concentrations and with the addition of 2.0 µM PO_4^{3-}.

ranged from 40 to 60 % and in no instance was APA 100% suppressed by PO_4^{3-} additions. This suppressive effect was consistent over all size fractions of APA examined in the western, central and eastern Bay. The only exception to this suppression effect, observed when high concentrations of Chl *a* was present, is shown for July 2003 (Fig. 6A, B). During the July 2003 sampling either no APA suppression (Gulf and Little Madiera Bay stations) or an enhancement (Rankin Bight) of APA was observed with PO_4^{3-} additions, but only at stations with Chl a > 1.0 µg L^{-1}.

DISCUSSION

The presence of APA in all size fractions examined throughout the Bay, during all sampling periods, supports the importance of DOP availability and cycling to the entire pelagic microbial community within this shallow, carbonate dominated system. Koch et al (2009) reported similar results for size fractionated APA in size fractionated microbial communities and related particulate APA to seasonal blooms of algae, while dissolved APA was relatively constant. Dissolved APA was more variable in the current study, but did not display any direct relationship with any of the chemical or biological variables examined.

Although the highest particulate APA value, both on a volumetric and biomass normalized basis, was measured in the highest biomass phytoplankton bloom observed (11.02 µg Chl *a* L^{-1}), particulate APA was highly variable over the entire range of measured Chl *a*, PO_4^{3-} and DOP concentrations during the study, suggesting that

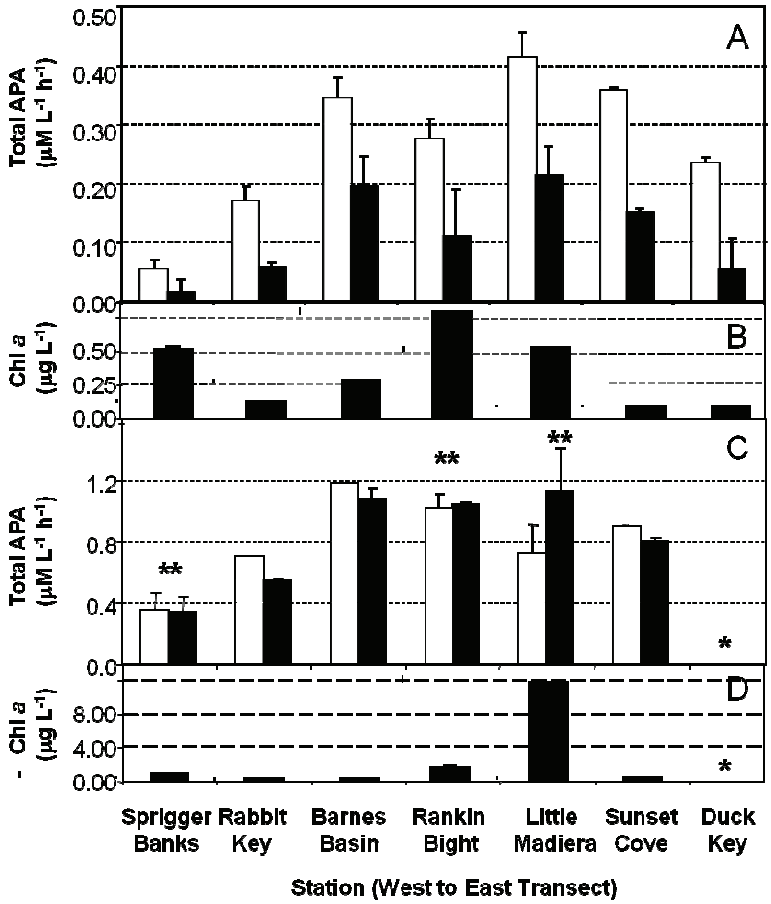

Figure 6. Influence of 2.0 μM PO_4^{3-} additions on total volumetric based alkaline phosphatase activity and its relationship with ambient Chl *a* concentrations during July 2003 and March 2004 sampling. A) Total volumetric based alkaline phosphatase activity for each station before (white bars) and immediately after (black bars) the addition of 2.0 μM PO_4^{3-} during July 2003 sampling, B) Total Chl *a* concentrations for each station during the July 2003 sampling, C) Total volumetric based alkaline phosphatase activity for each station before (white bars) and immediately after (black bars) the addition of 2.0 μM PO_4^{3-} during March 2004 sampling, and D) Total Chl *a* concentrations for each station during the March 2004 sampling. A star (*) indicate that a station was not sampling. Two stars (**) indicated that there was no significant difference in APA activity with 2.0 μM PO_4^{3-} additions at that site.

the physiological status of the microbial populations had a greater influence on APA than the magnitude of biomass present or ambient P concentration. This is supported by the inverse relationship between PO_4^{3-} uptake rates of the assemblage and APA activity (Fig. 5) and many studies that have linked the expression of APA in algae with cell quota and the nutrient status of the algae (Hoppe, 2003 and refs therein). The fact that neither Chl *a*, nor ambient PO_4^{3-} or DOP concentrations were a good predictor of APA activity in any size fraction examined of the microbial populations in Florida Bay suggests that management decisions regarding the significance of DOP inputs to Florida Bay based solely on ambient concentrations may be underestimating the significant of DOP cycling within the Bay.

The near continuous expression of APA by all size fractions throughout the Bay may be an adaptive strategy for living in a shallow, carbonate dominated estuary where geochemical reactions compete with biological uptake for available P (Orem et al. 1999; Nielson et al. 2006), resulting in consistently low concentrations of both PO_4^{3-} or DOP. Interestingly, APA was present regardless of the nutrient limitation status of assemblages (as indicated by response of the >0.7 μm fraction in coincident 48 hr nutrient addition bioassays, see Glibert et al. 2004, 2009). For example, values of between 33 and 100% of total APA were present in the particulate fraction at the Gulf station during all surveys throughout the study, despite a significance biomass response to inorganic and organic N additions in bioassays at this station. This suggests that the presence of measurable APA in microbial populations in Florida Bay may be directly linked to low ambient concentrations of PO_4^{3-} and DOP (which ranged from 0.02-0.08 and 0.02-0.59 μM respectively at the Gulf station during surveys) in addition to the nutrient status of the plankton.

Although no significant relationship between volumetric-based APA and either PO_4^{3-} or DOP (Fig. 3) was observed, overall concentrations of both P fractions were low throughout the Bay for the entire study: < 0.14 μM for PO_4^{3-} and <0.59 μM for DOP. A concentration of 0.5 μM PO_4^{3-} is often cited as the cutoff for induction of APA (Vargo and Shanley 1985; reviewed in Hoppe 2003). The hy-

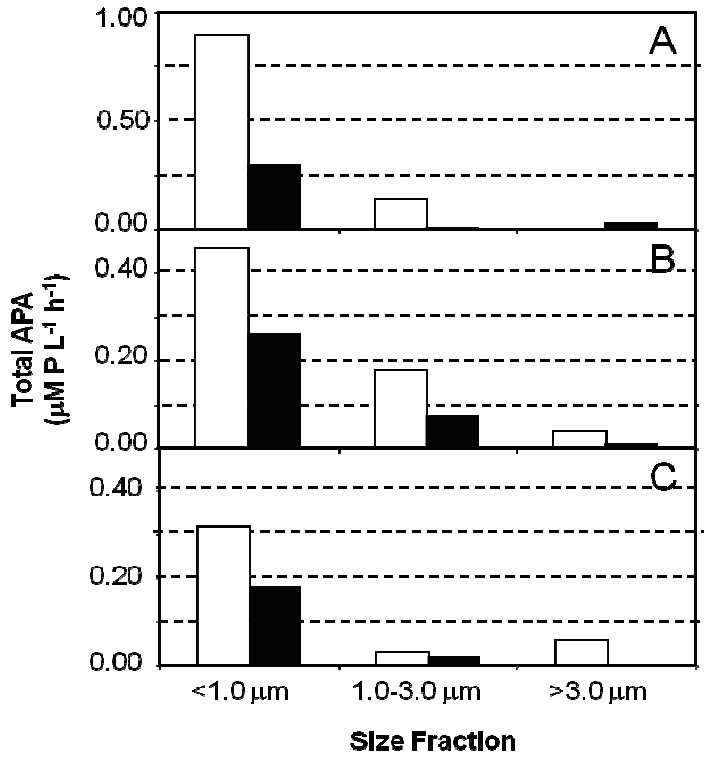

Figure 7. Size fractionated volumetric-based alkaline phosphatase activity volumetric-based alkaline phosphatase activity for A) western (Gulf Station), B) central (Barnes Key) and C) eastern (Sunset Cove) station water before (white bars) and immediately after (black bars) the addition of 2.0 μM PO_4^{3-} for the March 2004 sampling.

pothesis that ambient PO_4^{3-} or DOP concentrations are too low for total repression of APA with increasing concentrations of either nutrient is further supported by the effects of 2.0 μM PO_4^{3-} additions on APA of ambient assemblages. Repression of APA with PO_4^{3-} additions was evident at all stations tested at which Chl *a* concentrations were less than 1.0 μg L^{-1} in July 2003 and March 2004 (Fig. 6). Only at stations experiencing blooms, e.g. Rankin Bight and Little Madiera Bay in March 2004, was APA unaffected by PO_4^{3-} additions. This lack of repression is probably related to the higher amounts of phytoplankton biomass present, which likely were able to take up and assimilate the PO_4^{3-} addition too rapidly for it to repress APA activity in these populations. Additionally, co-limitation of APA by both P and carbon, as has been shown by Boyer et al. (2006) for bacterial populations in eastern Florida Bay, is not precluded based on the current analysis however, as significant APA activity was present throughout the Bay in the 0.2 -1.0 μm fraction (Fig. 2) which included bacteria.

Interestingly, PO_4^{3-} additions did not completely suppress APA in any population measured, even at low Chl *a* concentrations. This suggests that the different microbial populations comprising the > 0.2 μm fraction throughout the Bay are poised to utilize pulses of both DIP or DOP, regardless of their prior nutrient history or community composition. The lack of complete enzyme repression in the particulate fraction may be attributable to a diverse microbial community with a variable nutrient status or potentially to a fraction of the ambient microbial assemblage containing PO_4^{3-}-irrepressible APs. The APA of *Synechococcus* spp., which bloomed at the Barnes Basin station in October 2002 (Glibert et al. 2004), and in the eastern Bay stations from June 2005 through 2008 (Glibert et al. 2009), has been well documented (Grillo and Gibson 1979; Ray et al. 1991; Li et al. 1998; Glibert et al. 2004) and Wagner et al. (1995) has shown that *Synechococcus* possesses both PO_4^{3-} repressible and PO_4^{3-} irrepressible APs. Combined with its small size which reduces nutrient diffusion limitation of nutrient uptake and its ability to substitute out non-P containing lipids in membranes under P limitation (Van Mooey et al. 2009), *Synechococcus* is especially well adapted to compete with other bacteria and phytoplankton at the low inorganic P conditions observed in Florida Bay. Its lack of large internal nutrient storage pools, however, suggests that it may rely more heavily on recycled forms of P than other algae. The continual presence of measurable amounts of dissolved APA throughout the Bay may contribute to initiating and maintaining *Synechococcus* blooms through a continual supply of PO_4^{3-} from water column DOP hydrolysis. Conversely, *Synechococcus* itself may be a source of APA in the dissolved fraction as Li et al. (1998) found that dissolved APA produced by *Synechococcus* is stable for between 2 and 40 days.

The source of relatively high amounts of dissolved APA activity in Florida cannot be discerned

from the current study. Elevated concentrations of the enzyme have been reported in other coastal areas (e.g., the Red Sea, (Li et al. 1998) and the Bay of Biscay (Labry et al., 2005)). In Florida Bay, high levels of dissolved APA may indirectly relate to the shallow average depth of the Bay, which results in a water column subject to both frequent resuspension events as well as the continual effects of exposure to high levels of ultraviolet radiation. Sloppy feeding associated with zooplankton grazing has been shown to result in increased concentrations of dissolved APA (J. O'Neil, pers. comm.). Although some of the biomass responses in nutrient addition bioassays were suggestive of a grazing limitation of biomass (Fig. 2), the percentage of total APA in the dissolved form in these bioassays was highly variable and not suggestive of a direct correlation with ambient grazing activity

Although historically characterized by an east-west gradient in nutrient limitation of primary production and biomass which is further supported by 48 hr nutrient addition bioassay results in the current study, Florida Bay is also characterized by the year round presence of the enzyme AP in both dissolved and particulate fractions in the water column. The dichotomy of the presence of measurable amounts of APA in N limited microbial populations may be due in large part to the continually low ambient concentrations of both PO_4^{3-} and DOP throughout the Bay. Combined with the lack of a significant relationship between enzyme activity and Chl *a*, PO_4^{3-} and DOP, these results suggest that low ambient PO_4^{3-} and DOP concentrations are not necessarily a good indicator of either organic P utilization or P limitation, and that low DIN:DIP ratios may not necessarily imply N limitation in this system.

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WATER COLUMN NITROGEN CYCLING AND MICROBIAL PLANKTON IN FLORIDA BAY

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ABSTRACT

Water column ammonium potential uptake and regeneration rates (light and dark) were determined in isotope ($^{15}NH_4^+$) dilution experiments and compared to microbial plankton composition in Florida Bay (USA). Water samples were collected from four sites in August 2004, January 2005, and November 2006, and mean light and dark potential uptake rates (0.01 – 0.51 μM N h^{-1} and 0.01 – 0.48 μM N h^{-1}, respectively) significantly exceeded regeneration rates (0.01 – 0.10 and 0 – 0.09 μM N h^{-1}, respectively). Maximum potential uptake rates occurred in November during a cyanobacterial bloom and coincided with peak bacteria and heterotrophic nanoflagellate abundances. Combined with the lack of light/dark differences, these results indicate that planktonic bacteria may be responsible for a large portion of total NH_4^+ uptake. Potential uptake to regeneration ratios correlated ($p < 0.01$) with choreotrich ciliate abundance to chlorophyll *a* concentration ratios, suggesting that microzooplankton herbivory is important in water column N recycling. Sediment N regeneration rates were calculated from sediment-water interface nutrient fluxes and N transformations (described elsewhere). Sediment dissolved inorganic N fluxes were summed and added to denitrification rate estimates to determine areal sediment regeneration rates (107 – 595 μmol N m^{-2} h^{-1}). Water column NH_4^+ regeneration rates were depth-averaged and converted to areal rates for comparison with sediment regeneration rates. These depth-averaged regeneration rates were highest in November and ranged from 6.80 – 73.4 μmol N m^{-2} h^{-1}, accounting for 1.13 – 30.3 % of total system regeneration. These results suggest that nutrient regeneration in sediments play a major role in supporting primary production in Florida Bay, but this role diminishes during cyanobacterial blooms.

Keywords: nitrogen, uptake, regeneration, phytoplankton, bacteria, microzooplankton

INTRODUCTION

Primary production in aquatic systems often depends on nitrogen (N) regenerated within the microbial food web (e.g., bacterial remineralization and protist excretion; Eppley and Peterson 1979, Glibert 1988) and "new" N inputs from outside the system (e.g., allochthonous inputs; Dugdale and Goering 1967, Bode et al. 2002). The concept of new N entering oceanic systems often refers to upwelling of nitrate (NO_3^-; Dickson and Wheeler 1995, Raimbault and Garcia 2008). However, new N can include other sources, such as advection, diffusion, atmospheric deposition, or terrestrial runoff (Dugdale and Goering 1967, Eppley and Peterson 1979, Flint et al. 1986, Glibert 1993, Dickson and Wheeler 1995, Capone 2000). System dependence on regenerated N is related inversely to

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the quantity of new N inputs (Glibert 1988, Dham et al. 2002, Lomas et al. 2002), but this relationship is less clear in coastal systems where new N likely includes inorganic and organic reduced forms, such as NH_4^+ and urea (Paasche 1988). Low NO_3^- to total N uptake ratios in coastal systems indicate that primary production depends in large part on reduced N forms (Paasche 1988), including organic N (e.g., Glibert et al. 2004).

While diatoms are well-adapted to using NO_3^- (Glibert 1988, Yin et al. 1998, Lomas and Glibert 1999, Lomas and Glibert 2000, Heil et al. 2007), NH_4^+ is the preferred inorganic N source for most primary producers (McCarthy et al. 1977, Thompson et al. 1989, Berg et al. 2003). Ammonium also can be an important N source for heterotrophic bacteria (Glibert 1993, Vallino et al. 1996, Veuger et al. 2007). Ammonium uptake and regeneration rates in coastal systems are high compared to offshore waters due to higher biomass and primary productivity (Paasche 1988, Dickson and Wheeler 1995). Also, benthic regeneration can contribute significantly to water column NH_4^+ supply, especially in shallow, coastal environments (Flint et al. 1986, Paasche 1988, Ziegler and Benner 1999, Warnken et al. 2000, An and Gardner 2002, LaMontagne et al. 2002), but the relative importance of benthic versus water column regeneration of nutrients is not clear for most coastal ecosystems.

Florida Bay is a shallow (mean depth 1.4 m), subtropical, marine estuary with distinct wet (May – October) and dry (November – April) seasons. Florida Bay is bounded to the north by mainland Florida, to the east and south by the Florida Keys island chain, and to the west by the Gulf of Mexico. Physical exchange with the Atlantic Ocean is restricted to a few tidal channels along the Florida Keys (Smith 1998, Gibson et al. 2008), but exchange with the Gulf of Mexico, driven by wind and tides, occurs readily (Wang et al. 2004). However, a complex system of shallow basins separated by mud banks reduces inter-basin water exchange and can lead to long residence times within the basins (Kelble et al. 2007). Sediments consist of carbonate muds, and distinct dry and wet seasons affect system salinity and nutrient delivery from freshwater inputs (Boyer et al. 1999).

Large-scale seagrass die-offs began in 1987, and hypothesized causes include hypersalinity, sulfide toxicity, and abnormally high temperatures (Koch et al. 2007). A review of available literature summarized some of the ecological changes affecting Florida Bay and concluded that there was no clear causal relationship between these changes and human activities (Fourqurean and Robblee 1999). Other investigators have hypothesized that nutrient enrichment from cultural eutrophication is the underlying cause of seagrass die-offs and other ecological changes in Florida Bay (Lapointe and Barile 2004). This view was supported indirectly by description of a complex cascade of stressors starting with nutrient enrichment and ending with seagrass asphyxiation via an oxygen imbalance (Koch et al. 2007).

Florida Bay has experienced increasing occurrences of phytoplankton blooms (e.g., Lavrentyev et al. 1998, Boyer et al. 1999, Phlips et al. 1999, Boyer et al. 2006). These blooms consist primarily of picocyanobacteria from the genus *Synechococcus* and occur most frequently in the north-central region of Florida Bay (Phlips et al. 1999). The superior competitiveness of *Synechococcus* for scarce phosphorus (P) and relatively abundant ammonium (NH_4^+) in Florida Bay, combined with its abilities to thrive at low irradiances, resist viral infection, and adapt to salinity variations, may help explain the occurrence of these blooms (Lavrentyev et al. 1998).

The primary objective of this project was to measure light and dark water column NH_4^+ uptake and regeneration rates and relate them to water column characteristics and microbial plankton abundance in Florida Bay. A broader goal was to compare depth-averaged water column NH_4^+ regeneration rates to those at the sediment-water interface. This comparison may provide insights into nutrient sources important for primary production in Florida Bay.

METHODS

Study Site

Surface water was collected from four sites in Florida Bay in August 2004 (wet season), January 2005 (dry season), and November 2006 (transitional from wet to dry season). The third sampling event, originally scheduled for November 2005, was postponed by one year due to severe damage to the Keys Marine Laboratory from Hurricane Wilma in October 2005. Sampling sites were

located in the central bay (Rabbit Key; Latitude 24° 58.555N, Longitude 80° 49.183W), northwest region (Murray Key; Latitude 25° 06.357N, Longitude 80° 56.582W), north-central region (Rankin Key; Latitude 25° 07.437N, Longitude 80° 47.546W), and northeast region (Duck Key; Latitude 25° 10.764N, Longitude 80° 29.342W; Fig. 1). Sampling sites correspond to those described previously (e.g., Boyer et al. 1999, Cotner et al. 2000, Williams et al., 2008). All sites were shallow (< 1.2 m) with well-mixed water columns. In November 2006, a picocyanobacteria bloom was present at the Rankin and Rabbit Key sites.

Water Column Background Measurements

Water depth, salinity, temperature, photosynthetically active radiation (PAR), dissolved oxygen (DO) and chlorophyll *a* concentration (as fluorescence) were measured using a Hydrolab Datasonde 4 with Surveyor 4 data logger. The Hydrolab is equipped with a WET Labs WETStar Miniature fluorometer and circulating pump. Chlorophyll *a* measurements with the Hydrolab were verified with acetone extractions in a previous study, and a plot of Hydrolab versus acetone extraction values gave $r^2 = 0.9917$ with a line slope of 1.07, indicating good agreement between the methods (First 2002).

Water samples for nutrient analyses were filtered (0.2 µm syringe filter) in the field and frozen for later analysis on a Lachat Quikchem 8000 flow-injection auto-analyzer (NO_3^-+NO_2^-, NO_2^-, and o-PO_4^{3-}) and high performance liquid chromatography (NH_4^+; Gardner et al. 1995b). A salinity effect was discovered with the colorimetric sample peak integration for NO_2^- analyses, and these concentrations were corrected by quantifying the magnitude of this effect via a series of standard NO_2^- additions at various salinities. Sediment cores for nutrient fluxes, N transformation rates (denitrification, dissimilatory NO_3^- reduction to NH_4^+, and N_2 fixation), and sediment O_2 demand (SOD) also were collected at each site and sampling event and were used to determine areal sediment N regeneration rates (as the sum of net NH_4^+, NO_3^-, and NO_2^- fluxes and denitrification; Gardner and McCarthy, 2009).

Water Column Incubations and Sample Analyses

Water samples from each site were returned to the laboratory (Keys Marine Laboratory, Layton, FL) and enriched with 99 atom % $^{15}NH_4^+$ to a final concentration of ~8 µM. The samples were partitioned into triplicate light and dark polystyrene bottles (70 mL; Corning) and incubated at in situ light and temperature for ~24 hours. Dark bottles were wrapped with aluminum foil. In January 2005, a separate incubation was conducted using filtered (1 µm) site water. Initial (0 hours), intermediate (~14 hours; near dawn), and final (~24 hours) samples were filtered with 0.2 µm syringe filters (Osmonics) into 8 mL Wheaton glass vials and frozen. The first 3-4 mL of filtrate was discarded to prevent NH_4^+ contamination from the syringe filter. Total NH_4^+

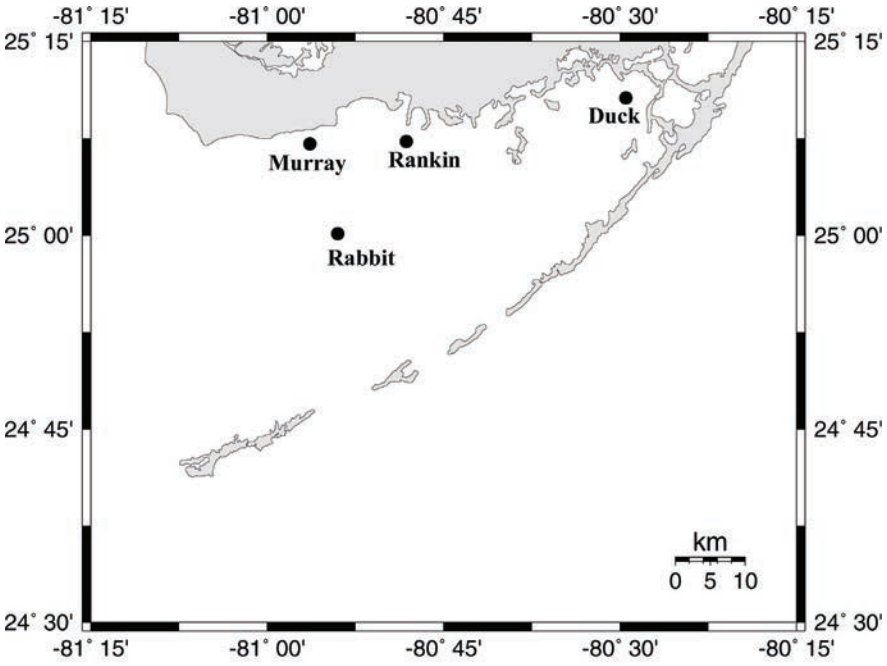

Figure 1. Location of sampling sites in Florida Bay.

concentration and atom % ^{15}N were determined using high performance liquid chromatography (HPLC; Gardner et al. 1995b). Potential NH_4^+ uptake and regeneration rates were calculated using the Blackburn/Caperon model (Blackburn 1979, Caperon et al. 1979). At the Rankin site in November 2006, the isotope addition was depleted (< 0.1 µM) at the final sampling, and rates determined from the intermediate sampling point (~14 hours) are reported. Only the 24-hour rates are reported for all other cases. No sampling event produced ambient NH_4^+ concentrations > 4.6 µM. Thus, substrate additions higher than the ambient concentrations were used in this study, and NH_4^+ uptake rates are qualified as "potential".

Volumetric water column NH_4^+ regeneration rates (in µmol N l^{-1} h^{-1}) were converted to depth-averaged areal rates (in µmol N m^{-2} h^{-1}) for comparison with areal sediment NH_4^+ regeneration rates. The shallow, well-mixed water column at the sampled sites allows the assumption that water column rates are uniform from the water surface to the sediment surface.

Microbial Plankton Counts

Water samples for enumerating microbial food web constituents were collected simultaneously with the isotope dilution experiments. Bacterioplankton were preserved in formaldehyde and immediately frozen in liquid N. In the laboratory, bacteria were stained with SYBR Green I and quantified by a FACSort flow cytometer (Marie et al. 1997; Jochem 2001). Heterotrophic nanoflagellates (HNF) were preserved in formaldehyde and counted on 0.8 µm membrane filters using epifluorescence microscopy after staining with DAPI. Microzooplankton (ciliates and dinoflagellates) preserved in Lugol's iodine were settled overnight in 50-100 ml chambers and counted under an inverted differential interference contrast microscope. Microzooplankton counts included both aplastidic and plastidic dinoflagellates, since mixotrophy is wide spread among these protists (e.g., Stoecker 1999).

Single factor analysis of variance (ANOVA) was used to identify statistical differences ($p < 0.05$) in rates among the sites, seasons, light/dark treatments, and potential uptake/regeneration rates. Statistical relationships between N cycling rates and microbial plankton abundances were analyzed using correlation and linear regression.

Table 1. In situ water column characteristics in Florida Bay. Values for August 2004 and January 2005 represent an average of two visits to each site. Sal = salinity. Temp = temperature. PAR = photosynthetically active radiation and is presented as the percentage of PAR at the water surface that reaches the sediment surface. DO = dissolved oxygen. Chl = chlorophyll. BDL = below detection limit (= 0.1 µM for NH_4^+).

Station	Date	Depth (m)	Sal	Temp (°C)	PAR %	DO mg l^{-1}	chl *a* µg l^{-1}	NH_4^+ µM	NO_3^- µM	NO_2^- µM	o-PO_4^{3-} µM
Rabbit	Aug-04	0.81	38.4	31.6	35	5.2	0.6	0.49	0.93	0.57	0.11
	Jan-05	0.95	38.3	23.0	36	4.6	1.5	0.61	0.89	0.38	0.04
	Nov-06	0.72	36.9	25.8	26	6.1	7.9	BDL	0.33	0.45	0.17
Murray	Aug-04	0.38	37.2	31.6	36	6.6	1.4	BDL	1.00	0.61	0.08
	Jan-05	1.24	33.7	23.5	17	5.1	4.3	0.35	0.63	0.29	0.04
	Nov-06	0.83	35.7	25.2	11	5.5	4.4	BDL	0.50	0.54	0.06
Rankin	Aug-04	0.18	40.2	31.8	59	4.7	1.7	1.49	1.08	0.61	0.06
	Jan-05	1.03	45.1	23.1	32	4.7	2.9	0.44	1.12	0.37	0.02
	Nov-06	0.74	31.7	25.3	7	7.4	7.0	BDL	0.25	0.42	0.12
Duck	Aug-04	0.93	41.5	31.1	49	5.1	0.0	1.17	1.18	0.58	0.10
	Jan-05	0.74	41.2	22.9	37	5.3	1.9	4.43	1.85	0.43	0.04
	Nov-06	0.78	31.4	24.9	30	5.0	1.6	0.42	0.28	0.45	0.05

RESULTS

Water Column Characteristics

Hydrological data are presented in Table 1. Water depths were very shallow (< 0.5 m) at Murray and Rankin in August, and all other water depths were between 0.7 and 1.2 m. Salinities were relatively stable at Rabbit (36.9 – 38.4) and Murray (33.7 – 37.2), but Duck and Rankin each had salinity > 40 in August and January and ~31.5 in November. Water temperatures were ≥ 23 °C, even in January, and exhibited a typical seasonal pattern with highest temperatures (~31.5 °C) in August and lowest temperatures in January (~23 °C). Water clarity, as the percentage of surface PAR reaching the sediments, also showed a seasonal pattern with highest water clarity in August (mean = 44.8 ± 5.7 %; mean ± standard error) and lowest in November (mean = 18.5 ± 5.6 %; $p = 0.02$), when the cyanobacteria bloom was observed. DO remained above hypoxic levels and ranged from 4.6 – 7.4 mg l^{-1}.

Chlorophyll *a* was < 4.5 µg l^{-1} except at Rankin and Rabbit in November (7.0 and 7.9 µg l^{-1}, respectively; Table 1), concomitant with observed cyanobacteria blooms. Seasonally, chlorophyll *a* values were significantly lower in August than November ($p = 0.02$) and marginally lower in August than January ($p = 0.056$). No significant differences were observed between sites despite the bloom conditions in November.

There were no significant differences between sites for ambient nutrient concentrations (Table 1). Dissolved inorganic N (DIN = $NH_4^+ + NO_3^- + NO_2^-$) concentrations were < 3.2 µM except at Duck in January (6.7 µM; Table 1), when NH_4^+ (4.4 µM) and NO_3^- (1.9 µM) concentrations peaked. NH_4^+ was the largest component of DIN at Rankin in August (47 %) and Duck in January (66 %), with NH_4^+ and NO_3^- essentially equivalent at Duck in August (40 %). NO_2^- was the largest component of DIN at all sites (39 – 63 %) in November despite low concentrations (range = 0.42 to 0.54 µM). In all other cases, NO_3^- (range = 0.25 – 1.85 µM) was the largest component of DIN (40 – 62 %). Orthophosphate concentrations ranged from 0.02 to 0.17 µM and were significantly lower in January than August ($p < 0.01$) and marginally lower in January than November ($p = 0.06$). DIN:o-PO_4^{3-} was lowest in November (12.6 ± 4.5 versus 30.1 ± 8.0 in August and 85.8 ± 30.6 in January) at all sites, but these differences were not statistically robust ($p = 0.11$ and 0.06, respectively).

Table 2. Light (L) and dark (D) potential NH_4^+ uptake (Up) and regeneration (Reg) rates (µmol l^{-1} h^{-1}) in the water column in Florida Bay. SE = standard error (n = 3).

Station	Date	Up (L)	Up (D)	Reg (L)	Reg (D)
Rabbit	Aug-04	0.053	0.041	0.043	0.041
	SE	0.004	0.005	0.004	0.004
	Jan-05	0.024	0.029	0.026	0.033
	SE	0.009	0.005	0.008	0.005
	Nov-06	0.281	0.204	0.102	0.090
	SE	0.007	0.004	0.007	0.007
Murray	Aug-04	0.041	0.026	0.025	0.023
	SE	0.009	0.003	0.005	0.005
	Jan-05	0.090	0.043	0.014	0.018
	SE	0.022	0.011	0.011	0.011
	Nov-06	0.057	0.041	0.021	0.025
	SE	0.004	0.010	0.005	0.013
Rankin	Aug-04	0.070	0.026	0.075	0.050
	SE	0.010	0.008	0.006	0.008
	Jan-05	0.014	0.006	0.021	0.017
	SE	0.011	0.009	0.009	0.008
	Nov-06	0.512	0.482	0.058	0.043
	SE	0.008	0.008	0.009	0.006
Duck	Aug-04	0.042	0.045	0.024	0.033
	SE	0.004	0.007	0.005	0.007
	Jan-05	0.036	0.024	0.040	0.036
	SE	0.016	0.021	0.013	0.018
	Nov-06	0.172	0.105	0.060	0.053
	SE	0.006	0.008	0.007	0.008

Potential NH_4^+ Uptake Rates

No clear trends were observed for potential NH_4^+ uptake rates across sampling sites or seasons (Table 2). Light and dark rates (μmol l^{-1} h^{-1}) were < 0.1 in all cases except in November at Rabbit, Rankin, and Duck. For Florida Bay as a whole, both light and dark potential uptake rates were significantly higher ($p < 0.01$) in November than August or January. Significant light/dark potential uptake differences occurred only at Rankin in August and Rabbit and Duck in November. At Rabbit and Rankin, seasonal differences in light potential uptake (November > August > January) were observed. A similar, but less significant, pattern (November > August ≥ January) was observed at Duck, and no significant seasonal differences were apparent at Murray (January ≥ November ≥ August). However, Murray was the only site that showed a significant effect from the size-fractionated incubation in January. Light potential uptake at Murray in the whole water (0.090 ± 0.022) was significantly higher ($p = 0.04$) than in the filtered water (< 1 μm; 0.025 ± 0.003; not shown).

NH_4^+ Regeneration Rates

No significant light/dark differences in NH_4^+ regeneration rates were observed at any time (Table 2). No consistent pattern in NH_4^+ regeneration rates was observed either between sites or between seasons, and rates (μmol l^{-1} h^{-1}) ranged from 0.014 ± 0.011 at Murray in January to 0.102 ± 0.007 at Rabbit in November. Seasonally, for Florida Bay as a whole, regeneration rates were significantly higher in November than January, but rates in August were not significantly different from either November or January. Regeneration rates at Murray were significantly lower than all of the other sites, which were not different from each other. Light regeneration rates were significantly lower than light potential uptake rates at all sites in November, Duck in August, and Murray in January. Regeneration rates were significantly lower than dark potential uptake rates at all sites except Murray in November, but regeneration was higher than dark potential uptake at Rankin in August. Regeneration/potential uptake ratios were highest and near 1 in January (0.96 ± 0.29) and August (0.77 ± 0.11) and lowest in November (0.30 ± 0.06). The filtered water incubation conducted in January did not show any significant effect on regeneration rates (not shown).

Depth-averaged water column NH_4^+ regeneration rates (μmol N m^{-2} h^{-1}) ranged from 6.8 at Rankin in August to 73.4 at Rabbit in November (Table 3). Depth-averaged regeneration at Murray was significantly lower than those at Rabbit ($p = 0.04$) and Duck ($p = 0.05$), but no other significant differences were observed between sampling sites. Mean Florida Bay depth-averaged regeneration in November was 45.1 ± 11.5 versus 23.3 ± 2.6 in January and 20.3 ± 4.3 in August, but these seasonal differences were not significant. However, the proportion of water column to total regeneration (depth-averaged water column regeneration plus sediment regeneration; Fig. 2) in November (0.22 ± 0.05) was higher than January (0.08 ± 0.02; $p = 0.04$). This proportion in August (0.10 ± 0.05) was not different from November or January.

Table 3. Net sediment (Sed) DIN fluxes (sum of NH_4^+, NO_3^-, and NO_2^-), net sediment N_2 fluxes (denitrification), sediment regeneration rates (Sed Reg; sum of sediment DIN flux and denitrification), and depth-averaged water column NH_4^+ regeneration (DA WC Reg) rates in Florida Bay. No sediment N_2 fixation was observed, so net N_2 fluxes represent an estimate of denitrification. Sediment regeneration rates from Gardner and McCarthy (submitted).

	Date	Rabbit	Murray	Rankin	Duck
Sed DIN Flux (μmol N m^{-2} h^{-1})	Aug-04	103	165	447	193
	Jan-05	40	172	33	209
	Nov-06	146	33	81	382
Sed Net N_2 Flux (μmol N m^{-2} h^{-1})	Aug-04	6.1	49.1	65.0	53.3
	Jan-05	156	210	119	357
	Nov-06	39.3	7.5	109	73.1
Sed Reg (μmol N m^{-2} h^{-1})	Aug-04	109	214	512	246
	Jan-05	196	382	152	566
	Nov-06	185	40	190	455
DA WC Reg (μmol N m^{-2} h^{-1})	Aug-04	35.2	9.2	14.1	23.0
	Jan-05	24.7	17.4	21.6	29.6
	Nov-06	73.4	17.4	42.9	46.8

Table 4. Microbial plankton abundances in Florida Bay. HNF = heterotrophic nanoflagellates. N/D = not determined.

Station	Date	Bacteria 10^9 l^{-1}	HNF 10^5 l^{-1}	Total ciliates 10^3 l^{-1}	Choreotrich ciliates 10^3 l^{-1}	Dinoflagellates 10^3 l^{-1}
Rabbit	Aug-04	0.69	2.32	2.32	0.52	30.7
	Jan-05	0.59	3.31	4.19	1.98	7.11
	Nov-06	N/D	N/D	N/D	N/D	N/D
Murray	Aug-04	1.08	5.30	6.11	0.58	4.91
	Jan-05	1.02	7.21	8.59	1.46	2.22
	Nov-06	0.86	4.65	0.70	0.48	4.77
Rankin	Aug-04	1.49	7.21	4.39	2.64	9.97
	Jan-05	0.86	13.0	7.61	1.20	29.6
	Nov-06	15.2	62.1	10.0	2.18	58.9
Duck	Aug-04	0.95	4.03	5.50	2.16	22.5
	Jan-05	0.57	2.21	5.27	1.68	2.94
	Nov-06	0.97	11.2	3.43	0.48	7.77

Microbial Plankton and Relationships With N Cycling

Bacterioplankton abundance ranged from 0.57 x 10^9 to 1.49 x 10^9 cells l^{-1} and reached its maximum of 15.2 x 10^9 cells l^{-1} during the *Synechococcus* bloom at Rankin Key in November (Table 4). The abundance of heterotrophic nanoflagellates followed a similar pattern (2.21 x 10^5 to 13.0 x 10^5 cells l^{-1}, maximum 62.1 x 10^5 cells l^{-1} at Rankin). Bacteria and HNF were positively correlated ($p < 0.001$) with light and dark NH_4^+ potential uptake rates. However, these correlations were strongly biased by the data obtained at Rankin Key during the bloom. Removing these obvious outliers resulted in the loss of correlation. The ratio of HNF to bacteria remained weakly correlated with the dark regeneration to potential uptake ratio ($p = 0.08$). Although dinoflagellates and ciliates also peaked at Rankin in November (59.9 x 10^3 cells l^{-1} and 10.0 x 10^3 cells l^{-1}, respectively), the abundance of both groups of microzooplankton was distributed more evenly among the sites and dates. Among ciliates, choreotrichs (i.e., ciliates from the order Choreotrichida; Small and Lynn, 1985; including *Lohmaniella*, *Strobilidium*, and *Strombidinopsis*) were the most common, and their contribution to total ciliate abundance ranged from 9 to 68%. They were the only microbial plankton group to show a stable positive relationship with N cycling data. Specifically, the ratio of choreotrich abundance to chlorophyll *a* concentration remained correlated with the regeneration to potential uptake ratio (light $r = 0.78$ $p < 0.001$, dark $r = 0.64$, $p < 0.05$), whether the November bloom data were included or not. In contrast, the latter N cycling ratio was negatively correlated with the chlorophyll *a* to DIN ratio ($r = -0.76$, $p < 0.05$, light rates only).

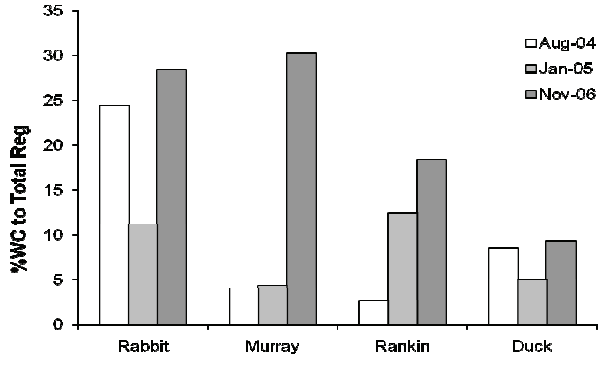

Figure 2. Percentage of depth-averaged water column NH_4^+ regeneration to total regeneration as the sum of depth-averaged water column NH_4^+ regeneration and sediment regeneration (sum of net sediment DIN flux and denitrification).

DISCUSSION

Water Column Characteristics

Mean DIN concentration (2.1 ± 0.5 µM) observed in Florida Bay during the sampling events in August 2004, January 2005, and November 2006 was less than 50 % of long-term mean DIN levels reported previously (~4.6 µM; Boyer et al. 1999). Present NO_3^- estimates were similar to long-term averages reported previously, but NH_4^+ concentrations were much lower than long-term median NH_4^+ estimates in Florida Bay (Boyer et al. 1999, Phlips et al. 1999). Further, long-term median NO_2^- estimates accounted for only ~3.5 % of DIN (Boyer et al. 1999), whereas NO_2^- comprised ~32 % of DIN in the present study (Table 1). These results support observations of rapidly increasing NO_2^- concentrations in the central basin of Florida Bay in the mid-1990's, which represented the last few years of the dataset evaluated (Boyer et al. 1999). However, previous observations that DIN was dominated by NH_4^+ in Florida Bay (Fourqurean and Robblee 1999, Cotner et al. 2000) were not supported in most cases in the present study, which found generally low NH_4^+ concentrations (< 1.0 µM; Table 1). Sediment core incubations conducted simultaneously with the present water column incubations revealed high NO_2^- and NH_4^+ effluxes (up to 100 and 800 µmol N m^{-2} h^{-1}, respectively) from sediments after $^{15}NO_3^-$ enrichment (Gardner and McCarthy, 2009). These results suggest that sediment processes are influencing water column DIN concentrations and available forms, but NH_4^+ scarcity in the water column shows that NH_4^+ released from sediments is rapidly taken up, assimilated, and/or transformed.

Previous studies have suggested that primary production in Florida Bay is P-limited (Fourqurean and Robblee 1999, Cotner et al. 2000). However, N limitation or co-limitation also has been observed in some cases (e.g., Lavrentyev et al. 1998). In the present study, available P (as o-PO_4^{3-}) was low (≤ 0.12 µM; Table 1), but DIN concentrations also were low in most cases. Nutrient limitation bioassays were not conducted, but DIN:o-PO_4^{3-} ratios below Redfield (i.e., N:P < 16) were observed at Rabbit (4.6) and Rankin (5.6) in November. Other investigators also have reported N:P ratios below 16 in Florida Bay (Glibert et al. 2004), thus implying some level of N limitation. As mentioned above, NH_4^+ is the favored inorganic N source for most primary producers, and NO_x assimilation requires intracellular reduction to NH_4^+ and is expensive energetically (Syrett 1981). This characteristic is particularly the case for cyanobacteria, which are superior competitors for reduced (Blomqvist et al. 1994) and organic N (Glibert et al. 2004) and often form blooms in Florida Bay (Lavrentyev et al. 1998, Phlips et al. 1999, Glibert et al. 2004). Thus, if NH_4^+:o-PO_4^{3-} ratios are considered, there were only 3 of 12 cases where this ratio exceeded 16, and none of these cases coincided with chlorophyll *a* concentrations > 3 µg l^{-1}. DIN:o-PO_4^{3-} may not be the best measure of nutrient limitation (e.g., Dodds 2003), but total N and P concentrations were not available for this project.

Potential NH_4^+ Uptake Rates

Significantly higher light and dark potential NH_4^+ uptake rates in November coincided with a picocyanobacteria bloom observed at all sites except Murray, which did not experience a phytoplankton bloom during the present study. Despite the clearly visible bloom conditions, measured chlorophyll *a* levels were not as high (maximum = 7.9 µg l^{-1} at Rabbit) as observed during other bloom events (> 20 µg l^{-1}; e.g., Phlips et al. 1999). A separate water sample collected from Rankin in November and analyzed using a grinding procedure found a chlorophyll *a* concentration of ~28 µg l^{-1} (Williams et al., 2008). Thus, it seems feasible that the Hydrolab fluorometer may have underestimated chlorophyll *a* from cyanobacteria. Note, however, that no significant relationships between chlorophyll *a* and potential NH_4^+ uptake rates were observed, although the small dataset generated here is not conducive to determining statistically significant differences between sampling sites and seasons. Excluding November, the observed range of potential NH_4^+ uptake rates in Florida Bay (0.014 – 0.090 µmol N l^{-1} h^{-1}; Table 2) is similar to phytoplankton N demand estimated from chlorophyll *a* concentrations, phytoplankton growth rates, and assuming Redfield ratios (0.021 – 0.063 µmol N l^{-1} h^{-1}; Burd and Jackson 2002).

With the exception of Rankin in August and Rabbit and Duck in November, light and dark potential uptake rates were not significantly different (Table 2). Light and dark water column incubations may provide information on the importance of

autotrophic versus heterotrophic processes to N cycling (e.g. Gardner et al. 2000). Light effects on heterotrophic nutrient regeneration often are less apparent than for autotrophic nutrient uptake (phytoplankton), since heterotrophs usually do not require light energy (Wheeler et al. 1989). Bacterial uptake can account for up to 78% of total NH_4^+ uptake in aquatic systems (Kirchman 2000, Gardner et al. 2004), and the results of ^{15}N incubations and the bacterial abundance peak observed at Rankin Key in this study suggest that bacterial uptake represented a large component of potential NH_4^+ uptake. A parallel study conducted simultaneously in Florida Bay found a significant positive relationship between NH_4^+ concentration and heterotrophic bacterial net growth rates (Williams et al. in 2008). However, some phytoplankton (Jochem 1999), especially cyanobacteria (Shi et al. 2007), can remain active in dark conditions. Therefore, dark potential uptake observed in the present study may include opportunistic autotrophic uptake as a consequence of N limitation, more specifically NH_4^+ limitation, in addition to heterotrophic NH_4^+ uptake.

Uptake of organic N compounds, such as urea and amino acids, was not evaluated in this study, but other investigators found substantial organic N uptake in the area near Rankin (Glibert et al. 2004) and a broader area of the southwest Florida shelf, including parts of Florida Bay (Heil et al. 2007). Amino acid additions stimulated particulate organic carbon accumulation in bacteria in Florida Bay (Cotner et al. 2000), which further supports the potential importance of organic N as a nutrient source for bacteria and bacteria to system nutrient uptake dynamics.

NH_4^+ Regeneration Rates

Water column NH_4^+ regeneration rates at Murray, which represents the most Gulf of Mexico influenced sampling site, were significantly lower than the other sites. Bacterial net growth rates also were lowest at this site, but other microbial food web components and activities did not differ from other sites (Williams et al. 2008). Sediment N transformations at Murray differed from other sites only in November, when the other sites were experiencing a cyanobacteria bloom (Gardner and McCarthy, 2009). Thus, intuitive explanations for the lower regeneration rates at Murray are not evident, except for a different hydrology via a more direct connection to the Gulf of Mexico and shorter residence times. This hydrology may provide organisms at Murray a more consistent supply of nutrients from the Gulf of Mexico, whereas the other sites depend more on nutrients from the mainland, groundwater, or internally regenerated nutrients, depending upon the timing of freshwater inflows.

In most cases, with November being a notable exception, balanced regeneration (light or dark) and dark potential uptake rates (Table 2) suggest that heterotrophic recycling (i.e., microbial grazing or bacterial remineralization) in the water column can sustain phytoplankton and bacterial NH_4^+ uptake requirements. In one half of the cases, where there are not significant differences between light potential uptake and regeneration, heterotrophic recycling may sustain combined bacterial and phytoplankton uptake. In November, water column regeneration could not sustain bacterial NH_4^+ uptake, except at Murray. At all sites in November, combined phytoplankton and bacterial NH_4^+ uptake likely was supplemented by new N from outside the system or sediment regeneration. This hypothesis is supported by observed similarity in spatial patterns between chl *a*, NO_2^-, and NH_4^+ in the central bay, which suggested that cyanobacteria blooms are supported by regenerated nutrients (Burd and Jackson 2002).

The only microbial parameter that showed a stable relationship with N cycling rates in this study was the abundance of choreotrich ciliates. These ciliates are flexible omnivores and can switch between herbivory and bacterivory (Fenchel 1987). Similar microzooplankton correlated with NH_4^+ regeneration rates in Lake Michigan (Gardner et al. 2004). In a parallel study, microzooplankton herbivory consumed a significant part of picoplankton primary production in Florida Bay, except during the bloom in November (Jochem and Lavrentyev, unpublished data). On the other hand, the abundance of bacteria and HNF increased by almost an order of magnitude in the bloom affected waters. The ratio between these organisms correlated with NH_4^+ regeneration rates in Lake Maracaibo (Gardner et al. 1998). It is plausible that, during cyanobacterial blooms in Florida Bay, N recycling in the water column is primarily driven by a heterotrophic rather than herbivorous food web.

Water Column versus Sediment Regeneration

Sediment denitrification may exacerbate or cause N limitation or co-limitation in marine systems (Seitzinger 1988, An et al. 2001), particularly in cases where new nutrients enter the system episodically (e.g., McCarthy et al. 2007b). In the absence of new N entering hydrologically isolated areas of Florida Bay, water column primary production (including cyanobacterial bloom persistence) is likely driven in part by sediment processes (i.e., organic matter mineralization, dissimilatory NO_3^- reduction to NH_4^+ (DNRA), and N fixation; An and Gardner 2002, Gardner et al. 2006). In dry seasons, primary production dependence on internal regeneration sources and N fixation likely increases.

In Florida Bay, depth-averaged water column NH_4^+ regeneration accounted for 11.9 ± 2.1 % (mean ± SE) of total regeneration, but this proportion was generally higher in November (~22 %; Table 3) during the cyanobacteria bloom and lowest in January (~8 %; dry season). A similar trend was observed in a hypereutrophic, shallow lake in China, where water column regeneration increased in importance during cyanobacteria bloom conditions (McCarthy et al. 2007a). Mean sediment regeneration in January (320 ± 95 µmol N m^{-2} h^{-1}) was higher than August (270 ± 60) or November (220 ± 86; Table 3), but these differences were not statistically significant for this small dataset. It is clear, however, that sediments play a major role in fueling system primary production, including seagrasses. This conclusion is reached even when denitrification is excluded as a sediment regeneration term, since this N must be fixed to re-enter the system in a form that is bioavailable to most organisms. With denitrification excluded, sediment DIN fluxes, which would include any DNRA, still accounted for ~76 % of total measured regeneration (Table 3). DNRA rates in Florida Bay sediments generally exceeded denitrification rates except in January, when denitrification exceeded DNRA (Gardner and McCarthy, 2009). Denitrification in January was fueled almost exclusively by NO_3^- from nitrification (Gardner and McCarthy, 2009), and ambient water column NO_3^- concentrations were low at all times during these sampling events (< 1.9 µM). These observations, when considered with the high sediment NH_4^+ effluxes in all seasons, suggest that aerobic nitrification cannot keep up with anaerobic organic matter decomposition. The result is surplus NH_4^+, which diffuses into the water column where it can be assimilated by phytoplankton and bacteria.

This hypothesis is complicated by the observation that water column NH_4^+ concentrations during the three sampling events were lower than long-term averages and generally did not comprise the largest proportion of DIN (Table 1). Florida Bay is shallow, and light usually reaches the sediment surface in sufficient quantity to support seagrasses, which can take up NH_4^+ and other nutrients through their roots (Stapel et al. 1996). Despite the well-documented seagrass die-offs in Florida Bay in recent years, the bay remains a seagrass-dominated estuary. Thus, it is likely that most NH_4^+ diffusing from the sediments is intercepted by seagrasses (roots and leaves), epiphytes, drift algae (Irlandi et al. 2004), and/or microphytobenthic organisms before reaching the water column. Light inhibition by cyanobacteria blooms in the water column may result in lower sediment regeneration and higher water column regeneration. This scenario may explain the significant increase in water column proportion of total measured regeneration in November in this study, but additional data is needed to determine if the tendencies suggested here are statistically robust for Florida Bay.

Large differences in potential NH_4^+ uptake and

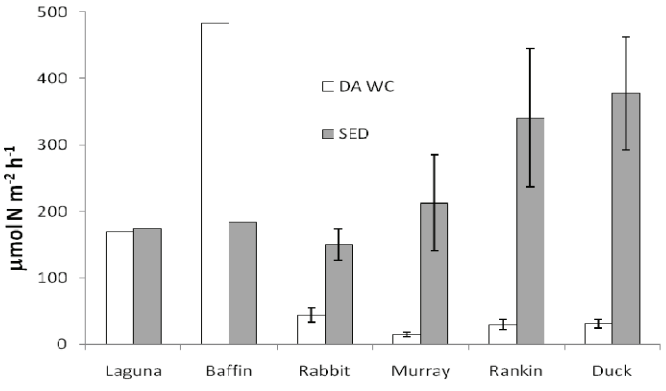

Figure 3. Depth-averaged water column NH_4^+ (DA WC) and sediment (SED) regeneration rates in Florida Bay compared to Laguna Madre ("Laguna") and Baffin Bay ("Baffin"), Texas.

regeneration rates, such as those observed in Florida Bay in November (Table 2), suggest that water column N dynamics are dominated by autotrophs and support N limitation or co-limitation observations during those periods (Gardner et al. 2000), with significant regeneration occurring in sediments (Flint et al. 1986, Ziegler and Benner 1999, Gardner et al. 2006). Similar findings were reported for south Texas estuaries, where primary production dependence on sediment regeneration was estimated at 90% for the Nueces estuary (Texas; Flint et al. 1986), although subsequent work reported similar contributions from water column and sediment regeneration in Nueces and Guadalupe estuaries (Texas; Benner and Yoon 1989). Sediment regeneration (sum of denitrification and DIN flux) rates in northern Laguna Madre and Baffin Bay (Texas) were 175 and 184 μmol N m^{-2} h^{-1}, respectively (An and Gardner 2000). Volumetric water column NH_4^+ regeneration rates from Laguna Madre and Baffin Bay were 170 and 483 μmol N m^{-2} h^{-1}, respectively (Fig. 3; Gardner and McCarthy, unpublished data). This comparison suggests that water column NH_4^+ regeneration is as important as sediment regeneration in Laguna Madre and more than twice as important in Baffin Bay.

Northern Laguna Madre is less populated by seagrasses than Florida Bay and has endured persistent blooms of Texas brown tide (*Aureomonas lagunensis*), which have stressed seagrasses and led to reduced water clarity (Buskey et al. 1996, 2001). Sediment regeneration is more rapid in seagrass-dominated systems versus unvegetated areas (Ziegler and Benner 1999), and Laguna Madre bottom area is 70-75% covered with seagrasses (Quammen and Onuf 1993). However, seagrasses are virtually absent in Baffin Bay (Blanchard and Montagna 1995), which is deeper and more turbid than Laguna Madre and Florida Bay. A similar importance of water column regeneration processes was found in Baffin Bay by other investigators (Cotner et al. 2004). The comparison between Florida Bay, Laguna Madre, and Baffin Bay (Fig. 3) illustrates the greater importance of water column regeneration, and concomitant decrease in importance of sediment regeneration, as water depths increase and clarity, via suspended solids or phytoplankton blooms, decreases.

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ESTIMATES OF PHYTOPLANKTON GROWTH FROM A LAGRANGIAN EXPERIMENT IN WESTERN FLORIDA BAY, USA

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ABSTRACT

Phytoplankton community growth rates were estimated from *in situ* changes in particulate standing stock and compared to rates derived from dilution gradient and dialysis cultures during a drifter deployment in northwestern Florida Bay. Diurnal changes in particulate organic carbon were balanced by declines at night, so over 24 hours there was no net growth of the *in situ* phytoplankton population. Dissolved organic nitrogen and total dissolved phosphorus, in contrast, decreased during the day and increased at night. We interpret this as an indication that dissolved organic matter is a potential nutrient source supporting phytoplankton growth. Concurrent dilution gradient and dialysis cultures indicated that *in situ* populations were viable with estimated growth rates of ca. 0.5 – 0.7 day^{-1}. When nutrient enrichments were omitted from the incubations in the dilution gradient series there was no net growth. The constant *in situ* phytoplankton standing stock over the two day drifter experiment was attributed to nutrient limitation.

Key *Words: Florida Bay, phytoplankton, growth rates, nutrients.*

INTRODUCTION

Florida Bay is a subtropical lagoon composed of shallow basins in which water properties vary from east-to-west across three distinct regions (Boyer et al. 1997). The eastern region (Fig. 1) has a mean depth of ca. 1.5 meters with a typical 'estuarine' salinity distribution that reflects the release of controlled freshwater sources along the northeastern boundary (McIvor et al. 1994) coupled to an inflow of saline waters from the central region and southeastern boundaries. The central region can be divided into northern and southern sections on the basis of salinity, nutrient, and plankton distributions. Long residence times, coupled with minimal freshwater inputs, and cyanobacterial blooms characterize the north central Bay where high salinities develop when evaporation rates are high (Nuttle et al. 2000). The properties of the south central region reflect inputs from the north central basins and marine waters that enter through inter-island passes between the Keys (Smith 1998). The western region is open to the broad southwest Florida shelf and consequently the water properties have a strong marine signature (Wang et al. 1994). Diatom blooms typically occur between late summer to winter in western Florida Bay (Phlips and Badylak 1996) and contiguous

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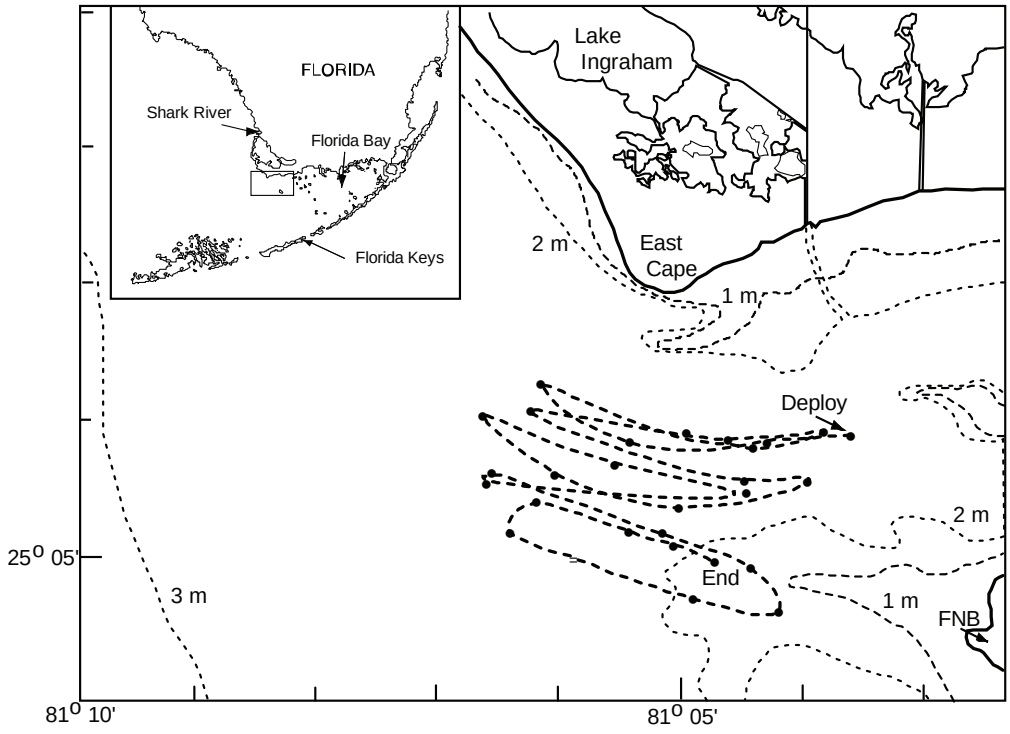

Figure 1. Map of the study area. Dashed line is the trajectory for one of four drifters that remained within 100 m of each other during the initial 48 hours of the deployment (June 17 and 18). Insert shows the location of the drifter study (box) in relation to Florida bay.

waters (Jurado et al. 2007).

Significant events have affected the Florida Bay ecosystem over the past two decades. These include an extensive die-off of the dominant seagrass *Thalassia testudinum* that began in 1987 (Robblee et al. 1991), the development of dense cyanobacterial blooms in the north central basins in the early 1990s (Phlips et al. 1999), and a significant reduction in sponge biomass between 1991 and 1993 (Butler et al. 1995). Several hypotheses have been proposed to explain the observed alterations in the ecosystem structure (e.g., Brand 2002, Peterson et al. 2006), most of which include impacts of anthropogenic regulation on freshwater inputs and/or decadal-scale variations in nutrient loading (Fourqurean and Robblee 1999). Based on the stochiometric relationships between available nitrogen and phosphorus and the stochiometry of particulate matter throughout Florida Bay, Fourqurean et al. (1993) hypothesized that phosphorus is the nutrient that potentially limited seagrass and primary production in the water column. Subsequently, bioassays by Tomas et al. (1999) revealed spatial differences in the nutrients that limit the growth of pelagic phytoplankton. Diatom blooms in the northwest Bay are consistently nitrogen and silicate limited (Jurado et al. 2007), while the cyanobacterial blooms in the north central region were commonly phosphorus limited, although nitrogen may also be a limiting factor. In the south central and eastern regions the primary limiting nutrient is phosphorus. Both dissolved organic phosphorus (DOP) and dissolved organic nitrogen (DON) contribute to high biomass in the north central region (Glibert et al. 2004).

Growth rates in nutrient-amended bioassays provide an indication that rates of nutrient supply likely vary across the Bay. Tomas et al. (1999) found population growth rates of 0.2 to 0.6 divisions day^{-1} in eastern Florida Bay with maximal rates of 3 to 4 divisions day^{-1} in the diatom-dominated waters of western Florida Bay at Sprigger Bank and Sandy Key. Typical concentrations of inorganic nitrogen and phosphorus are relatively low in the western Bay at < 0.5 mmol m^{-3} for dissolved inorganic nitrite + nitrite. Given that diatom -dominated blooms attain chlorophyll *a* concentrations of 10 mg m^{-3}, the *in situ* nutrient concentra-

tions could not sustain the high observed growth rates in the bioassays for > one day. Thus, the growth rates are an unrealistic estimate of *in situ* community growth rates, or alternatively, external (allochthonous) nutrient sources must sustain the diatom populations at the high turnover rates.

Fourqurean et al. (1993) first suggested that offshore waters of the Gulf of Mexico could be a major source of phosphorus to sustain the diatom blooms along the western margin. The Shark River system may also contribute nutrients since it has elevated TN:TP ratios (Rudnick et al. 1999). Surface drifters deployed at the Shark River display seasonal trajectories that vary with local wind fields. During the winter-spring season, when diatom blooms occur in western Florida Bay (Phlips and Badylak 1996), Lee et al. (2000) found drifter paths were typically in a SE direction ultimately exiting Florida Bay through the passes between the middle Keys where the strongest inflow of Gulf water is also found. These surface flows are the result of enhanced westerly and northwesterly winds that accompanied cold fronts, and support the hypothesis that the outflow from the Shark River could potentially contribute nutrients to sustain diatom blooms in the western Bay (Tomas et al. 1999, Jurado et al. 2007).

We conducted a series of growth rate studies in the surface waters of western Florida Bay in early summer 1998. The main objective was to evaluate phytoplankton growth rates and concurrent biomass levels to estimate nutrient requirements necessary to sustain the natural population of these primary producers. The natural populations were sampled during the deployment of surface drifters that tracked a water parcel in which phytoplankton growth and loss estimates were derived from *in situ* changes in morning and evening concentrations of chlorophyll, dissolved nutrients and particulate carbon, nitrogen, and phosphorus.

METHODS

Experimental design

Four surface drifters were deployed from the R/V *Calanus* on June 17 1998 south of East Cape Sable in northwestern Florida Bay (Fig. 1). The channel is bounded on the south by First National Bank and to the east by a shoal area designated the Middle Grounds. The platforms were configured as CODE drifters as described in Davis et al. (1985), but without vhf radios. Drifters were located visually every 2 hours between 0700 to 2100 hours Eastern Standard Time and positions recorded with a differential GPS system.

Surface water samples were collected at the drifter locations every 12 hours, once in the morning (0900) and evening (2100 hours local time). After the surface samples were returned to the vessel, seawater was filtered trough Millipore HA (0.45 µm) filters and frozen in acid-cleaned polyethylene bottles. Upon return to the laboratory, the samples were thawed and analyzed for dissolved inorganic (nitrate + nitrite) nitrogen via standard autoanalyzer methods (Lantry et al. 1995), and soluble reactive phosphorus (Karl and Tien 1992). Separate aliquots were filtered through pre-combusted GF/F filters, stored frozen in acid-cleaned bottles, and subsequently analyzed for Total Dissolved Phosphorus (TDP), Total Dissolved Nitrogen (TDN) and Particulate Phosphorus (POP) by the methods described in Solorzano and Sharp (1980a,b).

Chlorophyll *a* samples were filtered onto Whatman GF/F filters and stored over a dessicant at -20° C. The filters were extracted in absolute methanol for fluorometric determination of chlorophyll *a* and phaeopigment *a* according to Holm Hansen and Reimann (1976). Fluorescence was measured on the extracts before and after acidification with a Turner Designs 10-AU fluorometer. Particulate matter was filtered onto pre-combusted (400°C for 2 hours) 25 mm Whatman GF/F filters for particulate carbon (POC) and nitrogen (PON) analyses. The filters were stored at -20°C until they analyzed with a Carlo Erba Model 1106 CHN analyzer.

Growth and production estimates are based on the morning to evening changes in the concentration of particulate carbon and on measured phytoplankton growth rates. Growth rates of the natural populations were determined in two ways: 1) from dialysis cultures (Vargo 1984) initiated daily and incubated for 48 hours and 2) from two dilution gradient experiments (Landry and Hassett 1982) initiated on June 17 and 18 and incubated for 24 hours with and without nutrient additions.

Growth rates

Samples for growth rate studies by the dilution gradient method were prepared with seawater fil-

tered through a sterile GF/F filter to crate a dilution series of 1.0, 0.8, 0.5 and 0.2 of whole seawater:filtered seawater. The bottles were incubated in a Plexiglas tank with flowing seawater equipped with a blue Plexiglas filter and neutral density screening to simulate light conditions at which the samples were collected. Nutrient additions to the dilution gradient experiments were made with inorganic P as sodium phosphate (1 µmol kg^{-1}), sodium nitrate (10 µmol kg^{-1}) and sodium silicate (10 µmol kg^{-1}). A replicate set of duplicate bottles were incubated with undiluted seawater and the nutrient enrichments (+Nut), as well as with no enrichments (-Nut).

Table 1. The observed difference in particulate matter (POC, PON, POP) and dissolved matter (TDP, TDN) between the morning and evening samplings at the drifters. Positive values correspond to increased particulate matter with negative values corresponding to declines in dissolved materials. The values in parentheses are the increases in POC and PON that could be supported by the observed decreases in TDP if all of the P were utilized to produce POC and PON according to Redfield ratios (by weight).

Date	POC	PON	POP	TDP	TDN
17 June	34 (151)	4.5 (26)	0	-3.7	-134
18 June	31 (143)	6 (26)	0.9	-3.5	-109
19 June	0 (135)	0 (24)	2.4	-3.3	+ 73

Dialysis cultures were incubated in tanks of flowing seawater equipped with a wheel for gentle mixing and shaded with neutral density screening to approximate the average light climate where the sample was collected. Briefly, whole water samples were filtered through a 153 µm Nitex mesh to remove larger zooplankton. The screened sample was then used to fill duplicate dialysis tubes (48 Å pore size) which were tied at both ends with soft string leaving a small (3-4 mm dia.) bubble inside to facilitate mixing (Sakshaug and Jensen 1978). The tubes were then placed between two dissimilar sized wheels and rotated at approximately 10 rpm while submerged within the tank and incubated for 24 to 48 hours before being sampled for chlorophyll concentration.

Growth rates were calculated from the change in chlorophyll concentration in each replicate dialysis tube and dilution gradient bottle. Nutrient requirements were estimated based on measured C:N:P ratios and from the values typically used for the theoretical Redfield ratio of 41:7.2:1 by weight (Redfield et al. 1963). We have used the weight based ratios for C, N, and P since the changes we discuss are implicated for biomass, which is a mass quantity.

Potential nutrient input from the Shark River is derived from property-salinity relationships obtained during a synoptic transect of the river from the high salinity Florida Bay end member to freshwater.

RESULTS

Drifter trajectories

The four drifters exhibited a series of tidal oscillations south of East Cape Sable during the first forty-eight hours of the deployment (Fig. 1). The trajectories traced east-west ellipses that were displaced to the south during each successive tidal cycle. As the drifters moved progressively south, the trajectories placed them near the shallow water over the First National Bank after two and half days (Fig. 1). The southerly motion of the four

Table 2. Elemental composition as ratios (by weight) for particulate organic carbon (C), particulate organic nitrogen (N) and particulate phosphorus in the morning and evening samples collected at the drifters.

Date	Time	C:N	C:P	N:P
Jun 17	09:00	4.85	18.1	3.7
	21:00	5.30	29.5	5.5
Jun18	09:00	3.65	29.0	7.3
	21:00	5.63	29.8	7.2
Jun 19	09:00	5.19	50.0	9.6
	21:00	5.32	26.4	4.9
Redfield Ratio		5.7	41	7.2

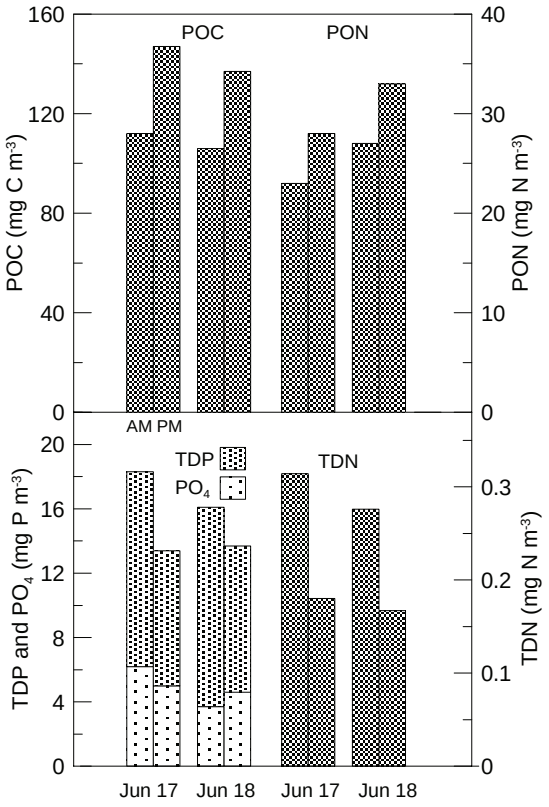

Figure 2. Concentrations of particulate matter as POC and PON (top panel) and dissolved materials, as TDP,PO_4 and DON (bottom panel), in morning (AM) and evening (PM) samples on June 17 and 18. Both TDP and PO_4 concentrations are on the same axis with the PO_4 concentrations corresponding to the lower stippled portion of the bar. For each day the morning sample corresponds to the left bar and the evening sample is the right bar, as indicated for June 17 POC. Both POC and PO_4 increase from morning to evening at the drifter locations, while the TDP and DON concentrations decrease (see text).

drifters indicated that the net flow along the western margin of Florida Bay, specifically at the mouth of the channel, was southeast at approximately 1.5 km d^{-1}. At the evening sampling (21:00) on June 19 one of the drifters grounded in the shallow waters on the southern edge of First National Bank. The four platforms were retrieved with a final sampling of water properties completed by 22:00 EDT.

Particulate Matter Composition

There were only minor variations in chlorophyll *a* concentration throughout the 60 hour deployment. Mean pigment concentrations in surface waters varied from an average of 0.68 mg m^{-3} in

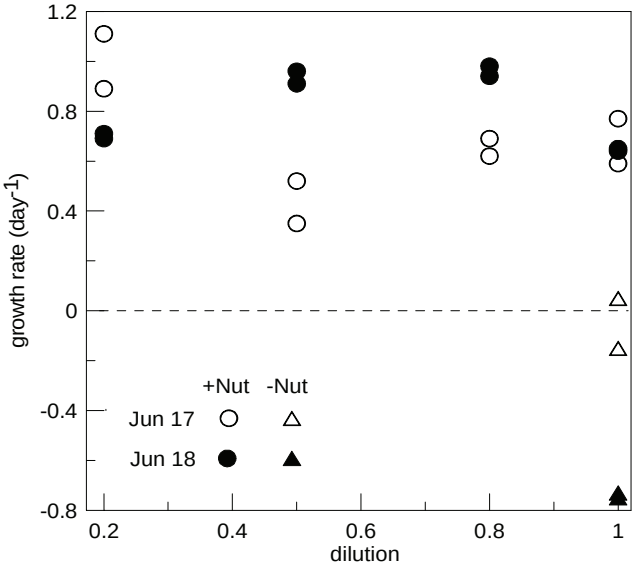

Figure 3. Growth rates in dilution gradient series with surface seawater collected at the drifters on June 17 and June 18. Unenriched controls exhibited little, or no, net growth.

the initial sample at 09:00 on June 17 to 0.50 mg m^{-3} in the final samples collected at 2100 on June 19. Particulate organic carbon and particulate organic nitrogen concentrations, in contrast, increased between the morning and evening sampling on each of the first two days (Fig. 2). The increases in POC and PON between the morning and evening samples was similar during each of the first two days, while the dissolved fractions of TDP and TDN decreased both dates during the day (Fig. 2, Table 1). The increase in POC was approximately 30 µg l^{-1} during the day on both dates, while PON increased by 4.5 to 6 µg l^{-1}. The observed increases in particulate C and N were, however, less than half of the POC and PON that could potentially be supported by the observed decrease in TDP if all of that phosphorus was utilized to produce POC and PON in accord with the Redfield ratios (Table 1).

The variability in morning-to-evening POP concentrations did not parallel the diel changes observed in POC and PON (data not shown) as no detectable changes were observed on June 18. On June 19, after the drifters had exited the channel, there were no consistent changes found in the concentrations of the particulate or dissolved properties between morning and afternoon samplings (Table 1).

Ratios of C:N (by weight) in particulate matter in western Florida Bay varied from a minimum of 3.65 to 5.32, generally less than the 5.7 ratio of the Redfield ratio (Table 2). For C:P, the ratios ranged from 18.1 to 50 with the majority less than the Redfield ratio of 41. The N:P ratio varied two-fold, from a minimum of 3.7 in the 09:00 sample on the first day of sampling to 9.6 in the morning sample from June 19 (Table 2). In total, the ratios suggest that the relative proportion of nitrogen was less than that of phosphorus, since the relative amounts of carbon and nitrogen in particulate matter were less than the canonical Redfield values.

Growth Rate Experiments

The growth response in the June 17 and June 18 dilution gradient experiments was nonlinear (Fig. 3). Growth rates computed from chlorophyll a increases in the duplicate bottles exhibited no consistent trend with increasing dilutions. When nutrients were added at a ratio of 1:10:10, the growth rates in the various dilutions ranged from 0.4 to 1.1 day^{-1} on June 17 and 0.8 to 1.0 day^{-1} on June 18, respectively. Conversely, if no nutrients were added to the bottles then the resulting growth rates were < 0.1 day^{-1} or negative (Fig. 3). These results indicate that the growth rate and biomass were nutrient limited in these surface waters, and that the mortality in the dilution gradient series was not a linear function of grazing pressure. Dialysis cultures begun on June 18 showed an increase in chlorophyll *a* concentrations in the replicate tubes which yields a mean net community growth rate of 0.75 day^{-1} (Table 2). This rate compares favorably with the average growth rate of 0.74 day^{-1} in the undiluted bottles (100% seawater) with nutrient enrichments.

Table 3. Growth rates for the plankton community derived from three independent methods on June 17 and 18. The diurnal increases in POC are from surface waters samples collected at the drifters in the morning and evening. The rates from the dilution gradient method were begun with surface waters collected in the morning sampling at the drifters on June 17 and 18. Rates of increase from the dialysis incubations are from surface waters collected on the morning of June 18.

Method	**Date**	**net growth rate (day^{-1})**
POC	June 17	0.54
	June 18	0.47
Dilution gradient	June 17	0.66 (+ N,P,Si)
		0.01 (- N,P,Si)
	June 18	1.01 (+ N,P,Si)
		0.0(- N,P,Si)
Dialysis culture	June 18 – 20	0.75

DISCUSSION

The central objective of this study was to evaluate phytoplankton growth rates in western Florida Bay, and interpret these rates in the context of nutrient demands of the pelagic primary producers. Surface drifters provided the means to follow a parcel of surface water and repeatedly sample particulate matter with minimal influence of advection, at least over a period of 48 h when the drifters remained in close proximity. The net southerly drift was similar to longer-term drifter deployments in western Florida Bay. Surface salinity maps, surface drifter trajectories (Wang 1998, Lee et al., 2002), and current meter deployments along 81° 05' W (Smith 2000), describe a net southeasterly flow in western Florida Bay. The sub-tidal southerly flow varies from 0.1 to 5 km d^{-1} with a net inflow principally in the northern two-thirds of the boundary and a net outflow along the southern third (Smith 2000). The southward flow has been hypothesized to deliver nutrients from freshwater sources that discharge onto the Shelf in the Ten Thousand Island region and the Shark River Slough into western Florida Bay (Lee et al. 2002). The nutrient flux into the western Bay could account for up to 85% of total phosphorus and 90% of the total nitrogen inputs to the Bay ecosystem (Rudnick et al. 1999). Quantifying these inputs and the relevant temporal and spatial scales at which the exchange occurs is essential to understand the impact of future changes in freshwater flow on Florida Bay (Sutula et al. 2003; Rudnick et al. 2005).

While the drifters were within the channel,

diel changes in particulate matter were consistent with a net increase in organic matter during the day and a decrease at night. The net increase in POC yield carbon-based growth rates on the order of 0.5 day^{-1}, which are less than those from the dilution gradient series with nutrient enrichments. One assumption in extrapolating growth rates from the observed diurnal increase in particulate matter is that photoautotrophic production relies upon nutrient uptake. A major source of the phosphorus and nitrogen that could support this growth is dissolved organic nitrogen and dissolved organic phosphorus. Both DON and DOP decreased during the day at concentrations which were greater than that required to support the observed increases in POC.

Bioassays with a variety of nitrogen and phosphorus sources found different nutrient sources likely support distinct taxa across the Bay (Glibert et al. 2004, Boyer et al. 2006). Dissolved organic nitrogen utilization occurs in western Florida Bay, although the time course of DON and DOP utilization by photoautotrophs is likely tightly coupled to heterotrophic metabolism. Direct enrichment of Bay water with dissolved organic matter indicates that utilization by photoautotrophs is dependent on heterotrophic activity (Boyer et al. 2006). If the diurnal pattern in POC observed at the drifters reflects DON and DOP utilization by photoautotrophs, then the supply of required nutrients in the western bay must be in steady-state over the course of each day.

Growth experiments conducted onboard the ship with the dilution gradient method and dialysis culture further indicate that without continued nutrient input, ambient nutrient concentrations in western Florida Bay are insufficient to maintain phytoplankton biomass for a day. The two dilution gradient experiments revealed no net growth unless nitrogen, phosphorus and silicon were added as enrichments. Net growth with enrichments ranged from 0.5 to 1.0 day^{-1}, with the majority of rates at all dilutions at 0.5 – 0.7 day^{-1}. These rates are slightly higher than the rates derived from changes in POC (Table 3).

The chlorophyll *a* response at various dilutions was nonlinear and thus no grazing estimates were calculated. Nonlinear responses have occurred in marine environments from estuarine to oceanic waters (e.g, Gallegos 1983, Landry 1993, Lessard and Murrell 1998, Worden and Binder 2003). Deviations from linearity are generally attributed to variability in predator-prey relationships such as feeding saturation or changes in the growth rates of grazers. For the purposes of estimating growth rates, the incubations on June 17 and 18 are simply interpreted as evidence that the resident phytoplankton populations were viable, and that growth rates with nutrient enrichments were in the range of 0.5 to 0.7 day^{-1}. The growth rates in the dialysis cultures further support the conclusion that nutrient supply is sufficient to sustain growth at ca. 0.5 day^{-1} as long as nutrients were continually supplied.

The dialysis culture method is based on the maintenance of a diffusion gradient from the outside flowing sea water across the dialysis membrane to the interior of the tube where nutrients are maintained at very low levels by the enclosed phytoplankton. This gradient across the dialysis membrane supplies a continuous flux of nutrients to the enclosed population. The net effect is to allow biomass within the tube to grow at a rate similar to *in situ* rates that are set by environmental factors other than nutrients. Because nutrients are supplied continuously, growth is unlimited until the biomass within the tube increases to a concentration where the nutrient uptake by the biomass within the tube is equal to the flux of nutrient across the membrane; this did not occur in our study. Therefore, the similarity observed between the growth rates from the dialysis culture and that in the dilution gradient bottles was not unexpected. The similarity in the rates also suggests that grazing by macro or microzooplankton was minimal in the populations enclosed within the dialysis tubes.

In summary, changes in the standing stock of particulate matter during the drifter deployment in western Florida Bay are interpreted as indicating that over the course of 24 hours there was no net growth in the *in situ* phytoplankton population. Since the dilution gradient and dialysis cultures show the *in situ* populations were viable, and likely had *in situ* growth rates on the order of 0.5 day^{-1}, the lack of a net increase in chlorophyll *a* indicates that the growth rates were essentially equal to losses. We conclude that the phytoplankton populations were in 'steady state' during June 17 and 18. The absence of an increase in the phytoplankton standing stock was likely due to nutrient limitation since nutrient enrichments yielded appreciable in-

creases in biomass and the growth rate in cultures. Furthermore, dissolved organic nitrogen and phosphorus are likely a major source of the nutrients required to sustain growth in western Florida Bay.

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COMPARATIVE NUTRIENT AND PHYTOPLANKTON DYNAMICS IN TWO SUBTROPICAL ESTUARIES: FLORIDA BAY, USA, AND MORETON BAY, AUSTRALIA

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ABSTRACT

The subtropical estuaries of Florida Bay and Moreton Bay are shallow, at equivalent latitudes, and both subjected to increasing eutrophication. However, these are highly contrasting systems. Similar studies on nutrient availability, phytoplankton composition, and rates of enzyme activities were conducted on a seasonal basis but in different years in these two systems. Florida Bay, a lagoonal estuary, supports picocyanobacterial blooms, which expanded in magnitude and duration upon an injection of phosphorus (P) and organic matter during a major disturbance event of 2005. Moreton Bay, an estuary at the transition between river-dominated and lagoonal, supports proportionately more diatoms and dinoflagellate blooms, and in response to a major storm, diatoms increased in abundance. Community composition and nutrient uptake pathways were, in both cases, related to the dissolved nutrient N:P ratios.

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INTRODUCTION

Relationships between nutrient availability and plankton dynamics are of critical importance to the fundamental understanding of aquatic ecosystem function and to the management of these ecosystems, especially those that have been, or continue to be, impacted by excessive nutrient loading. The status and trends in nutrient loading and phytoplankton response often guide both short-term and long-term management decisions with respect to water quality. Temperate coastal and estuarine systems have been well studied with regard to nutrient-phytoplankton dynamics, especially in Europe and North America (e.g., Baltic Sea, Nehring et al. 1984, Rönnberg and Bonnsdorf 2004; Kattegatt, Richardson and Jørgensen 1996; Chesapeake Bay, Fisher et al. 1992, Cooper and

Brush 1993, Kemp et al. 2005; Narragansett Bay, Borkman and Smayda 1998; Neuse River Estuary, Mallin et al. 1991, Boyer et al. 1993, Burkholder et al. 2006), but subtropical estuaries have been comparatively less well studied. These subtropical systems are, however, receiving increased nutrient loading like their temperate counterparts, and thus it is important to understand how they process these nutrients.

Here, we compare two subtropical estuaries, Florida Bay, USA, and Moreton Bay, Australia, with respect to seasonal patterns in nutrient availability and phytoplankton response. Both systems are adjacent to major metropolitan cities and agricultural lands and downstream of their major nutrient discharge. Florida Bay has been the focus of considerable recent scientific and management concern because of the widespread ecological changes within the system that have been hypothesized to be associated with ongoing eutrophication and land-use changes (James et al. 1991, Hunt and Nuttle 2007). Major anthropogenic changes to freshwater flow in the adjacent Everglades began nearly a century ago, when tidal passes were filled, and water flow was channelized (Wanless et al. 1994, Fourqurean and Robblee 1999). These changes resulted in large alterations of the salinity and nutrient regime. In recent decades, recurrent declines in seagrass distribution, increases in algal blooms and decreases in coral health have all been documented (Fourqurean et al. 1993, Phlips et al. 1995, Szmant and Forrester 1994, Glibert et al. 2004, 2009). Moreton Bay, on the other hand, has been considered historically oligotrophic, although recent increases in nutrient loading have led to macroalgal and microalgal blooms, loss of seagrass and other symptoms of ecosystem stress (Dennison et al. 1999, Abal et al. 2001, Watkinson et al. 2005, O'Neil and Dennison 2005).

We have applied a comparative approach to the study of nutrient and plankton dynamics in these systems. One of the outcomes of the comparative approach in studies of different ecosystem types is the potential of identifying commonalities in phytoplankton response to environmental forcings, one of the major goals of the International Global Ecology and Oceanography of Harmful Algal Blooms (GEOHAB) program (Anderson et al. 2005). The comparisons herein draw on both seasonally collected data during years of relatively normal flow, and also draw on previously published data on the response of nutrients and phytoplankton following a major flood event in Moreton Bay (Moss 1998, Heil et al. 1998, O'Donohue et al. 1998) and a series of hurricanes and other disturbances in Florida Bay (Rudnick et al. 2008, Glibert et al. 2009). This analysis shows that even though the systems were sharply contrasting in terms of hydrology, nutrient availability, and dominant nutrient forms, and in their response to disturbance events, commonalities existed in the responses by dominant plankton groups in each system to the relative availability of specific nutrient forms.

SITE DESCRIPTIONS

Florida Bay, a subtropical lagoon, is about twice the area of Moreton Bay, a river-dominated estuary, although both drain watersheds of comparable size and are characterized by carbonate sediments in their respective eastern regions (Fig. 1, Table 1). Moreton Bay is deeper than Florida Bay, ~7 m vs. ~1-2 m on average, respectively, but both systems have residence times that generally approximate 1- 1.5 months (Table 1). Florida Bay receives freshwater from drainage of the Everglades though Taylor and Shark River Sloughs, but much of this flow results from managed discharge rather than from natural hydrological conditions (Rudnick et al. 1999). Freshwater flow to Moreton Bay is dominated by several rivers, of which Brisbane River is the largest. Freshwater flow to both systems is seasonally pulsed corresponding to the wet season of the year. The watersheds of both systems contain heavily populated cities, Miami and Brisbane, although these watershed also drain considerable agricultural lands as well.

Both estuaries can be partitioned into regions that are differentially impacted by freshwater and by nutrient inputs. In Moreton Bay, the eastern region is characterized by oceanic salinities and relatively clear water. Western Moreton Bay is characterized by residence times on the order of weeks to months, and variable salinities from oceanic to fresh and turbid waters (Dennison and Abal 1999). Both regions of Moreton Bay are characterized by N limitation (O'Donohue and Dennison 1997; Glibert et al. 2006a). Florida Bay also displays a distinct east-to-west gradient, in which the eastern-part is generally considered to be P-limited while N

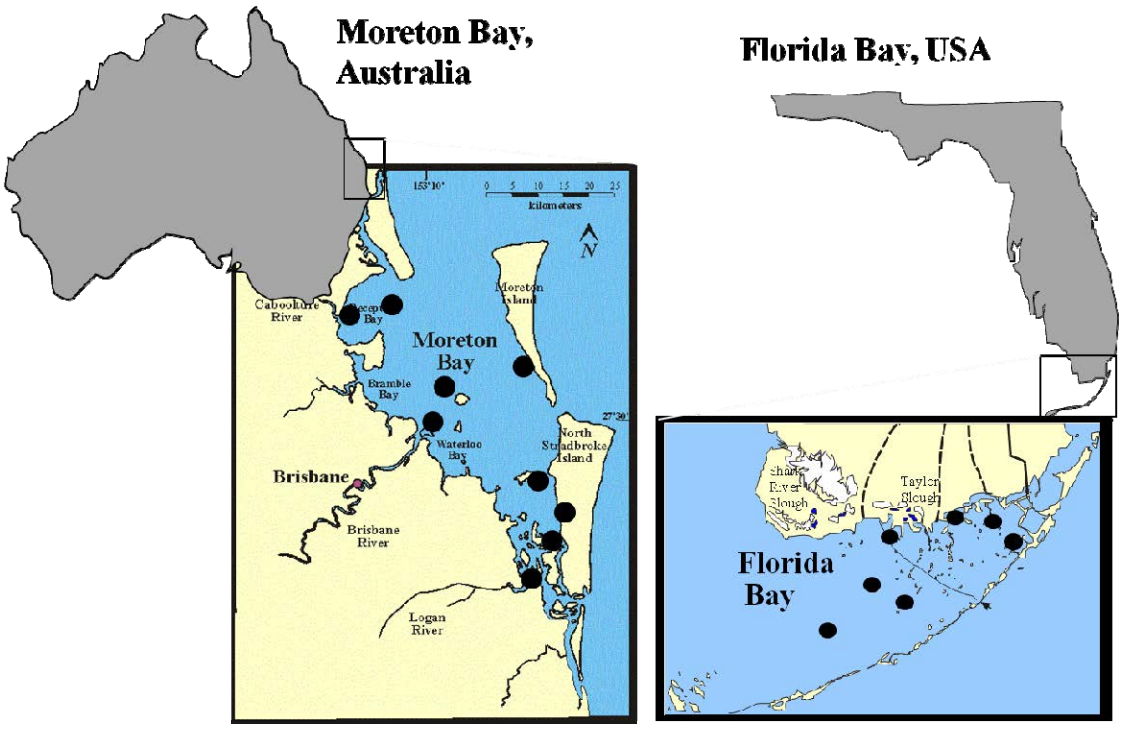

Figure 1. Maps of station locations in Moreton Bay and Florida Bay.

limitation, when it does occur, is more likely in the west. Traditionally most algal blooms in Florida Bay occur in the central basin where both N and P may be sufficient (Fourqurean et al. 1992, Hitchcock et al. 1998, Glibert et al. 2004). In Moreton Bay, the gradient of anthropogenic nutrient impact is generally from west to east, whereas in Florida Bay, the eastern region is generally considered more nutrient impacted than the Gulf of Mexico – influenced western bay region.

Table 1. Comparative features of the subtropical estuaries Moreton Bay and Florida Bay.

	Moreton Bay	**Florida Bay**
Coordinates	27.50^{o}S, 153.17^{o}E	24.83^{o}N 80.78^{o}W
Bay Size (km^2)	700	1,393
Avg. Depth (m)	7	1-2
Watershed Size (km^2)	21,220	27,971
Watershed Type	developed, Pine farms	developed, Agriculture, Everglades
Residence Time (d)	~45	~28
Major freshwater sources	Brisbane River Logan River Caboolture River	Taylor Slough Shark River Slough
Nearest City	Brisbane	Miami
City Size (M)	1.6	2.3

METHODS

The data presented are from seasonal studies conducted during years of near-normal flow in both systems. Comparisons of event responses are from data previously presented in the literature (Davies and Eyre 1998, Moss 1998, Heil et al. 1998, Rudnick et al. 2006, Glibert et al. 2009). Each system was studied seasonally in different years under different projects. Here the data corresponding to three seasons each are presented (Table 2). These data were selected for comparison from larger data sets as the methods applied in both cases were most comparable. In each case, stations were sampled once per day in order to collect and process samples at the same approximate time of day. For each site and each seasonal period of study, 7- 9 stations were sampled (Fig. 1). In both systems, stations were grouped to represent west, central (Florida only) or eastern regions of the respective bays. All samples were collected from just below the surface during mid- morning and returned to the laboratory within 1-2 hrs of sample collection.

Subsamples were split for various analyses. For dissolved and particulate nutrients, suspended sediments and chlorophyll *a* (Chl *a*) determination, samples were processed by filtering (GF/F filters) replicate aliquots and the filtrates and filters were retained for later determination. Methods of analysis are listed in Table 3 and described more fully in Glibert et al. (2004, 2006a). Activities of the enzyme alkaline phosphatase (APA) and urease were determined in Florida Bay but not Moreton Bay. APA was determined by measuring changes in sample fluorescence over time after the addition of a methyl fluoroscein phosphate substrate (Perry 1972). Changes in fluorescence upon substrate additions were followed in 15 min intervals for 2 hr with a Turner Designs fluorometer to confirm the linearity of the response. Urease activities were determined within 2 wks of sample collection on samples that were filtered onto GF/F filters and stored in liquid N_2. The method of Peers et al. (2000), following the modifications of Solomon et al. (2007) was used.

Dominant phytoplankton groups were enumerated differently in the two studies. In Moreton Bay, microscopic identification and enumerations of the net phytoplankton community were made using Lugol's preserved samples (Heil et al. 1998). In Florida Bay, phytoplankton pigments were determined using a high-performance liquid chromatograph (Van Heukelem and Thomas 2001). Our focus herein is on the dominant groups of microalgae: diatoms, dinoflagellates and cyanobacteria. We therefore used the ratios of Fucoxanthin:Chl *a* as a relative indicator of diatom abundance, Zeaxanthin:Chl *a* as a relative indicator of cyanobacterial biomass, and Peridinin:Chl *a* as an indicator of dinoflagellate biomass in Florida Bay, while recognizing that these ratios are not indicators of these algal groups exclusively. Inasmuch as laboratory calibrations were not conducted for representative species from Florida Bay, no attempt was made to convert these relative abundances to quantitative estimates based on available software packages (e.g., CHEMTAX; Millie et al. 1997).

RESULTS

Standing Stocks

During the periods of study, both estuaries exhibited strong seasonal signals and similar concentrations of inorganic N (sum of concentrations of NH_4^+, NO_3^- and NO_2^-), 1-4 μM-N (Fig. 2A,B). Conversely, concentrations of PO_4^{3-} were signifi-

Table 2. Dates of sampling on which the data for this paper were collected. Each period of sampling for each bay system encompassed 7-9 stations as shown in Figure 1.

	Moreton Bay	Florida Bay
Spring	September 18-28, 1997	March 15-21, 2003
Summer	January 27-February 7, 1998	July 24-August 1, 2003
Autumn/Winter	July 26-31, 1998	November 6-12, 2002

Table 3. Analytical methods used in each study region

	Moreton Bay	Florida Bay
Dissolved inorganic nutrients	Clesceri et al (1989)	Lane et al. (2000)
Urea	McCarthy et al. (1970)	Revilla et al. (2005)
Dissolved organic N	Antek analyzer	Bronk et al. (2000)
Dissolved organic P	no data	Solozano and Sharp (1980)
Particulate N	Control equipment analyzer	Control equipment analyzer
Chlorophyll *a*	Parsons et al. (1984)	Holm-Hansen et al. (1965)
Alkaline phosphatase activity	no data	Perry (1972)
Urease activity	no data	Peers et al. (2000); Solomon et al. (2007)

cantly higher at all seasons and at all sites in Moreton Bay than in Florida Bay, exceeding 1 μM-P in the western sites in the summer sampling period (Fig. 2C,D). Florida Bay had higher $Si(OH)_4$ concentrations than Moreton Bay, especially in the central region (Fig. 2E,F). Concentrations of $Si(OH)_4$ were highest in summer.

The range of concentrations of organic forms of nutrients also differed between these estuaries. Average concentrations of organic N (DON) exceeded 25 μM-N in Florida Bay in the central and eastern regions at all seasons, while in Moreton Bay, concentrations of DON remained <5 μM-N through the bay all year (Fig. 3A,B). Moreton Bay

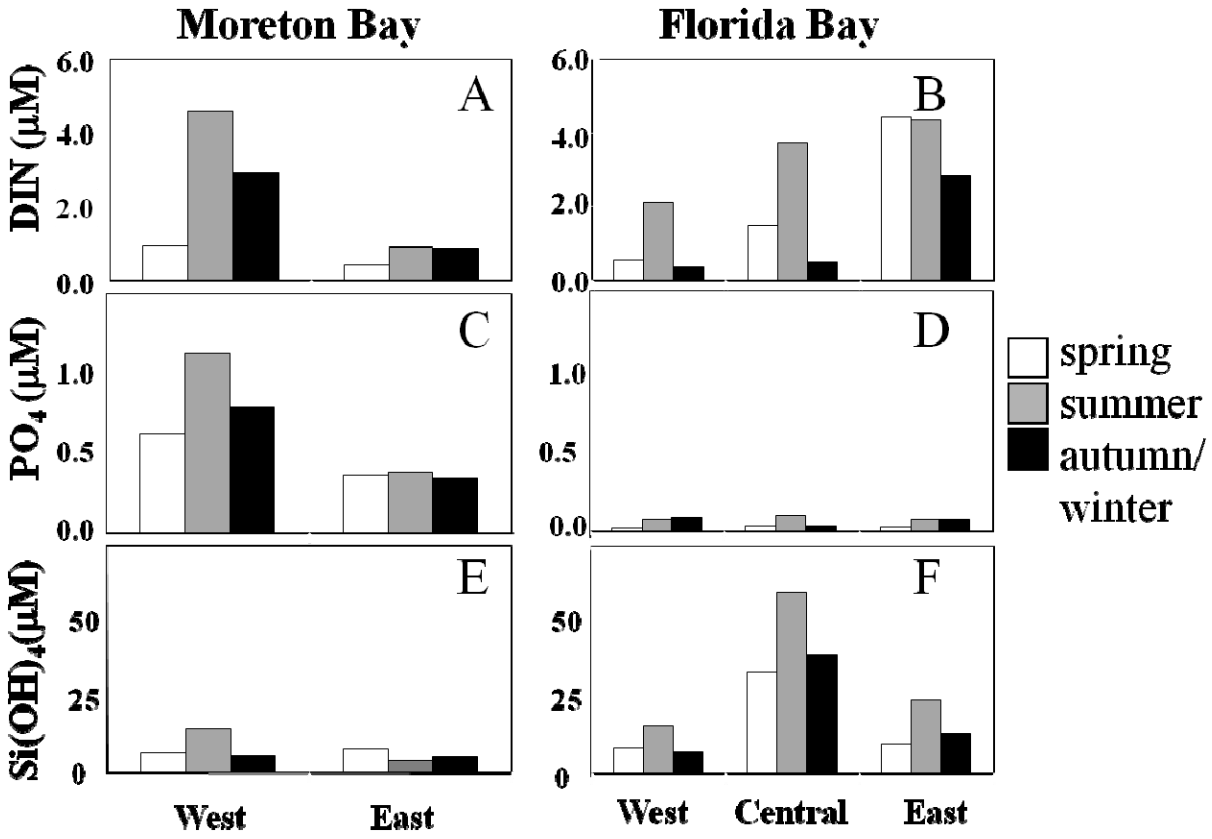

Figure 2. Mean concentrations of the inorganic nutrients in the western, eastern and central (Florida only) regions of Moreton Bay and Florida Bay for three seasons.

did have measurable concentrations of one component of the DON pool, urea, but these concentrations remained <1 μM-N. In Florida Bay urea was regionally variable, with the highest concentrations in the east where >1 μM-N was detected at all seasons (Fig. 3C,D). Highest urea concentrations were observed in summer in western Moreton Bay and in central and eastern Florida Bay. Organic P (DOP) in both estuaries showed a regional gradient, higher in the western region in both cases (Fig. 3E,F). Highest concentrations of DOP, >1 μM-P, were observed in Moreton Bay in summer.

Concentrations of suspended sediments also differed between these estuaries (Fig. 4). Concentrations of suspended sediments in Moreton Bay

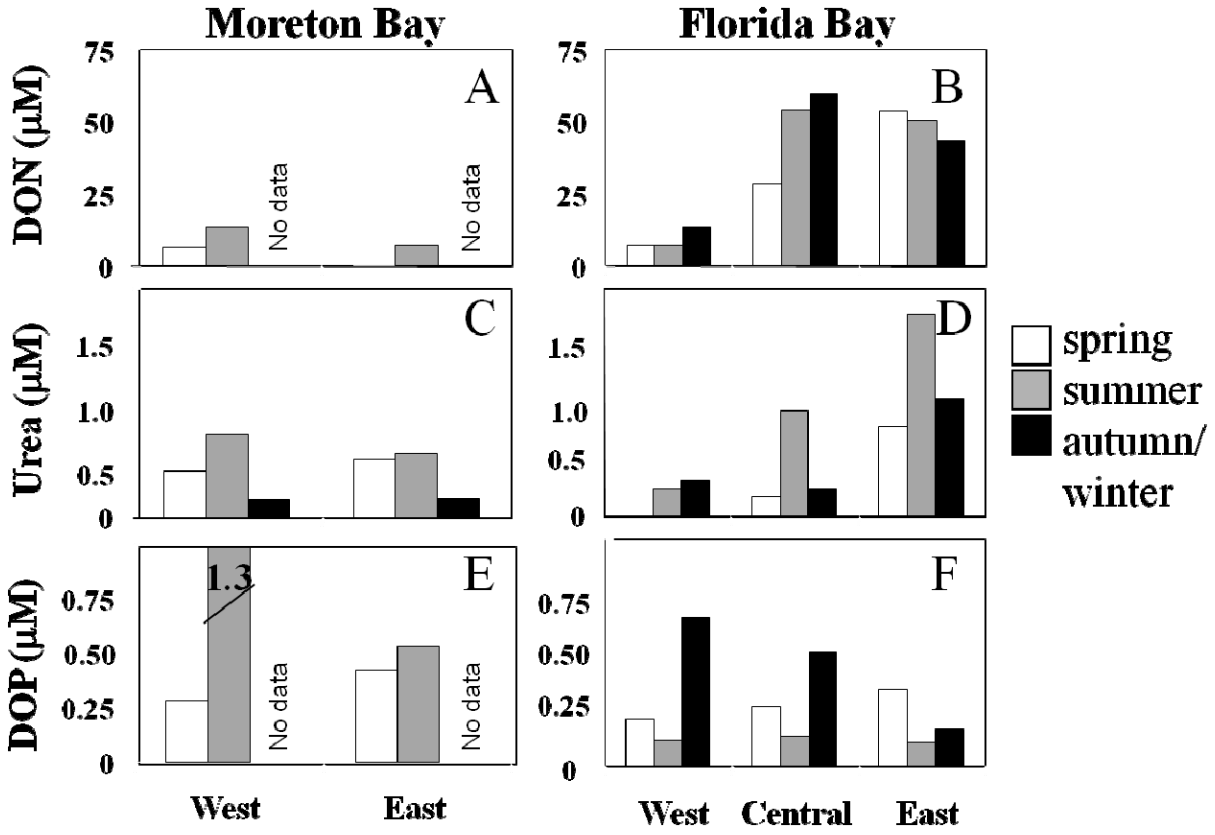

Figure 3. Mean concentrations of dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP) in the western, eastern and central (Florida only) regions of Moreton Bay and Florida Bay for three seasons.

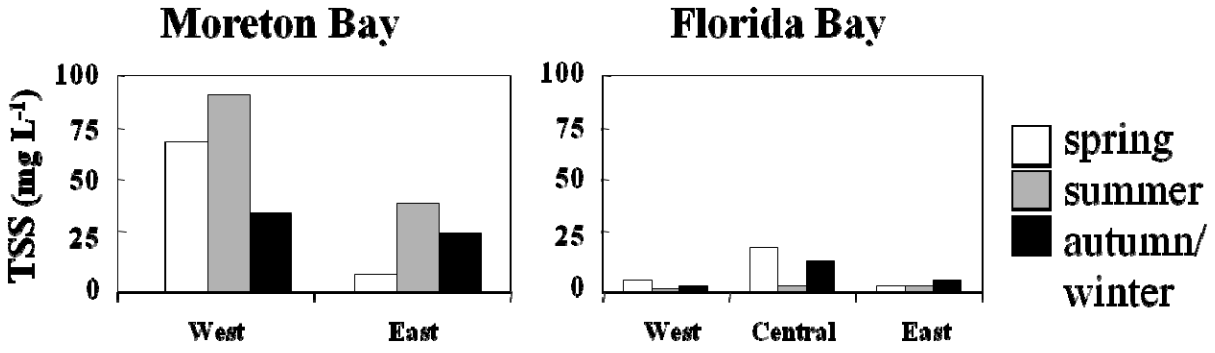

Figure 4. Mean concentrations of suspended sediments in the western, eastern and central (Florida only) regions of Moreton Bay and Florida Bay for three seasons.

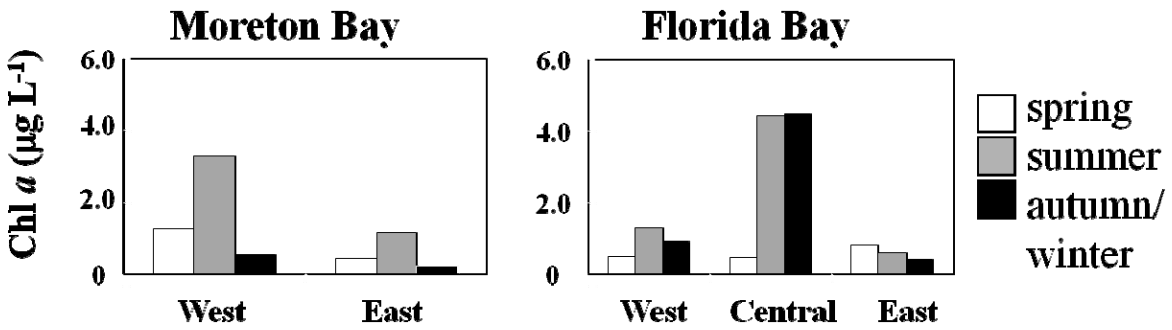

Figure 5. Mean concentrations of chlorophyll *a* in the western, eastern and central (Florida only) regions of Moreton Bay and Florida Bay for three seasons.

were up to an order of magnitude higher in Moreton Bay than in central Florida Bay. A strong seasonal signal in suspended sediment concentration was evident; highest suspended sediments in Moreton Bay were observed during the summer, but this was the season of lowest concentration in Florida Bay.

Both systems were characterized by comparable levels of Chl *a*, with maxima of ~4 μg L^{-1} in Florida Bay (Fig. 5). Seasonal variation in Chl *a* was also similar: both systems also had highest Chl *a* levels in the summer, although high levels were also sustained in Florida Bay in autumn/ winter. Very low Chl *a* levels, < 2 μ g L^{-1}, were found in the other regions of both bays throughout the year.

A comparison for each estuary of the ambient dissolved inorganic N:inorganic P (DIN:DIP) ratio and the N:P ratios of the particulate material (PN:PP: individual data not shown) reveals strong differences (Fig. 6). For Moreton Bay, every ratio for every region and season fell well below the Redfield ratio of 16:1 (Redfield 1958), indicating strong N limitation. In contrast, for Florida Bay almost every ratio for every region and season fell above the Redfield ratio, suggesting moderate to strong P-limitation.

Phytoplankton Composition and its Relation to Nutrient Availability

Moreton Bay net phytoplankton communities were dominated by diatoms and flagellates. [Note

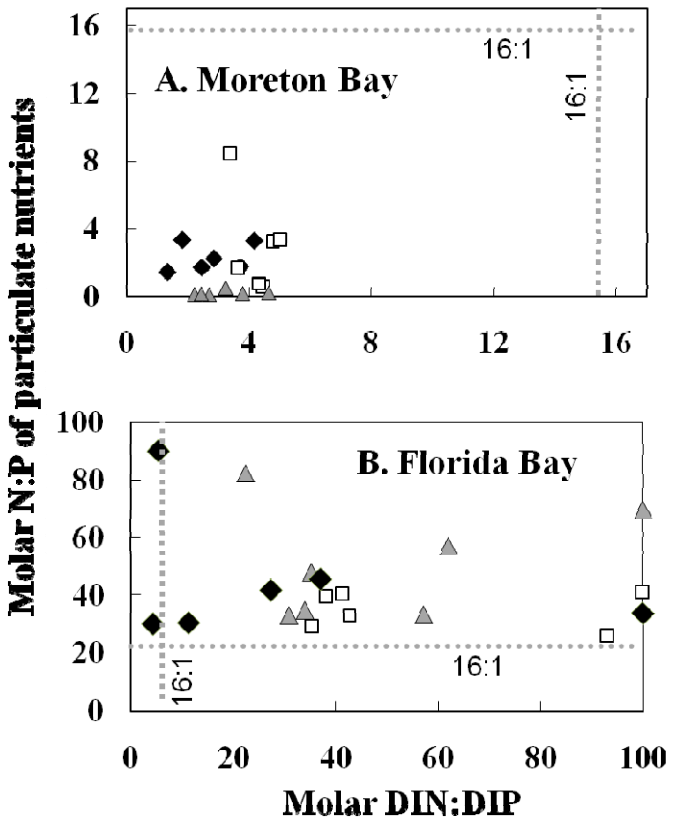

Figure 6. The relationship between ratios of dissolved inorganic nitrogen: dissolved inorganic phosphorus (DIN:DIP) and those of particulate nitrogen: particulate phosphorus (PN:PP) for all sites sampled in Moreton Bay (panel A) and Florida Bay (panel B). Data from spring are represented by squares, from summer by triangles, and from autumn/winter by diamonds. The dashed lines indicate Redfield proportions. For Florida Bay, DIN:DIP >100 are shown as =100.

that blooms of the benthic cyanobacterium *Lyngbya majuscula* are now common in Moreton Bay but were just beginning to be established at the time of the sampling reported herein (Roelfsema et al. 2005). Only large cyanobacteria such as *Trichodesmium*, not picocyanobacteria, were enumerated in Moreton Bay; they are not representative of the water column cyanobacterial populations in total and are thus not reported herein. Picoplankton were not measured in Moreton Bay.] When all data were combined, dinoflagellates were found to decrease in abundance as a function of the ambient DIN:DIP ratio, whereas diatoms were found to increase in abundance (Fig. 7). Thus, at DIN:DIP ratios < 4 (strong N limitation), the phytoplankton were characterized by a mixed assemblage, dominated by dinoflagellates, whereas at DIN:DIP ratios between 4-8, the community was almost exclusively composed of diatoms. In fact, a DIN:DIP ratio of ~4 appeared to be the transition point between dinoflagellate and diatom dominance.

The DIN:DIP ratio for Florida Bay spanned a considerably larger gradient than that found for Moreton Bay. The relative abundance of cyanobacteria (as Zeaxanthin:Chl *a* ratio) was strongly related to N:P ratio, declining as the ratio increased, i.e., as P limitation increased (Fig. 8A). The relative abundance of dinoflagellates (as Peridinin:Chl *a*) increased as the DIN:DIP ratio increased, a finding in sharp contrast to that observed in Moreton Bay (Fig. 8B). The relative abundance of diatoms (as Fucoxanthin: Chl *a*) also increased in Florida Bay as the DIN:DIP ratio increased, reaching a maximum in the region of DIN:DIP ratios ~40, and then decreasing again as the DIN:DIP ratio increased substantially above that of Redfield (Fig. 8C).

When the results of the comparative systems are combined, it appears that cyanobacteria were dominant in Florida Bay when both inorganic N and inorganic P were sufficient and roughly in Redfield proportion (DIN:DIP ~≤20, Florida Bay, central region). Diatoms were most common in both systems when the DIN:DIP ratios were closer to Redfield proportions, but were able to thrive in Florida Bay even when DIN:DIP ratios were quite elevated. Their relative proportions decreased when DIN:DIP was either very low, <4, or very high, >100. Dinoflagellates, on the other hand, did proportionately better when the DIN:DIP ratio was either very low (Moreton Bay) or very high (Florida Bay).

To examine the possibility of whether organic sources of N or P were used by different phytoplankton groups, the expression of the enzymes urease and APA were examined. These data are only available for Florida Bay. Based on their proliferation at DIN:DIP ratios well below Redfield, i.e., N limiting conditions, both cyanobacteria and dinoflagellates would be expected to have high capacity for organic N uptake, and thus would be pre-

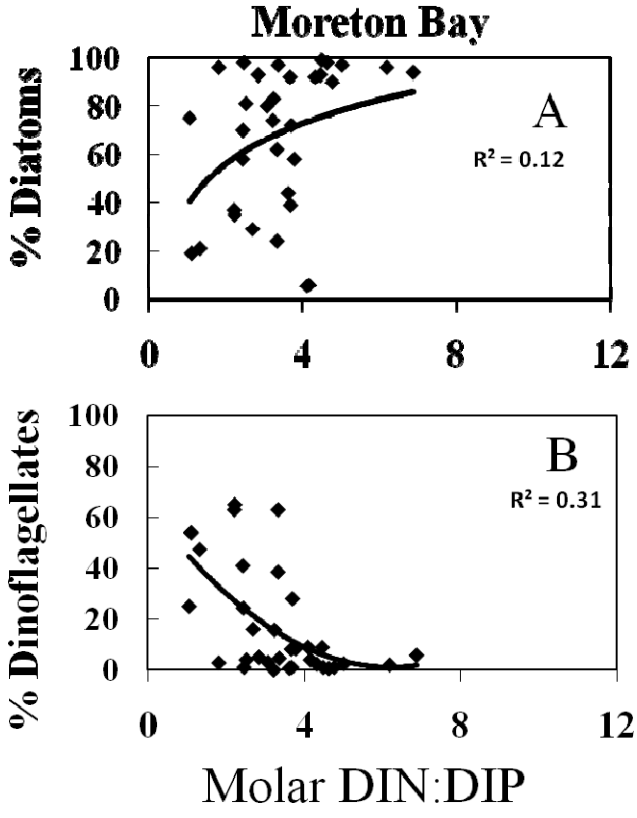

Figure 7. The percent dinoflagellates (upper panel) and percent diatoms (lower panel) for all seasons combined for Moreton Bay as a function of the molar DIN:DIP ratio. The R^2 is given for relationships that are significant at $P<0.05$.

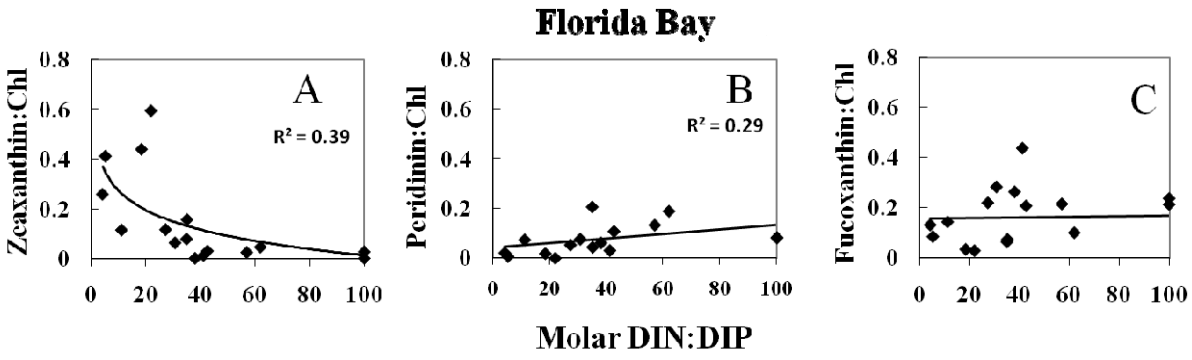

Figure 8. The contribution of diatoms (panel A), dinoflagellates (panel B) and cyanobacteria (panel C) for all seasons combined Florida Bay as a function of the molar DIN:DIP ratio. Community composition is represented as a ratio of the respective dominant accessory pigment to chlorophyll *a* as described in text. Data for DIN:DIP ratios >100 are represented as =100. The R^2 is given for relationships that are significant at $P<0.05$.

dicted to have high expression of urease activity. Conversely, based on their success under very high DIN:DIP condition, i.e. under P limitation, dinoflagellates would be expected to also have high capacity for organic P uptake, as would diatoms, and this should be expressed in their APA levels.

Indeed for Florida Bay, APA increased linearly as the proportion of diatoms increased (Fig. 9A). APA was also associated with the relative contributions of dinoflagellates to the community, but with no consistent trend (Fig. 9B). In the case of cyanobacteria (Fig. 9C), the rate of APA generally decreased as cyanobacterial abundance increased, but not significantly. Urease activity, in contrast, decreased with the relative proportion of diatoms and dinoflagellates (Fig. 9D,E), but increased significantly with the relative abundance of cyanobacteria (Fig. 9F).

DISCUSSION

Although both estuaries are subtropical, Florida Bay and Moreton Bay represent different classes of estuarine types, i.e. coastal lagoon and river-dominated (*sensu* Madden et al. 2010). These estuarine types have been hypothesized to support different types of algal blooms: coastal lagoons more typically support frequent summer- winter blooms of picoplankton (<3 μm in size), whereas riverine systems are often characterized by high-biomass spring blooms generally composed of large (>10 μm) diatoms (Glibert et al. 2010). The picoplankton blooms of lagoons are more likely to be sustained on regenerated forms of nutrients, such as NH_4^+, urea, or dissolved organic substrates, compared to riverine spring blooms which are often supported by NO_3^- (Glibert et al. 2010). Interestingly, Moreton Bay, while nominally river-dominated, does not show all of these common traits of a river-dominated system. Eastern Australian estuaries are typically characterized by a spring bloom in October (austral spring) of chain-forming diatoms followed by dinoflagellates as the seasons progress (e.g., Jeffrey and Carpenter 1974, Hallegraeff and Reid 1986, Jeffrey and Hallegraeff 1990), but the phytoplankton of Moreton Bay has previously been found to be composed of diatoms and dinoflagellates as co-dominants in spring (Heil et al. 1998), with diatoms dominant in the summer and dinoflagellates dominant in winter. Such was the case in the seasonal study presented here as well. Moreton Bay, consequently, functions more like a lagoonal estuary than a riverine system in terms of seasonality, but more like a river-dominated system in terms of the resulting phytoplankton biomass and community composition. It is worth noting, however, that picocyanobacteria have not been enumerated in Moreton Bay to date and thus their potential contribution to the plankton community is not known. These differences are highlighted here both with respect to normal seasonality and with respect to response to disturbance events.

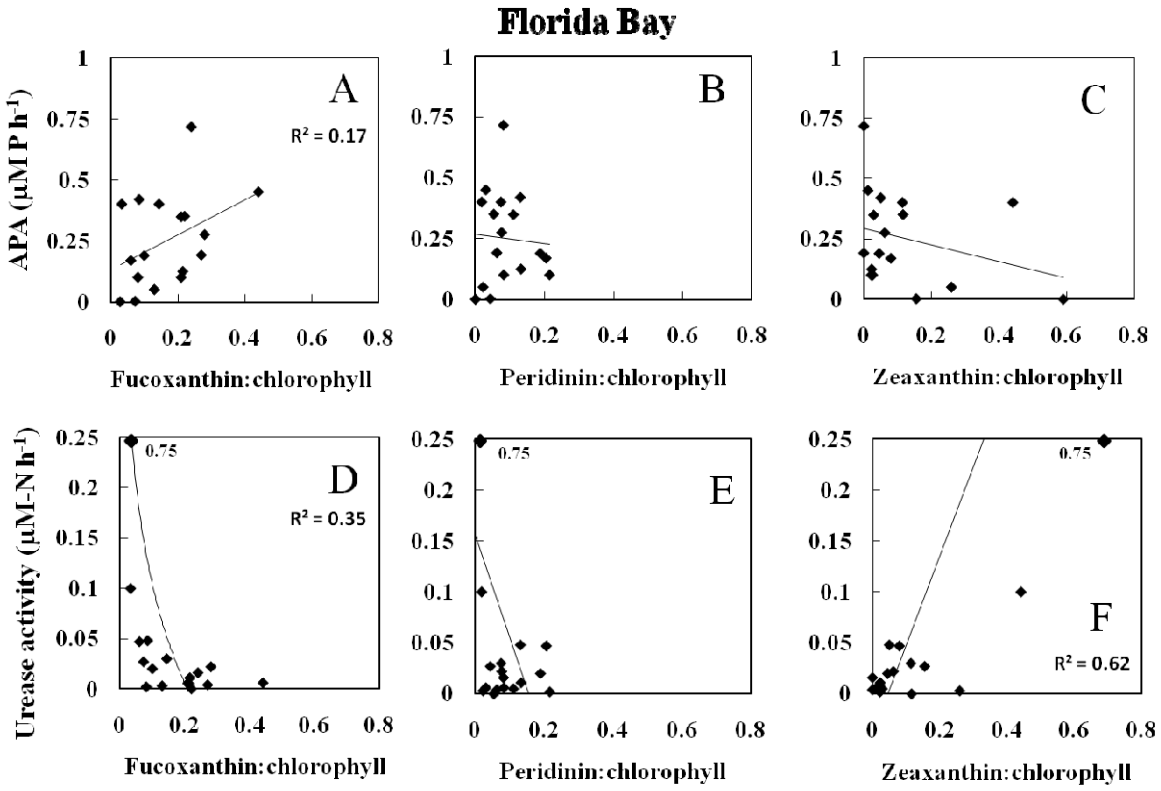

Figure 9: The relationship between alkaline phosphatase activity (APA; panels A-C) and urease activity (panels D-F) and community composition for all seasons combined for Florida Bay. Community composition is represented as a ratio of the respective dominant accessory pigment to chlorophyll *a* as described in text. The R^2 is given for relationships that are significant at $P<0.05$.

Phytoplankton Communities and Nutrient Composition

The ratio of DIN:DIP has previously been related to shifts in dominance of phytoplankton species groups within systems. For example, in Tolo Harbor, Hong Kong, where P loading increased due to population increases in the late 1908's, a distinct shift from diatoms to dinoflagellates was observed, coincident with a decrease in the ambient DIN:DIP ratio from 20:1 to <10:1 (Hodgkiss and Ho 1997, Hodgkiss 2001). Similar findings have been reported for Tunesian aquaculture lagoons (Romdhame et al. 1998) and for the prevalence of the heterotrophic dinoflagellate *Pfiesteria* in coastal estuaries along the eastern U.S. seaboard (Burkholder and Glasgow 1997, Burkholder et al. 2001, Glasgow et al. 2001). In Moreton Bay, the shift to dinoflagellates only occurred when the DIN:DIP ratio declined to <4, and in Florida Bay there was no clear discernable increase in dinoflagellates when DIN:DIP declined to less than Redfield proportions. The relationship with DIN:DIP in subtropical systems is thus complicated: dinoflagellates may show increase with DIN:DIP at both extremes of Redfield proportions. The species which thrive under the high DIN:DIP ratios of Florida Bay have yet to be comprehensively enumerated and studied.

One strategy for algae to thrive when inorganic nutrients are seemingly limiting is through the uptake of organic forms of N or P. Many algal groups do indeed depend on organic nutrients (see reviews by Antia et al. 1991, Berman and Bronk 2003). Phytoplankton groups differ in their requirements for, and ability to utilize, both inorganic and organic sources of nutrients. Moreover, the ability of algae to use different sources of nutrients depends on their physiological state at the time of nutrient

supply (i.e., nutrient history, growth rate, etc.), as well as other physical factors such as ambient temperature (Fan et al. 2003, Glibert and Burkholder 2006). Nevertheless, the similarity in relative species composition with nutrient ratio in these subtropical systems underscores that there is a degree of physiological regulation at the functional group level. Diatoms and dinoflagellates were apparently able to cope with inorganic P limitation through utilization of organic P sources via APA, while dinoflagellates and cyanobacteria were apparently able to cope with inorganic N limitation through uptake of organic N substrates such as urea and very possibly other forms (Glibert et al. 2004, 2006a). N_2 fixation must also be considered as an alternate N source, and would indeed be the case for the observed colonies of the diazotroph *Trichodesmium* in Moreton Bay (C. Heil unpub. data).

Cyanobacteria declined when P limitation was severe in Florida Bay. Cyanobacteria do not appear to have strong APA (e.g. Koch et al. 2009, Heil et al. 2009). However, it has recently been reported that *Synechococcus* spp. may be able to substitute non-P lipids under conditions of P limitation, making them able to survive under these conditions without needed to access alternate P sources (Van Mooey et al. 2009). Wager et al. (1995) has also shown that *Synechococcus* possesses both a P-repressible and a P-irrepressible AP.

The finding that different functional groups are sustained in inorganic nutrient limiting conditions by differential ability to use organic substrates has important management implications. As anthropogenically-derived organic nutrient, especially N, loading to coastal estuaries is increasing through the increased use of organic fertilizers, manures and other substrates (e.g., Glibert et al. 2006b), the potential to stimulate those organisms such as cyanobacteria and dinoflagellates with the capability to exploit those substrates is large. Moreover, these organisms have the ability to be sustained on DON produced *in situ*, potentially sustaining blooms for long periods of time (e.g. Glibert et al. 2009, 2010).

Expanding the Comparison: Response to Disturbance Events

Major disturbance events that are followed by phytoplankton blooms have been documented for both Moreton Bay and Florida Bay (Moss 1998, Heil et al. 1998, O'Donohue et al.1998, Rudnick et al. 2008, Glibert et al. 2009). These events resulted in the development of highly contrasting phytoplankton communities, however. The physiological capabilities of the different functional groups which dominate these systems provide the basis for understanding how these systems responded to major disturbances over the past decades.

In 2005 a major bloom of the picocyanobacterium *Synechococcus* sp. occurred in the historically clear, P-limited waters of the northeastern region of Florida Bay (Glibert et al. 2009). The initiation of this bloom roughly corresponded with a series of two types of disturbance. First, the hurricanes Katrina, Rita and Wilma passed through or immediately adjacent to the area, resulting in alteration of freshwater flow and a significant nutrient discharge to the eastern region (Rudnick et al. 2006, Glibert et al. 2009). Also, and about the same time, construction on a major causeway connecting the mainland and the Florida Keys occurred, with considerable construction-related clear-cutting and onsite mulching of mangroves in the area, resulting in input of organic matter (Rudnick et al. 2006, Glibert et al. 2009). A sharp increase in the loading of PO_4^{3-} to this region was also found, and this increase was strongly correlated with the resulting increase in Chl *a* documented in the same region (Rudnick et al. 2006). Following the trends shown here, an increase in PO_4^{3-} would decrease the DIN:DIP ratio, and an increase in the Zeaxanthin:Chl ratio would be expected. Indeed, the bloom was dominated almost exclusively by *Synechococcus* spp. This bloom was sustained for roughly 3 years, severely impacting seagrasses, sponges and other benthic communities in the system. The nutrients to sustain this bloom were apparently derived from the recycling of materials from the impacted benthic communities, nutrient release from episodic hypoxia, and the promotion of biogeochemical processes such as dissimilatory nitrate reduction to ammonia (DNRA) (Gardner and McCarthy 2005). This Florida Bay bloom continued to be composed almost exclusively of picocyanobacteria for at least a year, until flagellate and other microbial grazers began to develop (Glibert et al. 2009).

Moreton Bay experienced a 1 in 20 year flood event during a several-week period in 1996 (Davies and Eyre, 1998, Moss 1998, Heil et al. 1998). Dur-

ing the flood period it was estimated that N loads entering the bay were approximately equivalent to annual loads in a non-flood year, while flood loads of P were approximately one-third the annual loads in a non-flood year (Moss 1998). Concentrations of DIN in Moreton Bay were significantly elevated for about 3 weeks following the flood, but thereafter returned to baseline concentrations. Concentrations of PO_4^{3-} also increased, not as significantly as those of N, and also returned to baseline concentrations more slowly (Moss 1998). Phytoplankton biomass, in terms of Chl *a*, roughly doubled in concentration, to about 15 μg L^{-1}, but remained elevated for only a period of weeks (Moss 1998, Heil et al. 1998).

The phytoplankton bloom that developed in Moreton Bay in response to this event was initially composed primarily of diatoms, and in particular the species *Skeletonema costatum, Rhizosolenia setigera* and *Pseudo-nitzschia* spp. (Heil et al. 1998). High abundances of dinoflagellates were only found in the extreme northern bay region, above the Caboolture River. Numerous diatom species including *Rhizosolenia* sp. and *S. costatum* have previously been shown to take up NO_3^- over NH_4^+, although this finding has been for populations growing in cooler waters than those of subtropical or tropical estuaries (e.g., Probyn and Painting 1985, Probyn et al. 1990, Lomas and Glibert 1999a,b). Within about 5 weeks, flagellates comprised nearly half of the phytoplankton community of the central and northern regions of the bay, indicating rapid succession of species (Heil et al. 1998).

Based on an assumed C:Chl *a* ratio of 30 (Ayuka 1992, Gallegos and Vant 1996) and the Redfield ratio, it can be estimated that ~60 mg N m^{-3} was required to support the measured, integrated phytoplankton biomass of Moreton Bay following the flood. With a bay volume of 1,400 km^2 (Stephens 1992) and a mean photic depth during this time of 2m, 1.5 x 10^5 kg of N would be needed, compared to an estimated N load during the few days of the flood of ~3 x 10^5 kg of N day^{-1} (Heil et al. 1998, Davies and Eyre 1998). Consequently, much of the flood associated N load was converted to phytoplankton biomass in the Bay.

Thus from a nutrient standpoint, the Florida Bay hurricane/disturbance provided an initial injection of P, coupled with a likely increase in organic matter that synergistically initiated and sustained a picocyanobacterial bloom for months to years. Moreton Bay, by contrast, responded to a flood event for which the major nutrient enrichment was N, by the development of a short-lived (weeks) diatom bloom. In a review of hurricane impacts in the Neuse River Estuary, Paerl et al. (2007) also documented that the most common phytoplankton group to respond to elevated discharge and nutrients from these events were diatoms, chlorophytes and crytophytes and that dinoflagellate abundance was significantly reduced by such events. The slower-growing dinoflagellates prefer conditions of more moderate flow.

The response of both systems to major disturbance events also differed in the input mechanism and function that suspended sediments played in each event. In Australian estuaries, episodic flooding transports watershed and river derived suspended solids directly to coastal systems (Davies and Eyre, 1998), and deposition of these solids often results in severe coastal degradation and habitat destruction (Preen et al., 1995). The high particulate load in Australian estuaries also influences P dynamics in these estuaries (Harris 2001). A significant amount of watershed and river derived suspended solids accompanied the nutrient input into Moreton Bay during the flood event (Moss 1998). The Chl *a* maximum in western Moreton Bay was delayed for 2 weeks after the end of the flood event (Moss 1998), suggesting that this time was required for flood associated suspended solids to settle before the autotrophic algae had sufficient light availability to access flood nutrients for growth. High concentrations of both total suspended solids have been shown to reduce light penetration and prevent the utilization of available nutrient by phytoplankton in some coastal marine systems (Randall and Day 1981, Cloern 1987). Hurricane-associated winds in Florida Bay resulted in the introduction of significant resuspended solids, largely from benthic sources. These inputs had a briefer impact than observed in Moreton Bay due to the shallower average depth of Florida Bay. Nutrient inputs associated with freshwater inputs through the Taylor and Shark Sloughs do not have significant accompanying suspended solids inputs. It is interesting to note that although resuspended bottom sediments potentially have a significant, but brief impact in Florida Bay, the picocyanobacterial

bloom itself was sufficiently concentrated that it reduced light penetration, eventually resulting in mortality of the submerged aquatic vegetation.

A seasonal analysis of suspended sediments in each system further suggests that outside of major flood events such as occurred in 1998, impacts related to suspended solids were seasonal in nature in Moreton Bay and related to the 'wet' season inputs in summer. Comparatively, the highest average suspended solids in Florida Bay were evident in the spring period, and considerably lower in concentration than in Moreton Bay, which corresponds to a benthic, rather than riverine source.

In summary, the subtropical estuaries of Florida Bay and Moreton Bay were highly contrasting systems. At least during the periods of sampling covered herein, production and biomass in Florida Bay were more likely to have been limited by the availability of P, while N was the more likely limiting nutrient in Moreton Bay (Glibert et al. 2004, 2006a). Florida Bay, a lagoonal estuary, supported picocyanobacterial blooms, which also expanded in magnitude and duration upon an injection of P and organic matter during the disturbance event of 2005. Moreton Bay, an estuary at the transition between river-dominated and lagoon, supported large diatoms and dinoflagellate blooms, but diatoms appeared to develop preferentially following the flood event. These blooms could not be supported and soon dissipated. The fundamental differences between blooms in lagoons and river-dominated systems as previously described (Glibert et al. 2010) thus seem to be sustained in subtropical as well as temperate estuaries.

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SEASONAL AND REGIONAL VARIATIONS IN NET ECOSYSTEM PRODUCTION IN *THALASSIA TESTUDINUM* COMMUNITIES THROUGHOUT FLORIDA BAY

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ABSTRACT

Florida Bay is a unique, shallow ecosystem dominated by the seagrass, *Thalassia testudinum*, and characterized by a series of isolated basins, which often exhibit distinct water quality and environmental conditions. This study describes daily and seasonal variations in ecosystem-level photosynthetic production and respiration rates and how these processes are influenced by the presence of *T. testudinum*. Seasonal measurements were made over a 2-year period at four sites representing the major regions (Eastern, Northern, Central, and Western) in Florida Bay. Ecosystem production and respiration were also measured at an additional site in the Central region that has experienced seagrass dieback in recent years. Open-water dissolved O_2 concentrations measured continuously for consecutive days were used to estimate daytime apparent production (P_a), nighttime respiration (R), gross production (P_g), and net ecosystem production (NEP). Vertical profiles of dissolved O_2 concentrations throughout the water column within a seagrass bed showed distinct vertical gradients and diel patterns, with both nighttime low and daytime high O_2 concentrations occurring below the seagrass canopy. Rates of P_a and R were significantly higher in a *T. testudinum* community relative to adjacent bare sediments. In contrast, NEP did not differ between seagrass bed and bare sediments because of a consistent balance between P_a and R. Estimates of P_a and NEP varied both seasonally and regionally, ranging from 28.1 to 266 mmol O_2 m^{-2} d^{-1} and -56.3 to 116 mmol O_2 m^{-2} d^{-1}, respectively. In general, these variations are consistent with observed variability in light, temperature, salinity, and nutrient regimes. Highest rates were observed in *T. testudinum* communities in the Eastern and Western Regions compared to the rest of the Bay. Seagrass beds were net autotrophic (P_a:R > 1.0) at all sites throughout most of the year; however, net heterotrophic conditions (P_a:R < 1.0) were encountered in a few instances, possibly reflecting disturbance. The results of this study illustrate the importance of *T. testudinum* to NEP in Florida Bay and underscore the need to account for regional variations in annual biogeochemical budgets for Florida Bay.

Keywords: seagrass, ecosystem production, Florida Bay, *Thalassia testudinum*

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INTRODUCTION

There has been longstanding interest in measuring the balance between daytime net primary production (P_a) and respiration (R), or net ecosystem production (NEP = P_a - R), to assess the trophic status of seagrass communities (e.g., Odum 1956, Nixon 1972, Barron et al. 2004), which are often highly productive components of shallow, marine ecosystems. Whether seagrass communities are net autotrophic (P_a > R, positive NEP) or net heterotrophic (P_a < R, negative NEP) varies both seasonally and across systems (Murray and Wetzel 1987, D'Avanzo et al. 1996, Ziegler and Benner 1998). There are many factors that may regulate NEP in these communities, including inorganic nutrient availability, water residence time, and inputs and bioavailability of organic matter (Hopkinson and Vallino 1995, Kemp et al. 1997, Caffrey 2004).

Florida Bay is a unique marine ecosystem dominated by extensive seagrass communities, with *Thalassia testudinum* as the dominant species (Zieman et al. 1989). Although the predominance of calcium carbonate sediments generally leads to phosphorus (P) limitation for seagrass production, there are strong gradients in nitrogen- (N) and P-limitation, with the latter dominating eastern regions of the bay and N-limitation becoming more important in the bay's western regions (Fourqurean and Zieman 2002, Armitage et al. 2005). Sources of nutrients to Florida Bay include exchange with the Gulf of Mexico and Atlantic Ocean shelf, atmospheric deposition, and freshwater inputs (runoff and groundwater) from the Florida Keys and Everglades (Rudnick et al. 1999). Sources of N and P into Florida Bay from the Everglades represent a small (< 15%) fraction of total inputs and are predominately organic forms (Rudnick et al. 1999). Because shallow coastal ecosystems with high organic inputs tend to be net heterotrophic (Kemp et al. 1997), it is possible that net heterotrophy may occur in seagrass communities in Florida Bay where nutrients enter predominantly as organic compounds.

Since the late 1980's, regions of Florida Bay have been experiencing seagrass dieback (e.g., Robblee et al. 1991, Zieman et al. 1999). Indirect evidence suggests that the loss of seagrass has resulted in liberation of inorganic nutrients from the benthos to overlying water, allowing intense phytoplankton blooms to occur (Boyer et al. 1999, Fourqurean and Robblee 1999). In addition, the accumulation and degradation of plant material within dieback areas may have generated a large source of labile organic matter, fueling heterotrophic microbial activity (Bugden et al. 1998) and contributing further to net heterotrophy in dieback areas.

Given the predominance of seagrass communities and unique physiography of Florida Bay, the purpose of this study was to: 1) explore the role of *Thalassia testudinum* in ecosystem production by direct comparison with rates measured in adjacent unvegetated (bare) communities; and 2) to assess regional and seasonal variations in P_g, P_a, R and NEP in *T. testudinum* communities along an east-west transect in Florida Bay. Estimates of P_a and R were obtained from diel cycles in continuous measurements of O_2 concentrations in the open-water of five basins distributed across Florida Bay during several seasons in 2003 and 2004. Estimates of P_a and R in seagrass communities are typically obtained by measuring changes in dissolved oxygen (O_2) or total inorganic carbon concentrations in either the open-water (e.g., Odum 1956) or in chamber incubations (e.g., Ziegler and Benner 1998). Although both techniques have their strengths and drawbacks, the open-water approach provides a truly integrated measure of ecosystem P_a and R (Odum and Wilson 1962).

METHODS

Site Description

Florida Bay is a shallow, sub-tropical estuary bordered to the north by Everglades National Park and by the nearly contiguous islands of the Florida Keys to the south and east. This partially-enclosed, marine ecosystem is open to the Gulf of Mexico to the west and has limited exchange with the Atlantic Ocean through a series of channels between the Keys (Smith 1994). Inputs of freshwater runoff from the Everglades (Shark River Slough, Taylor Slough, and C-111 Canal) are relatively small due to water management practices upstream. Tidal range throughout the bay is also minimal, as physiographic formations (e.g., mudbanks and mangrove islands) limit exchange with the Gulf of Mexico (Holmquist et al. 1989). A series of natural, car-

bonate mudbanks divides the bay into numerous shallow (< 2 m) basins, further reducing water exchange within the system (Powell et al. 1989a).

Measurements of community O_2 cycling in *T. testudinum* beds were made in representative basins located in 4 regions (Northern, Western, Central and Eastern) of Florida Bay (Fig. 1). These sites were chosen to provide representative spatial coverage in distinct zones identified in previous studies (e.g., Zieman et al. 1989, Boyer et al. 1997) and to investigate rates along an east-west transect in Florida Bay. The Little Madeira site, located in northern Florida Bay, is heavily influenced by relatively N-replete, organic-rich freshwater inputs from Taylor River Slough (Rudnick et al. 1999). Sunset Cove represents the eastern Region of Florida Bay, which tends to be relatively P-limited, but may receive nutrient inputs from human activities in the Florida Keys (Lapointe and Clark 1991). In western Florida Bay, the site in Rabbit basin is influenced by exchange with the relatively P-rich water of the Gulf of Mexico, whereas the site in Rankin basin, represents the intermediate nutrient conditions and high evaporation rates typical of central Florida Bay (Rudnick et al. 1999). An additional site, Barnes, which is located in the southern part of the Central Region, experienced an active seagrass dieback event in August 2003. For the Sunset Cove area, rates were also measured in a bare area (unvegetated, ~500 m^2 diameter) adjacent to the *T. testudinum* covered site to provide a comparison between the two communities. The average depth of the *T. testudinum* beds in Rankin and Little Madeira was ~1 m, while mean depths at the other sites were ~2 m.

Open-water Measurements of P_a and R

Continuous measurements of water temperature, salinity, and dissolved O_2 concentrations in the open water were obtained using instrument packages (YSI 600 XLM) equipped with thermistors, conductivity sensors and pulsed-O_2 electrodes. At each site, one instrument was deployed at 0.75 m above the sediment surface within the *T. testudinum* beds just above the seagrass canopy. Measurements were recorded at 10-min intervals over a series of 3-12 day periods in August and November 2003, and January, March, June, and November 2004. On two occasions (June and August 2004), multiple sensors were deployed within a 50 m radius in the *T. testudinum* bed in Sunset Cove to estimate spatial variability in O_2 concentrations.

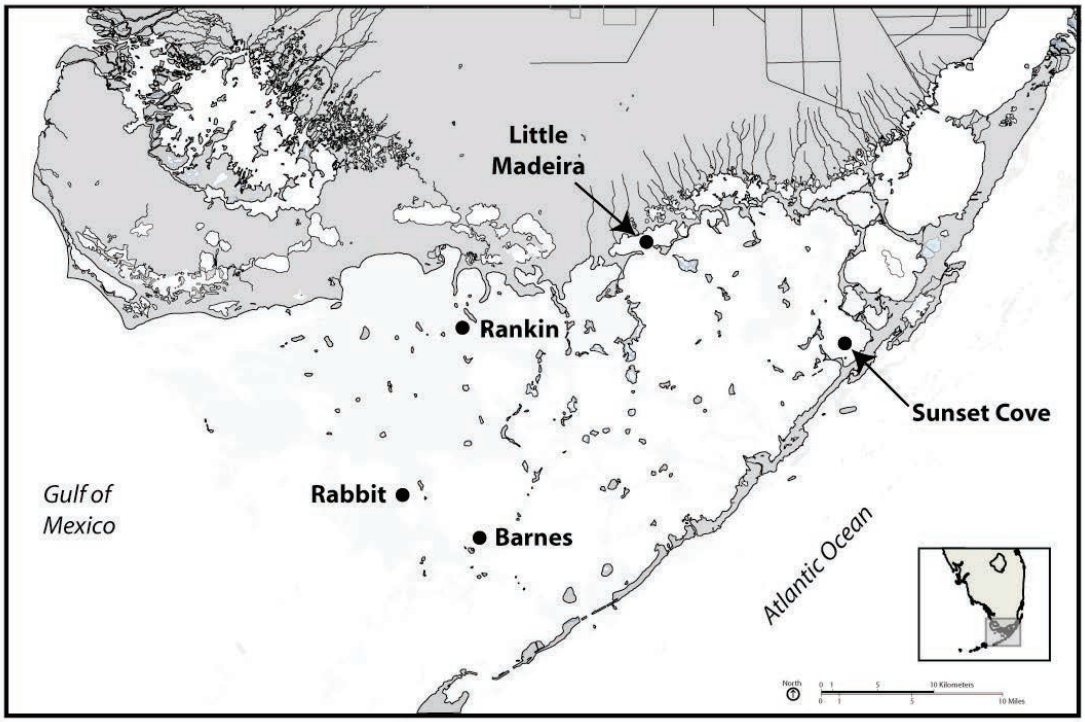

Figure 1. Map of field study sites in Florida Bay.

Despite day-to-day variability in light and temperature, continuous measurements made over 3-10 d periods tend to provide representative estimates of P_a and R (Kenney et al. 1988). This approach has been used successfully in shallow water systems dominated by seagrass (Ziegler and Benner 1998), macroalgae (D'Avanzo et al. 1996), and benthic microalgal communities (Kenney et al. 1988).

Records of O_2 concentrations were used to estimate daytime production and nighttime respiration. Nighttime respiration (R) was estimated from the slope of the decline in O_2 concentrations from the daily maximum to minimum concentrations near, respectively, dusk and dawn. Apparent daytime production (P_a) was estimated from slope of the increases in O_2 from the daily minimum to the maximum concentration (Hagy, J., pers. comm.). Gross daytime production (P_g) was calculated from the sum of R and P_a (Caffrey 2004), assuming that rates of respiration were constant throughout the daytime and nighttime hours. In November 2003, programming errors in the YSI instruments resulted in O_2 concentrations being recorded at 10-h rather than 10-min intervals at two sites (Rabbit and Sunset Cove). For these sites, P_a and R were estimated using changes in O_2 concentrations between two measurements, where the measurements coincided approximately with dawn and dusk. This technique has been shown previously to provide reasonable estimates of metabolic rates in seagrass beds (Odum and Hoskins 1958). In this manner we obtained 3 estimates of both P_a and R over the course of the deployment.

Vertically-integrated rates were computed by multiplying volumetric rates by water column depth. Rates were corrected for O_2 exchange across the air-water interface using an air-sea exchange coefficient of 0.5 g O_2 m^{-2} h^{-1} $(atm)^{-1}$, a value within the range of those reported for other shallow coastal bays (Kremer et al. 2003, Caffrey 2004). Although air-sea exchange coefficients vary with wind speed and tidal currents (Raymond and Cole 2001), a constant value for air-sea exchange was used in the absence of local wind data (Kemp and Boynton 1980). The difference between P_a and R was used to calculate net ecosystem production (NEP) for a given day.

Seasonal and annual estimates of rates were also calculated for each site. Rates from August 2003 and January, March and November 2004 were integrated over a 3-month period to estimate seasonal rates for summer, winter, spring, and fall, respectively. For the Barnes site, rates from early June 2004 were used in the absence of data from March 2004 and, consequently, this rate may overestimate the actual mean spring rate. Data from benthic flux cores in January 2004 at Sunset Cove were used to estimate winter rates at this site. While benthic flux cores give reasonable estimates of P_a and R (e.g., Nagel 2007), they may underestimate open-water rates (Ziegler and Benner 1998). Annual rates were estimated from seasonal rates integrated over the entire year. Photosynthetic and respiratory quotients of 1.0 (1 mol CO_2 = 1 mol O_2) were used to convert rates from oxygen to carbon units (Kemp et al. 1997).

Vertical variations in dissolved O_2 concentrations were measured in both the *T. testudinum* and bare communities in Sunset Cove during morning, early afternoon, and late evening in June 2004. Oxygen concentrations were measured at depths of 0, 50, 100, 160, 170, 180 and 210 (bare only) cm from the water surface with a hand-held dissolved O_2 meter (YSI™ Model 55). Within the seagrass bed, depths at 160, 170, and 180 cm corresponded roughly with the top of the canopy, half way below the canopy and the sediment surface, respectively.

Photosynthetically available irradiance (PAR) was measured at each site using a hand-held LICOR LI-1000 2π quantum sensor. PAR attenuation coefficients were estimated from measurements made at approximately 0.25- to 0.5-m depth intervals beginning at the water column surface at least 1 time during peak daily irradiance. Ambient irradiance in the air was recorded continuously at 15-min intervals at the National Park Service station in Key Largo, near the Sunset Cove site. PAR was also monitored continuously in the *T. testudinum* bed in Sunset Cove using a submersible Odyssey™ Light Meter (Dataflow Systems PTY, LTD) that was deployed adjacent to the YSI sensor. Continuous measurements of irradiance were integrated hourly and summed over a 24 h period to yield a daily integrated irradiance (mol m^{-2} d^{-1}).

In addition to environmental conditions (e.g., salinity, temperature, and PAR) measured *in situ*, rates measured in this study were also compared to contemporaneous seasonal and regional water quality data obtained from the SERC-FIU Water Quality Monitoring Network (Boyer and Briceno 2005)

for sites at Little Madeira, Rankin, Rabbit, and Barnes. Rates at Rankin and Rabbit were also compared to Braun-Blanquet seagrass abundance data measured at these sites in 2003-04 by the FWRI/ UNCW South Florida Fisheries Habitat Assessment Program.

Regional and seasonal differences in metabolism rates and environmental conditions as well as between the bare and *T. testudinum* communities in Sunset Cove were investigated by two-way analysis of variance (ANOVA). Post-hoc pairwise comparisons were made using the Tukey HSD test at $\alpha = 0.05$. Effects of environmental conditions measured *in situ* on rates were determined using regression analysis. A multivariate pair-wise correlation matrix was used to assess relationships between regional water quality data (Boyer and Briceno 2005) and metabolism rates. All statistical analyses were performed using JMP IN™ software.

RESULTS

Mean water temperatures in *T. testudinum* beds in Florida Bay ranged from ~20° to 31° C over the annual cycle (Table 1), with highest values observed in summer months. Salinities at all sites ranged from ~5 to 48, with the highest salinity recorded at Rankin in June 2004 (Table 1). Little Madeira exhibited the largest range (~5 to 36) in salinities as well as the lowest values recorded across basins. Seasonally, lowest salinities at all sites were observed in November 2003, whereas highest salinities were observed in June 2004 (excluding Sunset Cove) (Table 1). These patterns are similar to those reported previously (Boyer and Briceno 2005) and may be attributable to higher than average precipitation in Fall 2003 and lower than average precipitation throughout 2004. Light attenuation coefficients ranged from 0.3 to 5.6 m and were typically higher at Little Madeira and Rankin relative to the other sites (Table 1). The highest light attenuation coefficients at all sites were observed in November (2003 and 2004).

Dissolved O_2 concentrations exhibited clear diel patterns and varied both seasonally and regionally (Fig. 2). Concentrations ranged from 116 to 325 μM O_2 throughout the study (Table 1) and minimum and maximum O_2 concentrations typically occurred just after dawn and just before dusk, respectively (e.g., Fig. 2). Concentrations were highest in January and March 2004; however, the daily range in concentrations was greatest in summer months (e.g., Fig. 2a). Percent saturation of O_2 also varied seasonally (Table 1), ranging from 50% to 166% saturation. Lowest mean values of both O_2 concentration and percent saturation were recorded at Barnes in August 2003 and November 2004 (Table 1). At Little Madeira, patterns in O_2 concentrations exhibited frequent dramatic shifts in concentration over short time

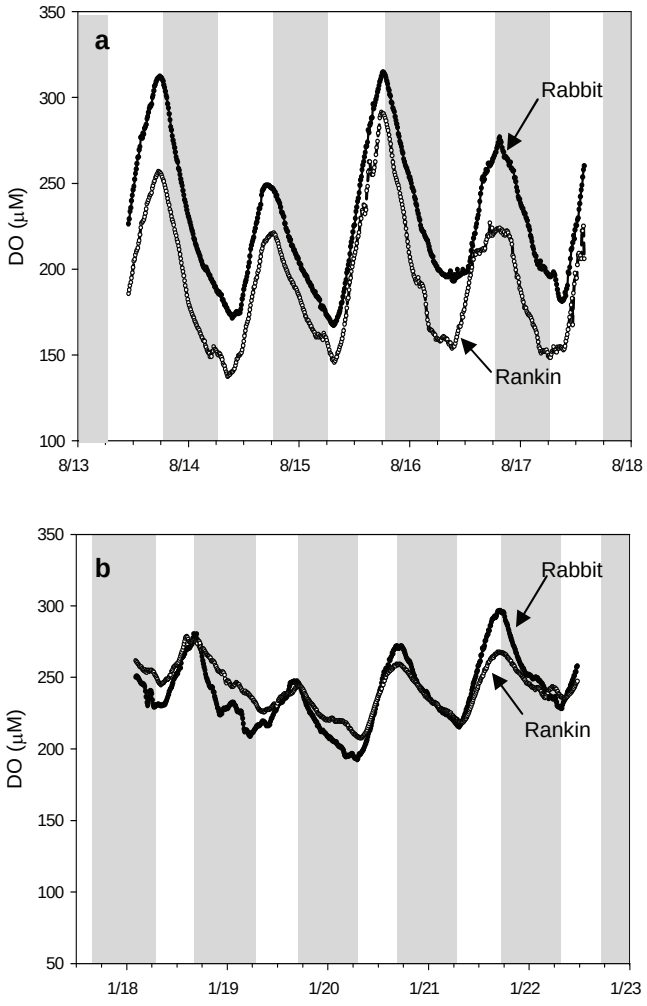

Figure 2. Example of diel variation in continuous O_2 concentrations (μM) in Rankin (open symbol) and Rabbit (solid) in August 2003 (a) and January 2004 (b). Shaded areas indicated nighttime hours.

Table 1. Mean (± SE) dissolved O_2 concentrations (μM), percent O_2 saturation (% Sat), range in % saturation, temperature (°C), salinity, and light attenuation coefficients (k_d, units = m^{-1}) in *Thalassia testudinum* beds throughout Florida Bay in 2003-4. "nd" indicates that no data are available for that month.

Site	O_2	% Saturation	Range	T	Salinity	k_d
Little Madeira						
Aug '03	226 ± 0.82	102 ± 0.41	82 - 134	29.8 ± 0.04	11.3 ± 0.05	nd
Nov '03	240 ± 0.54	94 ± 0.21	62 - 105	24.1 ± 0.03	4.93 ± 0.03	1.91
Jan '04	269 ± 0.65	104 ± 0.24	79 - 128	20.1 ± 0.04	15.4 ± 0.12	0.82
Mar '04	245 ± 0.70	100 ± 0.28	62 - 135	23.0 ± 0.05	16.2 ± 0.03	1.27
Jun '04	181 ± 1.26	94 ± 0.68	56 - 147	30.5 ± 0.03	35.4 ± 0.01	0.91
Nov '04	204 ± 0.61	92 ± 0.27	58 - 114	26.4 ± 0.02	36.0 ± 0.08	5.65
Rankin						
Aug '03	194 ± 1.34	102 ± 0.74	72 - 155	29.7 ± 0.03	39.9 ± 0.01	nd
Nov '03	228 ± 0.76	97 ± 0.35	76 - 120	23.9 ± 0.03	20.0 ± 0.04	0.76
Jan '04	246 ± 0.89	105 ± 0.37	84 - 127	20.6 ± 0.05	32.0 ± 0.01	0.44
Mar '04	229 ± 0.87	100 ± 0.41	78 - 136	21.2 ± 0.04	33.6 ± 0.02	0.95
Jun '04	188 ± 1.17	105 ± 0.69	66 - 159	30.5 ± 0.03	47.8 ± 0.02	0.95
Nov '04	nd	nd	nd	25.7 ± 0.02	36.4 ± 0.04	0.84
Rabbit						
Aug '03	231 ± 1.45	121 ± 0.80	86 - 166	29.9 ± 0.03	38.5 ± 0.01	nd
Nov '03	219 ± 7.16	99 ± 3.38	78 - 128	24.3 ± 0.25	31.8 ± 0.06	0.87
Jan '04	247 ± 0.61	107 ± 0.27	90 - 123	20.4 ± 0.03	33.5 ± 0.01	0.26
Mar '04	241 ± 0.90	106 ± 0.72	85 - 126	22.9 ± 0.05	35.0 ± 0.01	0.41
Jun '04	202 ± 0.98	108 ± 0.54	74 - 146	30.4 ± 0.02	41.1 ± 0.02	0.38
Nov '04	186 ± 0.67	89 ± 0.34	73 - 108	26.3 ± 0.02	36.1 ± 0.02	0.64
Barnes						
Aug '03	163 ± 0.81	85 ± 0.45	64 - 112	29.7 ± 0.03	38.4 ± 0.01	nd
Nov '03	245 ± 1.03	112 ± 0.31	90 - 137	24.2 ± 0.03	31.9 ± 0.01	0.55
Jan '04	246 ± 0.90	106 ± 0.42	87 - 138	20.4 ± 0.03	32.3 ± 0.01	0.66
Mar '04	213 ± 0.99	94 ± 0.24	77 - 125	22.3 ± 0.05	34.2 ± 0.01	0.48
Jun '04	207 ± 0.74	111 ± 0.41	77 - 145	30.2 ± 0.02	42.4 ± 0.01	0.51
Nov '04	166 ± 0.89	81 ± 0.46	52 - 112	26.3 ± 0.02	39.1 ± 0.02	0.54
Sunset Cove						
Aug '03	175 ± 1.35	90 ± 0.72	50 - 131	29.9 ± 0.03	36.3 ± 0.00	nd
Nov '03	246 ± 6.87	108 ± 2.95	88 - 125	23.5 ± 0.20	26.7 ± 0.09	0.27
Jan '04	nd	nd	nd	nd	nd	0.89
Mar '04	263 ± 0.70	116 ± 0.33	86 - 154	23.2 ± 0.04	28.2 ± 0.01	0.26
Jun '04	202 ± 1.11	106 ± 0.61	66 - 153	31.1 ± 0.02	36.3 ± 0.04	0.51
Nov '04	195 ± 0.71	97 ± 0.36	52 - 142	26.2 ± 0.02	40.4 ± 0.01	1.03

intervals, and these changes were significantly (r^2 = 0.82, $p < 0.01$) and negatively related to shifts in salinity. Spatial differences in concentrations and rates (P_a, R) at 3 stations each separated by 50 m across the *T. testudinum* bed in Sunset Cove were not significant in June or August 2004 (data not shown).

Clear temporal patterns were observed in vertical profiles of O_2 concentrations throughout a day in the water column overlying the *T. testudinum* and bare sediments in June 2004. At both sites, concentrations in the top 1 m of the water column increased from morning to late evening (Fig. 3). Concentrations varied little with depth at the bare site over the day, excluding a sharp decrease beginning ~20 cm above the sediment surface in the

Table 2. Rates (mean ± SE) of NEP, P_a, P_g, and R (mmol O_2 m^{-2} d^{-1}) in *Thalassia testudinum* beds throughout Florida Bay in 2003-4. "n" indicates the number of days over which the parameters were measured, and "nd" indicates that no data are available for that month. An '*' next to sites in November 2003 indicates that rates were calculated from changes between minimum and maximum O_2 concentrations available for those sites (see methods).

Site	P_a	R	P_g	NEP	n
Little Madeira					
Aug '03	73.9 ± 14.1	36.7 ± 7.84	117 ± 22.7	37.1 ± 8.00	4
Nov '03	39.1 ± 8.96	38.8 ± 10.8	71.2 ± 15.0	0.27 ± 11.0	7
Jan '04	56.2 ± 8.14	32.3 ± 8.15	82.6 ± 11.0	23.9 ± 11.0	6
Mar '04	72.7 ± 9.44	57.3 ± 7.44	130 ± 9.75	15.5 ± 13.8	12
Jun '04	nd	nd	nd	nd	nd
Nov '04	66.3 ± 9.88	53.7 ± 11.2	113 ± 11.35	12.6 ± 17.2	12
Rankin					
Aug '03	101 ± 22.1	83.8 ± 8.41	196 ± 31.9	20.3 ± 14.1	4
Nov '03	62.1 ± 8.57	62.0 ± 4.81	114 ± 9.16	-0.94 ± 9.81	7
Jan '04	72.5 ± 10.0	50.3 ± 3.41	114 ± 8.57	22.3 ± 13.8	5
Mar '04	68.0 ± 10.5	55.4 ± 3.51	123 ± 12.3	7.50 ± 9.79	5
Jun '04	99.8 ± 10.5	81.6 ± 5.65	208 ± 10.5	18.2 ± 13.6	5
Nov '04	nd	nd	nd	nd	nd
Rabbit					
Aug '03	224 ± 40.0	146 ± 20.3	398 ± 57.5	77.6 ± 32.7	4
*Nov '03**	126 ± 9.28	117 ± 12.3	229 ± 2.78	23.8 ± 15.8	3
Jan '04	85.8 ± 12.3	60.4 ± 5.19	135 ± 10.9	25.4 ± 15.4	5
Mar '04	110 ± 12.0	110 ± 8.83	220 ± 10.7	-35.0 ± 17.2	6
Jun '04	208 ± 5.95	133 ± 4.37	385 ± 8.32	74.4 ± 7.32	7
Nov '04	89.0 ± 9.20	111 ± 3.50	188 ± 7.83	-22.5 ± 11.5	4
Barnes					
Aug '03	107 ± 16.1	113 ± 9.02	242 ± 13.6	-20.0 ± 22.2	6
Nov '03	129 ± 23.7	68.1 ± 16.8	185 ± 34.6	61.1 ± 18.8	5
Jan '04	82.6 ± 8.09	81.2 ± 8.34	149 ± 5.99	1.38 ± 15.1	6
Mar '04	nd	nd	nd	nd	nd
Jun '04	166 ± 11.7	86.2 ± 6.01	281 ± 17.1	79.8 ± 10.2	7
Nov '04	69.0 ± 9.77	107 ± 5.71	164 ± 12.3	-37.6 ± 14.8	3
Sunset Cove					
Aug '03	247 ± 15.3	181 ± 16.6	461 ± 6.71	33.6 ± 39.4	4
*Nov '03**	128 ± 16.9	101 ± 18.2	229 ± 22.0	26.7 ± 27.4	3
Jan '04	nd	nd	nd	nd	nd
Mar '04	217 ± 13.5	111 ± 9.21	329 ± 22.5	106 ± 5.54	12
Jun '04	238 ± 16.2	170 ± 16.6	464 ± 34.4	68.8 ± 14.4	8
Nov '04	176 ± 19.0	149 ± 15.2	305 ± 26.9	27.6 ± 19.0	11

early evening (Fig. 3c). Oxygen concentrations were also relatively uniform in the top 1 m of the water column overlying the *T. testudinum* bed. However, sharp gradients in O_2 concentrations occurred in mid-afternoon and at dusk, with lowest concentrations observed at the sediment surface (Fig. 3b and c). Lowest O_2 concentrations occurred in late evening below the seagrass canopy, when there was a 3-fold drop in concentrations relative to above the canopy (Fig. 3c).

Estimates of P_a, R, and P_g in *T. testudinum* communities varied seasonally as well as among basins. Rates of P_a were typically greater than estimates of R and ranged from 28.1 to 265 mmol O_2 m^{-2} d^{-1} and 23.4 to 203 mmol O_2 m^{-2} d^{-1}, respectively (Table 2). Throughout the year, rates of P_g, P_a, and R were significantly higher ($p < 0.05$) in summer (June and August) compared to other months. Across all of the basins, the *T. testudinum* community in Sunset Cove exhibited significantly higher ($p < 0.05$) rates of P_g and P_a compared to other sites (Table 2). Lowest rates of P_g, P_a, and R

were observed at Rankin and Little Madeira (Table 2). Excluding November 2003, rates of P_g both and R were significantly higher ($p < 0.05$) in the *T. testudinum* community at Sunset Cove compared to rates in the adjacent bare community (Fig. 4) and estimates of P_g, P_a, and R were all significantly greater ($p < 0.02$) below the seagrass canopy relative to above the canopy (Nagel 2007). In addition to seasonal variability, day-to-day variations in rates were observed at each site throughout the year, and this variability was more pronounced in estimates of P_a relative to R.

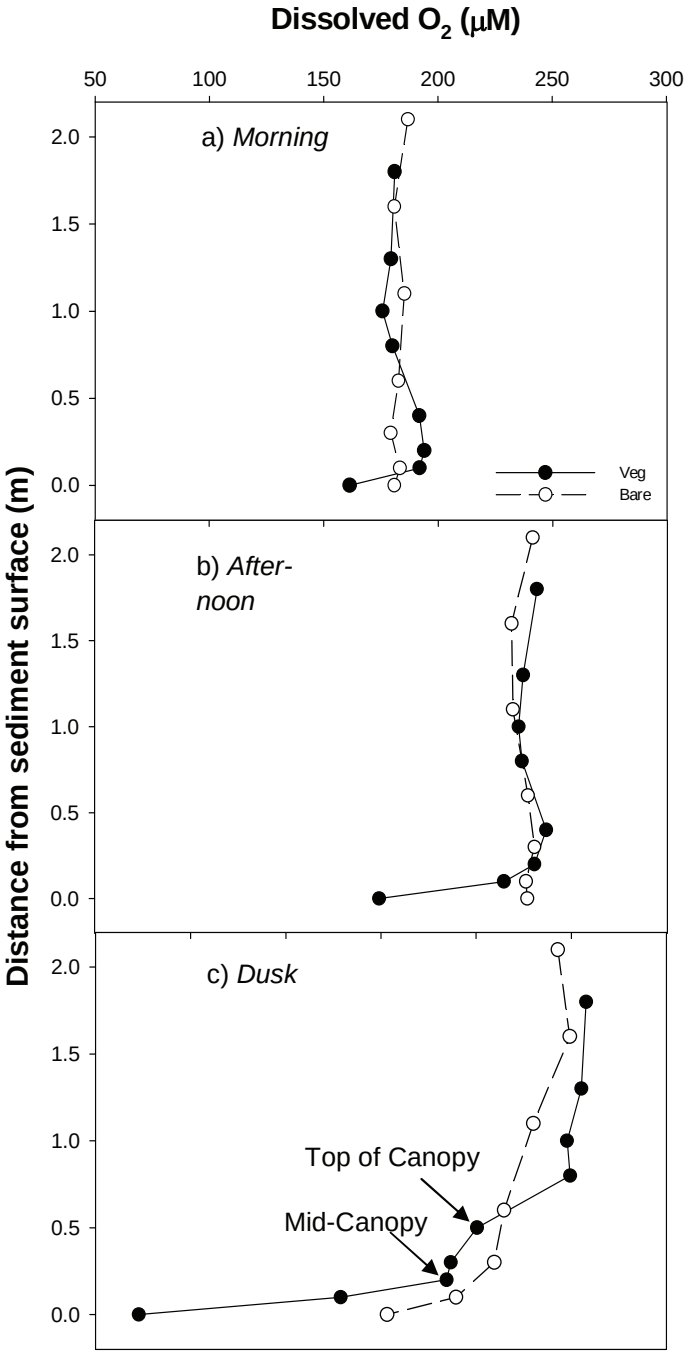

Figure 3. Vertical profiles of water column O_2 concentrations (μM) in a *Thalassia testudinum* bed and adjacent bare area at Sunset Cove in June 2004 during the morning (a), mid-afternoon (b), and at dusk (c). Depth on the y-axis is presented as distance from the sediment surface in meters.

In general, environmental conditions measured *in situ* explained much of the variability in estimates of P_g, P_a, and R, but relationships differed among sites. For example, P_g was positively related ($p < 0.03$) to PAR at Rankin, Little Madeira, and Sunset Cove (e.g., Fig. 5), but these relationships were marginally insignificant ($p < 0.10$) at Rabbit and Barnes. Similarly, variations in temperature significantly ($y = 3.05x - 10.5$, $r^2 = 0.58$, $p < 0.0002$) explained variability in R at Rankin, as well as at Rabbit and Sunset Cove. Varying air-sea exchange coefficients used to calculate rates had little influence (< 5% change) on rates at sites > 1 m depth and only a marginal effect (< 25% change) on rates at sites of 1 m in depth (Nagel 2007).

Seasonal and regional variations in ecosystem metabolism rates measured in this study were compared to water quality data at Little Madeira, Rankin, Rabbit, and Barnes (Boyer and Briceno 2005). These comparisons revealed that variations in surface temperature and salinity were significantly and positively correlated ($p < 0.05$) with P_g, P_a, and R (Table 3). In contrast, these rates were negatively correlated ($p < 0.05$) with turbidity (Table 3). Of all of the sites, phytoplankton abundance, as determined from chlorophyll-*a* concentrations, was highest at Rankin, and there was a strong positive correlation ($r = 0.91$, $p < 0.0001$) between turbidity and chlorophyll-*a* (Boyer and Briceno 2005) at this site. Although the highest degrees of light attenuation observed across the sites consistently occurred at Little Madeira (Table 1), P_g was not correlated with water clarity at this site ($r = 0.005$). For two of our study sites, Rankin and Rabbit, where seagrass

density (as Braun-Blanquet) data were available, P_g was positively correlated to plant abundance ($r = 0.79$, $p < 0.05$, Spearman's Rho correlation, data not shown).

Estimates of NEP also varied regionally (Table 2), ranging from -56.3 to 116 mmol O_2 m^{-2} d^{-1}, with highest rates of NEP observed in Sunset Cove. Like P_a, NEP varied seasonally, however there were no consistent seasonal patterns across basins. Mean NEP values were typically positive or not significantly different from zero, indicating either net production of O_2 or no net change in O_2 (Table 2). On some occasions, mean seasonal values of NEP were negative, indicating net consumption of O_2 (Table 2). Mean ratios of P_a:R within each basin, which followed patterns similar to those for P_a, were greater than 1.0 at all sites throughout the year, excluding August 2003 in Barnes, and highest ratios were observed in Little Madeira (Fig. 6a). Across all sites, there was a positive linear relationship between P_a and R and the slope of the linear regression line was also greater than 1.0 (Fig. 6b). There was no significant difference ($p > 0.05$) in NEP and ratios of P_a:R between the *T. testudinum* and bare communities in Sunset Cove. Annual estimates of NEP ranged from 4.5 to 136 mol C m^{-2} yr^{-1} and Sunset Cove exhibited the highest rates (Table 3).

DISCUSSION

Diel Patterns in O_2 Concentrations

Clear diel patterns in dissolved O_2 concentrations were observed in the water column over *T. testudinum* communities during this study, and these diurnal variations are consistent with those observed in previously reported studies (Odum 1957, Murray and Wetzel 1987, Moriarty et al. 1990, Leverone 1995, Ziegler and Benner 1998). Minimum O_2 concentrations, however, typically occurred after sunrise with the onset of net O_2 production often not evident until 2-3 h after dawn. This time lag in O_2 production could arise from mechanisms related to plant physiology and physical conditions. The light compensation point, or the minimum irradiance at which net photosynthesis is first evident, does not likely coincide with dawn, and the time of day at which the light compensation point is reached may depend on water clarity over the seagrass bed. Furthermore, O_2 evolution from the plant leaves to the surrounding water column may not occur simultaneously with the initiation of plant photosynthesis due to temporary lacunal storage of O_2 (Kemp et al. 1986). High respiratory demand of belowground tissues during nighttime hours could result in low partial pressure of O_2 in the plant lacunae until some time after photosynthesis begins (Greve et al. 2003, Borum et al. 2005). Depletion of O_2 in the lacunae to sufficient levels in response to the O_2 deficit created during the night could lead to a delay (up to 30 min) in evolution of O_2 to the water column (Kemp et al. 1986). Another source of delay may arise from the lag time associated with vertical mixing of O_2 between the plant canopy, where metabolic rates appear to be highest (Fig. 3), and the overlying water column, where the instrument was located (Koch and Gust 1999, Binzer et al. 2005). Thus, some combination of these factors likely contributed to measured daily time-delays between initial O_2 production and observed increases in water column O_2 concentration.

Peak O_2 concentrations also occurred during daytime hours, with the initial decline in concentrations beginning in the afternoon several hours after peak irradiance. The decline in O_2 concentrations during daylight hours is likely the result of heterotrophic processes overtaking autotrophic production during the day, and this is supported by the decline in hourly rates of daytime net production, P_a, during early afternoon (data not shown). Potential causes of this decline in O_2 production could stem from carbon or nutrient limitation during peak photosynthesis or corresponding high rates of photorespiration (Touchette and Burkholder 2000). However, these processes were not measured and would require further investigation to ascertain how these processes contributed to the observed depression in rates.

Diel variations in O_2 concentrations in the water column of seagrass beds have been widely used to estimate production and respiration in seagrass communities (Odum and Hoskins 1958, Odum and Wilson 1962, Nixon 1972, Ziegler and Benner 1998). While this technique is not without criticism, measurements of dissolved O_2 concentrations are relatively simple and provide reasonable estimates of total ecosystem production compared to other methods (Ziegler and Benner 1998). In this

Table 3. Pair-wise correlation matrix and coefficients for P_a, R, and P_g (mmol O_2 m^{-2} d^{-1}) measured in this study relative to total nitrogen (TN), dissolved inorganic nitrogen (DIN), total organic nitrogen (TON), total phosphorus (TP), chlorophyll-*a* (CHLA), total organic carbon (TOC), salinity, temperature, and turbidity in the water column overlying *T. testudinum* communities in Florida Bay 2003-04. Coefficients in bold indicate a significant difference of $p < 0.05$. Water quality data were provided by the SERC-FIU Water Quality Monitoring Network which is supported by SFWMD/SERC Cooperative Agreement #C-15397 as well as EPA Agreement #X994621-94-0.

	P_a	R	P_g	TN	DIN	TON	TP	CHLA	TOC	Salinity	Temp	Turbidity
P_a		**0.76**	**0.97**	-0.31	-0.34	-0.25	-0.21	-0.13	-0.37	**0.58**	**0.49**	**-0.53**
R			**0.88**	-0.22	-0.42	-0.15	-0.16	-0.14	**-0.45**	**0.70**	**0.45**	**-0.63**
P_g				-0.29	-0.37	-0.23	-0.20	-0.14	-0.37	**0.67**	**0.57**	**-0.57**
TN					-0.09	**0.99**	**0.69**	**0.45**	0.40	-0.16	-0.04	**0.61**
DIN						-0.24	**-0.46**	-0.15	0.25	-0.41	-0.04	0.26
TON							**0.74**	**0.46**	0.35	-0.09	-0.03	**0.56**
TP								**0.75**	**0.48**	0.03	-0.02	**0.57**
CHLA									**0.62**	0.01	0.21	**0.71**
TOC										-0.15	0.27	**0.76**
Salinity											0.39	**-0.48**
Temp												0.07
Turbidity												

study, estimates of daytime apparent production, P_a, were calculated from the rate of change in O_2 concentrations between the daily minimum and maximum concentrations, rather than between concentrations at dawn and dusk. This approach produces relatively conservative estimates of P_a and may slightly overestimate 24-h production.

Exchange of gases across the air-sea interface presents another potential source of error in calculating P_a and R using the open-water approach (e.g., Gazeau et al. 2005). Air-sea exchange coefficients vary with wind speed and fetch, and both of these factors may vary in Florida Bay over the course of a day. For this study, we assumed that air-sea exchange rates were constant for all sites over the deployment of the sensors, and thus we did not account for variability in air-sea O_2 exchange associated with varying wind speed and direction (Kremer et al. 2003). Higher wind velocities increase turbulence, and thus, air-sea exchange, which tends to cause underestimation of both P_a and R (Ziegler and Benner 1998). Wind fetch may also factor into air-sea exchange in Florida Bay depending on the relative position of nearby mangrove islands to the direction of the wind. Although wind speed and direction were not measured directly in this study, a sensitivity analysis suggested that effects of variable air-sea exchange coefficients on calculations of P_a and R were relatively small for these communities.

Influence of *Thalassia testudinum* on Ecosystem Production

Throughout the year, the *T. testudinum* community in Sunset Cove exhibited significantly higher rates of both P_a and P_g relative to adjacent bare sediments. While seagrasses are largely responsible for this difference, other benthic autotrophs, including epiphytes and benthic micro- and macroalgae, found in these communities may have contributed to the observed differences (Murray and Wetzel 1987, Moncreiff et al. 1992, Kaldy et al. 2002). Benthic microalgae exhibited high rates of productivity in areas without seagrass in Sunset Cove, and they have been shown to contribute significantly to ecosystem production in seagrass communities as well (Jensen and Gibson 1986, Santos et al. 2004). However, benthic microalgae were significantly less abundant within the seagrass bed (Burton-Evans 2005), presumably be-

Table 4. Seasonal (mol C m^{-2} mo^{-1}) and annual (mol C m^{-2} yr^{-1}) estimates of NEP, P_a, P_g, and R in *Thalassia testudinum* beds throughout Florida Bay.

Site	P_a	R	P_g	NEP
Little Madeira				
Winter	1.69	1.13	2.48	0.72
Spring	2.23	1.18	3.99	0.47
Summer	2.27	0.97	3.60	1.14
Fall	1.19	1.76	2.16	0.01
Annual	22.1	15.1	36.7	7.01
Rankin				
Winter	2.18	1.51	3.41	0.67
Spring	2.09	1.70	3.77	0.23
Summer	3.10	2.57	6.03	0.62
Fall	1.88	1.88	3.46	-0.03
Annual	27.7	23.0	50.0	4.48
Rabbit				
Winter	2.57	1.81	4.06	0.76
Spring	3.39	3.37	6.74	-1.07
Summer	6.87	4.49	12.2	2.38
Fall	3.83	3.55	6.93	0.72
Annual	50.0	39.7	89.8	8.37
Barnes				
Winter	2.48	2.44	4.47	0.04
Spring	5.09	2.64	8.60	2.45
Summer	3.29	3.47	7.41	-0.61
Fall	3.92	2.06	5.62	1.85
Annual	44.3	31.8	78.3	11.2
Sunset Cove				
Winter	6.52	3.34	9.88	3.18
Spring	7.31	5.20	14.2	2.11
Summer	7.59	5.56	14.1	1.03
Fall	3.88	3.07	6.96	0.81
Annual	75.9	51.5	136	21.4

cause of shading. Thus, their contribution to P_a and R in seagrass beds may have been relatively unimportant. Plankton contribution to ecosystem P_a and R was also relatively minor (< 5%) based on light and dark bottle measurements of O_2 production and consumption within this community (Cornwell, Madden, pers. comm.). However, the relative importance of phytoplankton would likely rise with any increases in nutrient inputs to Florida Bay (Armitage et al. 2006).

Net ecosystem production, NEP, was positive throughout the year in both the *T. testudinum* and bare communities, indicating net autotrophy. In contrast to elevated rates of P_a and R in the *T. testudinum* community compared to bare sediments, NEP did not differ significantly between the two communities (Fig. 4). This pattern may be explained by higher respiratory demands within the seagrass bed relative to the bare community. Enhanced deposition and trapping of organic matter and senescing plant material (Ward et al. 1984, Gacia and Duarte 2001) combined with elevated production of dissolved organic matter (Ziegler and Benner 1999, Barron et al. 2004) in seagrass communities stimulates heterotrophic microbial processes in both the sediment and overlying water column (Chin-Leo and Benner 1991, Velimirov and Walenta-Simon 1993), resulting in higher community respiratory demand. In addition, the physical structure of seagrass leaves provide habitat for epiphytic bacteria (Kirchman et al. 1984, Barnabas 1992) and other heterotrophic organisms (e.g., Hall and Bell 1993, Edgar et al. 1994), which also contribute to higher respiratory demand within these communities (e.g., Barron et al. 2004, Gacia et al. 2005). Strong gradients in O_2 concentrations within the seagrass canopy (Fig. 3) in combination with higher rates of R below the canopy (Nagel 2007) provide evidence of the higher respiratory demand in the *T. testudinum* community relative to adjacent bare sediments.

Regional Variations and Regulating Factors for P_g and NEP

Rates of gross primary production, P_g, observed in *T. testudinum* communities in Florida Bay were relatively high compared to rates reported for other seagrass communities but were

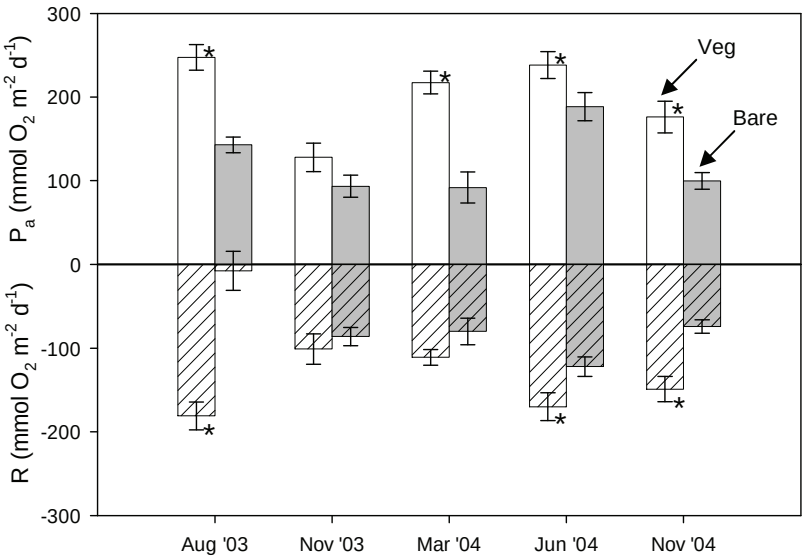

Figure 4. Seasonal variations in mean (± SE) rates of P_a (open) and R (hatched bars) in a *T. testudinum* bed (white bars) and bare area (gray bars) in Sunset Cove in 2003-2004. An '*' indicates a significant difference at $p < 0.05$ between the sites for each rate.

similar to rates reported in *T. testudinum* communities elsewhere (Odum and Hoskins 1958, Odum and Wilson 1962, Ziegler and Benner 1998). Despite seasonal variability, clear regional patterns in P_g emerged (Table 2). The high rates of P_g observed at Rabbit (Western Region) may be explained by higher inputs of relatively P-rich water of the Gulf of Mexico compared to other regions in the bay (Boyer et al. 1999) which stimulate production. Rates were also high at Sunset Cove in the Eastern Region, a region typically considered to be P-limited. Situated in close proximity to the Florida Keys, Sunset Cove may receive nutrient inputs in the form of freshwater runoff or groundwater seepage from the Keys leading to high rates of P_g that may not be representative of other communities in the Eastern Region. However, water column nutrient concentrations, and in particular P, decrease rapidly offshore of the Keys (Lapointe and Clark 1991). Furthermore, N:P ratios of plant tissues in Sunset Cove were higher (~50) than ratios of plants at Rabbit (~30) (Nagel 2007), suggesting that seagrasses in Sunset Cove were relatively P-limited (e.g., Fourqurean and Zieman 2002).

Previous studies have suggested that the relative abundance or spatial coverage of seagrasses tend to explain much of the seasonal and regional variability in P_g (Duarte 1989). For the two of our sites where plant cover data were available (Rankin and Rabbit), we found that sites with greater *T. testudinum* abundance where generally more productive and that P_g was positively correlated with plant abundance, with a considerable fraction (~80%) of the variation in P_g explained by plant abundance. Other environmental factors, including water quality conditions, may regulate P_g in Florida Bay seagrass communities. In a comparison with seasonal and regional water quality data (Boyer and Briceno 2005) rates of P_g were strongly correlated with temperature, turbidity, and salinity (Table 3). Surface temperature appears to have driven much of the seasonality observed in rates, as variations in temperature were significantly correlated with P_g. Variations in P_g were negatively correlated with turbidity, suggesting the importance of water clarity in driving productivity at seasonal and regional scales, even in this shallow subtropical system (e.g., Fourqurean et al. 2003). Furthermore, day-to-day and seasonal variations in irradiance measured in this study explained 15-65% of the variability in P_g observed at study sites (e.g., Fig. 5). While much of the turbidity within Florida Bay was likely related to tripton (non-algal particulate matter) concentrations (e.g., Kelble et al. 2005), phytoplankton abundance (e.g., Phlips et al. 1995) may have contributed to light attenuation at Rankin. At Little Madeira, where light attenuation was high and the water was often turbid and darkened with colored dissolved organic matter, a lack of correlation between P_g and water clarity suggests that *T. testudinum* plants in this very shallow Little Madeira site may be adapted to low-light conditions (e.g., Kraemer and Hanisak 2000). It is not clear whether phytoplankton blooms (Phlips et al. 1995, Glibert et al. 2004), sediment resuspension

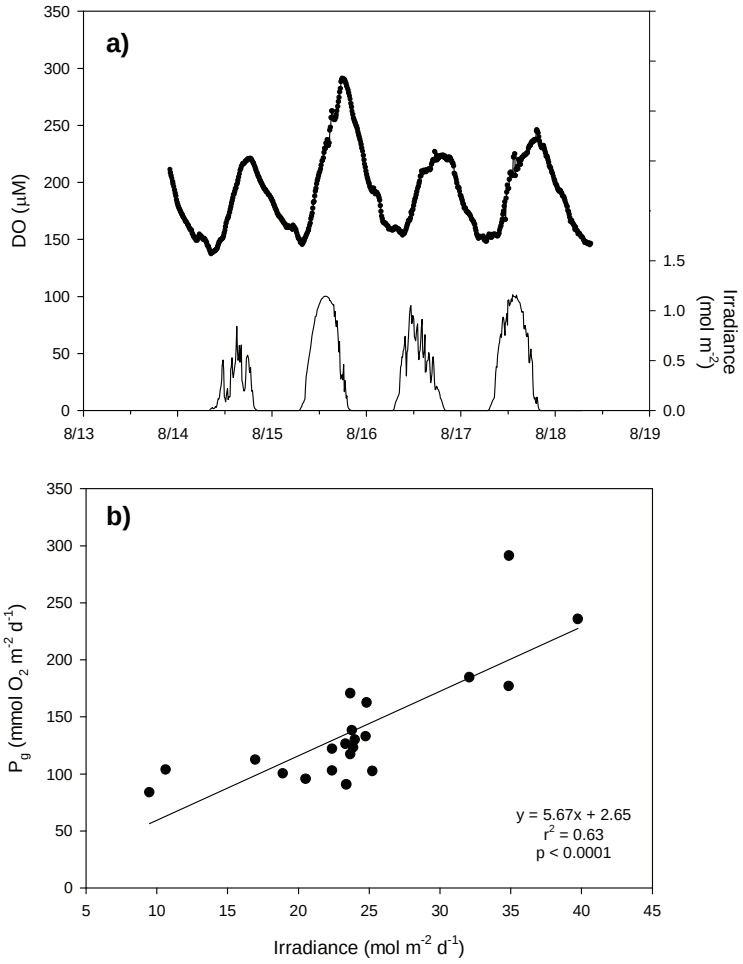

Figure 5. (a) Diel variations in dissolved O_2 concentrations (μM) (bold line) and irradiance in a *T. testudinum* bed in Rankin during August 2004. (b) Linear regression of P_g (mmol O_2 m^{-2} d^{-1}) on irradiance in Rankin throughout 2003-4.

(Lawrence et al. 2004) or high concentrations of chromophoric dissolved organic matter (Stabenau et al. 2004) contributed to increased turbidity throughout this study, However the reduction of water clarity associated with projected hydrological changes to the Everglades-Florida Bay watershed (Fourqurean et al. 2003) may lead to light-limiting conditions for plant production within this ecosystem in the future.

Short-term variations in salinity may have influenced negatively rates of P_g at Little Madeira. While *T. testudinum* is tolerant of prolonged exposure to salinities much higher (~ 60) than those observed in this study (Koch et al. 2007), rapid changes in salinity may temporarily increase plant respiratory demands (Jagels 1983), leading to short-term reductions in plant production. Rapid (~2-3 h) increases in salinity were frequently observed in Little Madeira, and these changes coincided with dramatic decreases in O_2 concentration and percent saturation (data not shown). This basin is a fairly isolated embayment with freshwater input from Taylor River Slough to the north and limited exchange with an adjacent basin to the South. The relatively rapid increases in salinity suggest increased water exchange with the adjacent basin leading to the intrusion of higher salinity water into Little Madeira (e.g., Sanford and Boicourt 1990). This rapid (~2-3 h) increase in salinity may have induced physiological stress (Koch and Erskine 2001) on the seagrass plants in Little Madeira, resulting in the observed temporary depression in P_g.

While nutrient availability certainly regulates plant production in Florida Bay to some extent (e.g., Powell et al. 1989b, Fourqurean et al. 1992), analysis of water column nutrient concentrations did not reveal any significant correlations between nutrient availability and P_g (Table 3). In fact, P_g was negatively correlated, albeit weakly (Table 3), with total N and P concentrations across all of the sites. This pattern suggests that plant metabolism in these communities may regulate nutrient concentrations in the overlying water column rather than vice versa. The high leaf affinity for nitrogen at low water column concentrations (Lee and Dunton 1999) results in reduced nutrient efflux from these communities and leads to lower overall nutrient concentrations in the water column. This pattern is supported by previous observations in the *T. testudinum* community in Sunset Cove, as plant leaves assimilated ammonium from the water column within and directly above the canopy, reducing concentrations by as much as 50% over the diel cycle (Nagel 2007). Given the relatively oligotrophic waters of Florida Bay, sediment nutrients may be a better predictor for P_g in this system as *T. testudinum* likely obtains most of its nutrients needed to support growth via assimilation from the sediment

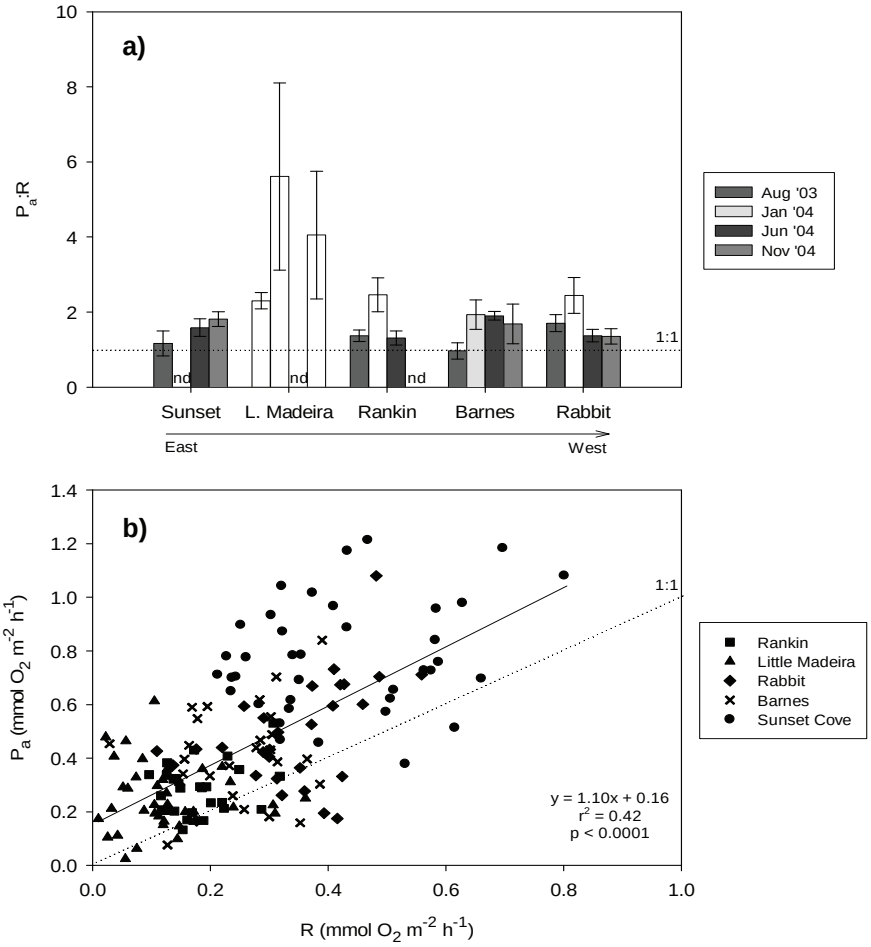

Figure 6. (a) Seasonal variations in mean (± SE) ratios of P_a:R in *T. testudinum* communities along an east-west transect in Florida Bay. (b) Linear regression of P_a:R for all *T. testudinum* communities in Florida Bay. The dotted lines in both (a) and (b) represent a P_a:R ratio of 1.

through belowground tissues (Lee and Dunton 1999, Nielsen et al. 2006).

Although seagrass communities are often considered highly productive, several studies have reported instances of both a balance between P_a and R (D'Avanzo et al. 1996, Ziegler and Benner 1998, Santos et al. 2004, Gacia et al. 2005) and net heterotrophy (Barron et al. 2004, Caffrey 2004) in seagrass ecosystems. This apparent incongruity arises from high plant respiratory demands combined with enhanced heterotrophic activity within these communities. There were five (of 30) instances in this study where NEP was significantly less than zero (Table 2). Four of these were associated with unusually high turbidity during wind-driven resuspension of bottom sediments in March or November (Kelble et al. 2005, Nagel pers. obs.). In August 2003, strong negative NEP observed for Barnes coincided with an outbreak of seagrass dieback at Barnes at that site (Nagel, pers. obs.). The dieback event was accompanied by numerous yellowing leaves and necrotic lesions as well as reduced plant abundance and leaf photosynthesis (Nagel 2007), symptoms shown to be associated with reduced photosynthetic capacity in *T. testudinum* (Durako and Kuss 1994). The negative NEP daily rates combined with P_a:R ratios < 1 suggest that heterotrophic processes dominated ecosystem metabolism during the dieback event. However, seagrass dieback had only a temporary impact on NEP in Barnes, since rates rebounded to positive values in subsequent months. This pattern of apparent seasonal recovery from seagrass dieback at Barnes was also observed in 2002-2003, when plant biomass increased significantly in winter following a dieback event observed during the summer (Nagel 2007). While the cause of the dieback in Barnes is not clear, prolonged dieback events and further loss of seagrasses could result in a shift from net autotrophy to net heterotrophy within this basin.

While instances of net heterotrophy did occur at a few sites in this study (Table 2), *T. testudinum* communities in Florida Bay were generally net autotrophic throughout most of the year, and this is consistent with reports for other seagrass communities (Hemminga and Duarte 2001, Barron et al. 2004, Gazeau et al. 2005). The magnitude of both NEP and values of P_a:R varied regionally. *T. testudinum* communities in Sunset Cove and Rabbit exhibited the highest rates of NEP, but P_a:R ratios

indicated that these communities were only slightly autotrophic or were in balance with respect to P_a and R (Fig. 6a). Although lowest overall rates of P_a and R were observed in Little Madeira, P_a:R ratios at this site were the highest observed in any region. With inputs of organic-rich nutrients from Taylor River Slough and high light attenuation relative to other basins (Kelble et al. 2005), it might be expected that heterotrophic processes dominate NEP in Little Madeira (Kemp et al. 1997). However, the clearly net autotrophic nature of this site suggests that organic matter inputs from the Everglades may relatively refractory with little influence in regulating NEP at this site. Projected changes to the hydrological regime in the Everglades will redirect and increase water flow through Taylor River Slough into Little Madeira (Fourqurean et al. 2003), which could increase organic matter inputs and the relative importance of heterotrophic processes to NEP in Little Madeira in the future.

The results of this study provide a novel contribution to rates and patterns of P_a, R, and NEP in Florida Bay. Despite regional variability in rates, annual estimates of NEP (Table 4) and P_a:R ratios (Fig. 6b) suggest that communities in Florida Bay dominated by *T. testudinum* were generally net autotrophic over the course of the year. These data on ecosystem level production and respiration in Florida Bay provide an initial basis for developing a bay-wide organic carbon budget. Projected changes in Everglades hydrology could alter the balance between production and respiration within this system, and this study provides a baseline against which future estimates of ecosystem production in Florida Bay could be compared.

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CARBON ($\delta^{13}C$) AND NITROGEN ($\delta^{15}N$) ISOTOPIC DISCRIMINATION IN MANGROVES IN FLORIDA COASTAL EVERGLADES AS A FUNCTION OF ENVIRONMENTAL STRESS

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ABSTRACT

Isotope signatures of mangrove leaves can vary depending on discrimination associated with plant response to environmental stressors defined by gradients of resources (such as water and nutrient limitation) and regulators (such as salinity and sulfide toxicity). We tested the variability of mangrove isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) across a stress gradient in south Florida, using green leaves from four mangrove species collected at six sites. Mangroves across the landscape studied are stressed by resource and regulator gradients represented by limited phosphorus concentrations combined with high sulfide concentrations, respectively. Foliar $\delta^{13}C$ ratios exhibited a range from -24.6 to –32.7‰, and multiple regression analysis showed that 46% of the variability in mangrove $\delta^{13}C$ composition could be explained by the differences in dissolved inorganic nitrogen, soluble reactive phosphorus, and sulfide porewater concentrations. ^{15}N discrimination in mangrove species ranged from –0.1 to 7.7‰, and porewater N, salinity, and leaf $N:P_a$ ratios accounted for 41% of this variability in mangrove leaves. The increase in soil P availability reduced ^{15}N discrimination due to higher N demand. Scrub mangroves ($\leq$1.5 m tall) are more water-use efficient, as indicated by higher $\delta^{13}C$; and have greater nutrient use efficiency ratios of P than do tall mangroves (5 to 10 m tall) existing in sites with greater soil P concentrations. The high variability of mangrove $\delta^{13}C$ and $\delta^{15}N$ across these resource and regulator gradients could be a confounding factor obscuring the linkages between mangrove wetlands and estuarine food webs. These results support the hypothesis that landscape factors may control mangrove structure and function, so that nutrient biogeochemistry and mangrove-based food webs in adjacent estuaries should account for watershed-specific organic inputs.

Keywords: stable isotopes, Florida coastal Everglades mangroves, plant stress, nutrient biogeochemistry, phosphorus limitation

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INTRODUCTION

Mangroves are among the most productive plant communities in the world and occupy a large proportion of the tropical coastal landscape (Lugo and Snedaker 1974, Twilley et al. 1996; Twilley 1997). Coastal ecosystems are important fishery regions, most likely because of available food and essential habitat that promote high levels of secondary productivity. A central paradigm of coastal ecology over the past four decades is that outwelling of organic matter from coastal wetlands represents a major source of energy that supports much of the secondary production of estuaries and nearshore waters (Odum 1968). However, different approaches to test the "outwelling hypothesis" in mangrove wetlands have found inconsistent results that question this popular concept (Lee 1995).

Early conclusions that mangrove detritus may be a significant source of food to consumers were based on three approaches: (1) habitat utilization and gut content analysis (Odum and Heald 1972, 1975, Yáñez-Arancibia et al.1993); (2) statistical correlations between nearshore fisheries and wetland areas (Macnae 1974, Turner 1977, Sasekumar et al. 1992, Primavera 1996); and (3) ecosystem mass balance (Twilley 1985). However, using natural isotope abundance to trace organic matter through food webs at different locations in the tropics, mangroves seem to play a secondary role as a source of organic matter that fuels adjacent aquatic ecosystems (Rodelli et al. 1984, Zieman et al. 1984, Stoner and Zimmerman 1988, Harrigan et al. 1989, Fleming et al.1990, Rezende et al. 1990, Ambler et al.1994, Hemminga et al. 1994, Rao et al. 1994, Newell et al. 1995, Primavera 1996, Loneragan et al. 1997, Marguillier et al. 1997, Wiedemeyer 1997, France 1998, Hayase et al. 1999, Lee 2000). Thus, the utilization of mangrove detritus as food for secondary consumers is still not clear. The major difficulty has been demonstrating that detritus exported from mangrove forests is actually utilized by food webs in the estuary.

Natural abundance of stable isotopes provides a powerful method to trace sources and transfers of organic matter through food webs (Peterson and Fry 1987). Natural isotopes can also trace the presence of environmental stress on plant metabolism (Yoneyama et al. 1991, Montoya and McCarthy 1995). It has been hypothesized that environmental stress derived from drought, limited nutrients, and hypersalinity can change the $\delta^{13}C$ and $\delta^{15}N$ signatures of mangrove leaf tissue (Farquhar et al. 1982, Naidoo 1985, Lin and Sternberg 1992a,b, Medina and Francisco 1997, Kao and Chang 1998, Fry et al. 2000, McKee et al. 2002). However, food web studies generally do not consider this variability to estimate trophic relationships in mangrove-dominated estuaries (Rodelli et al. 1984, Stoner and Zimmerman 1988, Rezende et al. 1990, Hemminga et al. 1994, Rao et al. 1994, Primavera 1996, Marguillier et al. 1997, Bouillon et al. 2000, Dehairs et al. 2000). Variations in plant isotope composition could be a confounding factor in obscuring linkages between mangrove detritus and estuarine food webs, particular in landscapes with strong environmental gradients.

A conceptual model of mangrove productivity uses resource, regulator and hydroperiod gradients to describe landscape patterns in community structure (Twilley and Rivera-Monroy 2005, 2009). Resource gradients include soil nutrients and available light that influences mangrove structure and function (Lugo and Snedaker 1974), while salinity (Ball 1988) and sulfides (McKee 1993) are regulators that can influence zonation of mangrove structure and community composition (Huston 1994). Nutrient stress reduces mangrove productivity affecting the photosynthetic capacity (Clough and Sim 1989, Feller 1995). Its effect cannot be separated from the supply of fresh water under natural conditions (Medina and Francisco 1997). Thus, structural development of mangrove communities, measured as tree height, can be inversely related to arid climates and availability of fresh water (Cintron et al. 1978, Castaneda-Moya et al. 2006) as well as to the lack of soil fertility (Feller 1995).

Mangroves in many locations of south Florida are limited by phosphorus concentrations (Koch 1997, Koch and Snedaker 1997, Davis et al. 2003). Landscape distribution of total phosphorus parallels forest structure and confirms the impact of this stressor along the Shark River estuary and the Taylor River estuary (Chen and Twilley 1999). These estuaries offer an excellent opportunity to test carbon and nitrogen isotopic discrimination as function of environmental stress associated with resource and regulator gradients. A better understanding of how mangrove isotope concentrations vary across landscapes in response to environ-

mental stress should improve our estimates of trophic linkages between mangrove wetlands and primary consumers of coupled estuarine ecosystems. Our hypothesis is that carbon and nitrogen isotope signatures are significantly different among mangroves forests of Florida Coastal Everglades adjacent to Florida Bay and express the intensity of environmental stress.

MATERIALS AND METHODS

Study Area

The study area is located in the mangrove-dominated sites of the Florida coastal Everglades (Long Term Ecological Research sites) of Everglades National Park (ENP, Fig. 1). Landscape transects have been established in two Everglades drainage basins, the Shark River Slough (SRS) and the Taylor Slough panhandle region (TS/Ph; http://fcelter.fiu.edu). The SRS transect is anchored at a canal inflow point along the Tamiami Trail (SRS1) and extends through the freshwater marsh (SRS2-3) to the mangrove estuary along Florida's southwest coast (SRS4-6). The TS/Ph transect is anchored at two main canal inflow points (TS/Ph1 and 4), and extends to Florida Bay (Fig.1). Three sampling sites were established in mangroves along the SRS transect (SRS4-6) and three sites along the TS/Ph transect (TS/PH6-8) (Table 1).

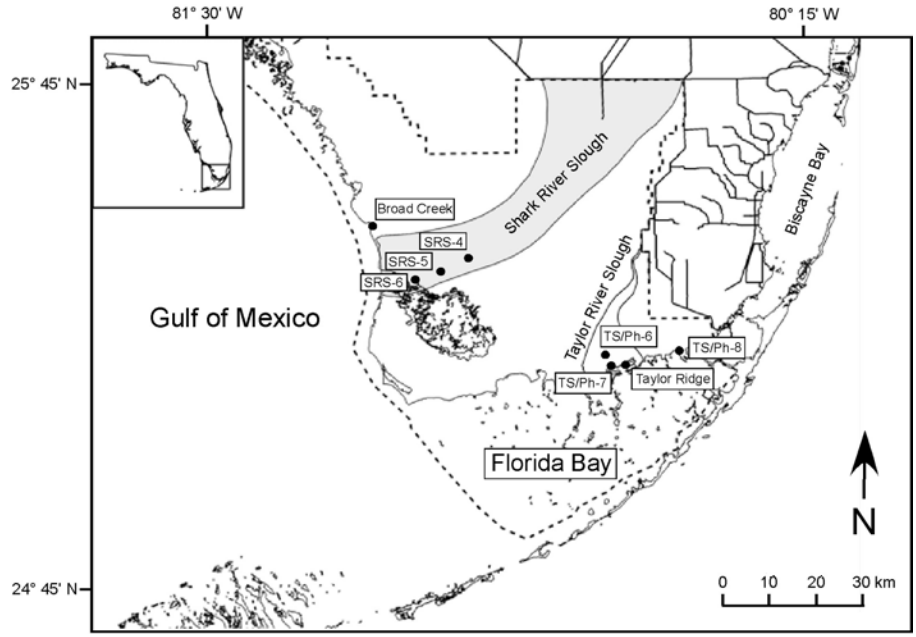

Figure 1. Everglades National Park showing locations of SRS4, SRS5, and SRS6 along the Shark River Estuary and TS/Ph6, TS/Ph7, and TS/Ph8 along Taylor Slough.

The entire Florida coastal Everglades landscape is oligotrophic (Chambers and Pederson 2006). The climate is subtropical, with distinct wet (June-November) and dry (December-May) seasons. Mangrove wetlands are distributed in a continuous band along the coast and extend inland from the Gulf of Mexico for 10-20 km to the upland limit of periodic seawater influence (Egler 1952; Ewe et al. 2006).

Analytical Techniques

Leaves of each mangrove species were sampled in May and October 2001; and January, May and October 2002. Mature, fully developed green leaves were removed manually from the canopy of the trees (1.5 to 2 m heights) at each site. Senescent leaves (yellow leaves free of structural damage) were collected from branches and from the forest floor. Fresh litter samples (brown leaves) and decayed litter (black leaves with loss of structural integrity) were collected from the soil surface. For each mangrove species, we collected at least 10 leaves of each stage (green, senescent, litter, decayed) and then obtained a mixed sample by species and decomposition stage.

Leaves were washed with ultrapure water to eliminate soil contamination and then dried at 60 ° C to constant mass. Dried samples were ground to a fine powder using a Wiley Mill. Samples for isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) and C and N concentrations were analyzed by continuous-flow gas isotope ratio mass spectrometry. An elemental analyzer (Carlo-Erba NC2500) was interfaced via a ConFlo II device to a gas isotope ratio mass spectrometer (Delta^{plus}, Finnigan MAT). $\delta^{13}C$ and $\delta^{15}N$ abundances were expressed relative to the conventional standards (PDB limestone for carbon and atmospheric air for nitrogen), using the following equation: $\delta X = ([R_{sample}/R_{standard}]-1)$

Table 1. General description of the attributes of each sampling site. TS/Ph=Taylor Slough Panhandle transect; SRS=Shark River Slough transect.

Habitat Attribute	TS/Ph6	TS/Ph7	TS/Ph8	SRS4	SRS5	SRS6
Location	80.651°W 25.216 °N	80.642°W 25.197 °N	80.524 °W 25.233 °N	80.964 °W 25.41 °N	80.032 °W 25.377 °N	81.078 °W 25.365 °N
Distance from the coast	3.5 km	1 km	0.1 km	20 km	8 km	4 km
Soil type	peat	peat	peat	silty peat	silty peat	silty peat
Mangrove canopy height	scrub ≤1.5m	scrub ≤1.5m	scrub ≤1.5m	≤5m	~8m	~10m
Mangrove composition	*Rhizophora mangle*. *Conocarpus erectus*. *Laguncularia racemosa*.	*Rhizophora mangle*. *Conocarpus erectus*. *Laguncularia racemosa*.	*Rhizophora mangle*. *Conocarpus erectus*. *Laguncularia racemosa*.	*Rhizophora mangle*. *Conocarpus erectus*. *Laguncularia racemosa*.	*Avicennia germinans*. *Rhizophora mangle*. *Laguncularia racemosa*.	*Avicennia germinans*. *Rhizophora mangle*. *Laguncularia racemosa*.

*1000[‰]; where X=^{13}C or ^{15}N, and R is the ratio of ^{13}C:^{12}C or ^{15}N:^{14}N. Replicate analyses of individual samples usually showed agreement better than 0.3‰.

Total phosphorus was analyzed by ashing subsamples of ground leaves and dissolving ash in 1N HCl. Extracts were analyzed in duplicate by colorimetric techniques using ascorbic acid reduction and assayed on a Thermo Spectronics Helios Delta analyzer at 885 nm. Concentrations of tissue nutrients are reported in milligrams of nutrient per gram of sample dry mass (mg/gdm). Relative indices of retranslocation for nitrogen and phosphorus were calculated using the following equation: RCR = ((G-S)/G)*100; where RCR = relative canopy retranslocation in %; G = concentration of nutrient in green leaves; and S = concentration of nutrient in senescent leaves.

Edaphic conditions at each site were analyzed by measuring total carbon, total nitrogen, and total phosphorus of soil. In addition, pore water was analyzed for dissolved inorganic nitrogen, soluble reactive phosphorus, salinity, and sulfide concentrations. Porewater samples to a depth of 30 cm were collected at eight stations within each-sampling site during the same dates described above for leaf collections. Salinity was measured *in situ* with an YSI-conductivity meter and values are expressed in mass units (g/kg). Samples for nutrient analyses were frozen for return to the laboratory. N and P were determined by standard methods using an Autoanalyzer ASX-500 series Lachat instruments at the Analytical Service Laboratory, Center of Ecology and Environmental Technology, University of Louisiana at Lafayette, USA. Sulfide concentrations were determined using an electrode Lazar Model IS-146.

At each sampling site, four soil samples from 0 to 10 cm depth were collected with a 15 cm diameter core. Each sample was divided into 2 cm sections. C, N and P analyses of each section were performed as described above for plant material. Isotopic analyses (δ^{13}C and δ^{15}N) were assayed only on the 0 to 2 cm section. Soil-subsamples used for δ^{13}C analysis were treated with 0.1N HCl to remove carbonates and then redried prior to analysis.

Plant data were analyzed using one-way

ANOVA to test for the effect of site on each parameter measured. The design was incomplete and unbalanced because not all species occurred at each site. A significant factor effect was followed by a Tukey's multiple-comparison test. Pore water and soil data were analyzed using two-way ANOVA to test for the effect of site and sampling date. Data that did not meet normality or variance homogeneity assumptions were transformed, but the untransformed means (±1 SE) are presented. Relationships among plant, soil, and porewater variables were examined by correlation and multiple regression analyses.

RESULTS

Soil Nutrient Resources

Nutrient concentrations revealed a soil fertility gradient determined mainly by variations in total phosphorus concentrations (soil-P). Differences in soil-P concentrations among sites were significant, with a clear increase seaward along both Shark River and Taylor Slough transects (Fig. 2a). Average soil-P concentrations (mg/cm^3) at Taylor Slough sites were 0.04 ±0.03, 0.07 ±0.06, and 0.15 ±0.16 for TS/Ph6 to 8, respectively. The seaward gradient along the Shark River transect was similar at 0.07 ±0.01, 0.11 ±0.01, and 0.18 ±0.1 for SRS4 to 6, respectively. This latter gradient parallels the tree-height gradient (short, intermediate, and tall) described for Shark River mangrove sites (Chen and Twilley 1999). Soil total nitrogen concentrations (soil-N) were not significantly different among the six sites (Fig. 2b). Average soil-N concentrations ranged from 1.53 ±0.33 mg/cm^3 in TS/Ph7 to 2.98 ±0.39 mg/cm^3 in SRS4. Carbon content was lower in Taylor Slough than at Shark River, especially at sites TS/Ph6 and TS/Ph7 where averages were 33.8 ±3.5 and 35.1 ±2.9 mg/cm^3, respectively.

Soil N:C$_a$ (*1000) ratios did not vary much among sites, except at SRS5 which had the lowest average of 13 ±21 (Fig. 2c). N:P$_a$ ratios were significantly different among sites with a trend to de-

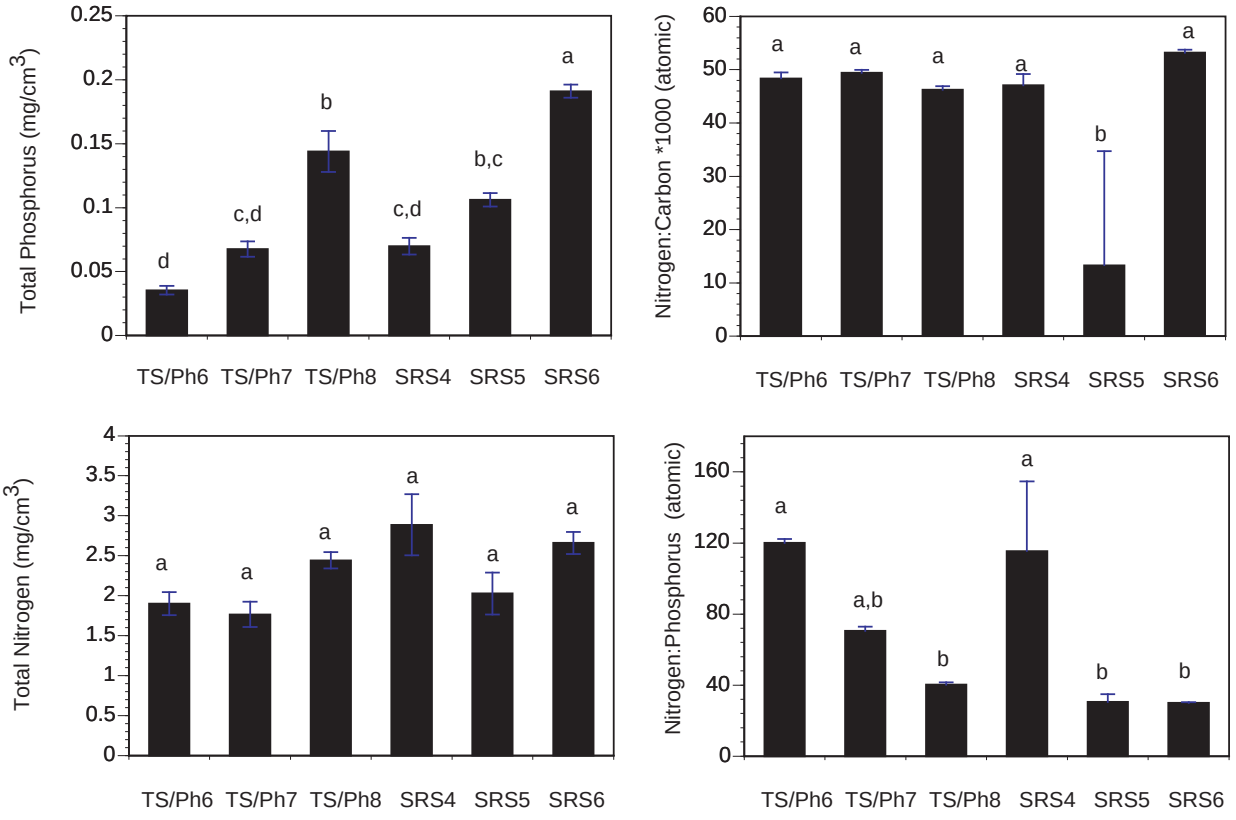

Figure 2. Soil nutrient density including total phosphorus, total nitrogen, and the ratios of N:C and N:P. Bars not connected by same letter are significantly different. Values are the mean ± 1 SE (n = 20).

crease from upland to seaward locations. In Taylor Slough mean N:P_a ratios for stations TS/Ph6 to 8 were 120 ±12, 72 ±13, and 43 ±14, respectively (Fig. 2d). TS/Ph6 was significantly different from TS/Ph7 (contrast: $F_{1, 45}$ =4.2 *P*=0.05) and TS/Ph8 (contrast: $F_{1, 45}$=10.4 *P*=0.002). Along the Shark River transect, N:P_a ratios were 114 ±16, 30±14, and 30 ±16 at SRS4, SRS5, and SRS6, respectively. SRS4 was significantly different from SRS5 (contrast: $F_{1, 42}$ =15.8 *P*=0.0003) and SRS6 (contrast: $F_{1, 42}$ = 13.9, *P*=0.0006).

Porewater concentrations of nitrate plus nitrite (NO_x) were frequently below detection limits, so values for NO_x and NH_4 were summed to determine dissolved inorganic nitrogen (DIN) concentrations (Fig. 3a). These values showed both temporal and spatial gradients along transects of both basins. In general, DIN concentrations were higher in TS/Ph sites with averages ranging from 4.3 ±0.4 to 46.2 ±5.3 μM. At Shark River sites, DIN concentrations fluctuated between 0.6 ±0.4 and 7.4 ±0.6 μM. Soluble reactive phosphorus (SRP) concentrations in porewater were significantly different among sites especially along the Shark River transect (Fig. 3b). During the wet season (October 2001) there was a significant increase in SRP concentrations from the upland sites towards the mouth of the estuary (Fig. 3b). The maximum average concentration measured at SRS6 (2.24 ±0.18 μM) was 10 times greater than the minimum (0.27 ±0.03 μM) recorded during the same season at SRS4.

Soil Regulators

The seasonal pattern of salinity (±SE) was similar along both transects; however, there were significant spatial differences between the two basins (Fig. 4a). The upper region of the Shark River estuary at SRS4 ranged from oligohaline conditions during the wet season (1.0 ±0.1) to mesohaline (8.7 ±0.1) during dry months (October 2001, 2002). The mid and lower regions of the Shark River estuary (SRS5 and SRS6) ranged from mesohaline (7.5 ±0.2 and 15.7 ±0.5, respectively) to polyhaline conditions (32.0 ±0.5 and 34.8 ±0.6, respectively). This seasonal pattern was similar at Taylor Slough sites that varied from mesohaline (6.8 ±1.8, 9.7 ±2.3, and 10 ±2.4) to polyhaline conditions (29.3 ±1.6, 29.3 ±2.1, and 19.5 ±0.9) for TS/Ph6 to 8, respectively.

Table 2. Summary of ANOVA results for carbon and nitrogen stable isotopes of mangroves green leaves collected along Taylor Slough (TS/Ph) and Shark River (SRS).

	Means by site								
	TS/Ph6	TS/Ph7	TS/Ph8	SRS4	SRS5	SRS6	n	*F*	*P*
$\delta^{13}C$									
R. mangle	-25.9	-26.4	-25.8	-29.2	-30.3	-31.2	65	84.80	<0.0001
L. racemosa		-29.2	-29.9	-30.4	-30.7	-29.8	45	2.20	0.0086
A. germinans		-27.4			-30.3	-30.3	22	6.20	0.0085
C. erectus	-28.5	-27.9	-27.3	-29.2			37	6.26	0.0018
$\delta^{15}N$									
R. mangle	-1.0	3.0	3.4	3.8	1.9	3.2	65	19.82	<0.0001
L. racemosa		0.8	-0.4	4.6	2.8	5.1	45	39.93	<0.0001
A. germinans		5.0			3.7	4.6	22	2.78	0.0871
C. erectus	1.5	2.7	-0.4	-0.1			37	8.89	0.0002

Table 3. Pearson correlation coefficients for variables measured in plant tissue ($\delta^{13}C$, $\delta^{15}N$, N-plant, P-plant), soils (N-soil, P-soil), and porewaters (DIN, SRP, sulfides, salinity). Total nitrogen (N), total phosphorus (P), dissolved inorganic nitrogen (DIN), soluble reactive phosphorus (SRP). Number of observations in parenthesis. * $P \leq 0.05$, ** $P \leq 0.01$, ns = no significant.

	green mangrove leaves				soil		porewater		
	$\delta^{13}C$	$\delta^{15}N$	N-plant	P-plant	N-soil	P-soil	DIN	SRP	sulfide
$\delta^{15}N$	-0.21**								
	(167)								
N-plant	-0.24**	0.32**							
	(171)	(167)							
P-plant	-0.40**	0.53**	0.69**						
	(166)	(161)	(164)						
N-soil	-0.15 ns	0.11 ns	0.10 ns	0.12 ns					
	(100)	(98)	(100)	(98)					
P-soil	-0.30**	0.21 ns	0.31**	0.30**	0.26*				
	(76)	(74)	(76)	(74)	(75)				
DIN	0.41**	-0.17*	-0.14 ns	-0.21**	-0.26**	-0.45*			
	(171)	(165)	(169)	(164)	(98)	(74)			
SRP	-0.22**	0.22**	0.10 ns	0.40**	-0.07 ns	-0.05 ns	0.01 ns		
	(171)	(165)	(169)	(164)	(98)	(74)	(171)		
sulfide	0.49**	-0.28**	-0.40**	-0.52**	-0.13 ns	-0.19 ns	0.36*	-0.10 ns	
	(163)	(157)	(161)	(159)	(100)	(76)	(161)	(161)	
salinity	0.09 ns	0.05 ns	0.03 ns	0.19**	-0.17 ns	-0.03 ns	0.25**	0.22**	0.02 ns
	(173)	(167)	(171)	(166)	(100)	(76)	(171)	(171)	(163)

Sulfide concentrations were significantly greater at the mangrove sites in Taylor Slough compared to mangrove soils in Shark River (Fig. 4b). On average, TS/Ph8 had the highest concentrations (mM ±SE) ranging from 0.64 ±0.04 to 3.25 ±0.71; followed by TS/Ph6 (0.29 ±0.04 to 1.5 ±0.06), and TS/Ph7 (0.19 ±0.01 to 1.2 ±0.1). Along the Shark River transect, sulfides at SRS5 varied from 0.08 ±0.02 to 0.28 ±0.03, SRS4 from 0.02 ±0.01 to 0.07 ±0.01, and SRS6 from 0 to 0.02 ±0.01.

Plant Tissue Nutrients

There were significant variations in total phosphorus concentrations of plant tissue (plant-P) among species, sampling sites, and decomposition stage. *A. germinans* had the highest plant-P concentration, which for green leaves was ≥ 50% higher than the concentration found in *R. mangle* and *L. racemosa*, and >60% higher than *C. erectus*. Senescent leaves had lower plant-P concentrations than green leaves. There was a small increase in plant-P concentration from senescent leaves to litter (<20%), and plant-P concentrations decreased again in decayed leaves. Among sites, the differences were significant. Mangrove leaves from Shark River sites had higher plant-P concentrations than leaves from Taylor Slough sites. Average plant-P concentrations (mg/gdm) were 0.95, 0.67, 0.48, along the Shark River transect (SRS6, SRS5, SRS4, respectively), and 0.37, 0.27, 0.24 at Taylor Slough (TS/Ph7, TS/Ph8, TS/Ph6, respectively).

Average total nitrogen concentrations of plant tissues (plant-N) were significantly different among species, decomposition stages, and sites. Across all sampling, green leaves of *A. germinans* had higher plant-N concentrations (19 mg/g) than *R. mangle* (10.8 mg/g). These concentrations were higher than those of *C. erectus* (8.7 mg/g), whereas *L. racemosa* had the lowest plant-N concentrations (7.9 mg/g). The four species showed a similar pattern of plant-N concentrations among compartments, with highest concentrations in green leaves, lowest in senescent leaves and an increasing concentration during the decomposition process. This pattern was consistent at all sampling sites. Plant-N concentrations in green leaves were similar among sampling sites; however, Shark River sites had higher averages (1.1, 1.2, 1.5 mg/g, for SRS-4 to 6, respectively) than Taylor Slough sites (0.9, 0.85, 0.85 mg/g for TS/Ph6 to 8, respectively).

Table 4. Summary of multiple regression analysis between isotopic ratios (C, N) of green mangrove leaves and dissolved inorganic nitrogen (DIN), soluble reactive phosphorus (DIP), sulfides, salinity, total soil phosphorus (P-soil), total soil nitrogen (N-soil), and N:P atomic ratios.

Source		DF	MS	*F*	*P*	Parameter	*t*	*P*	r^2 partial	r^2 model
variable $\delta^{13}C$:	model	9	9.94	5.66	<0.0001					0.46
	error	55	1.75							
Independent variables										
DIN		1				0.05	3.77	0.0004	0.20	0.20
SRP		1				-0.66	-4.37	<0.0001	0.19	0.39
Sulfides		1				0.82	2.74	0.0079	0.07	0.46
Salinity		1				-0.01	-0.58	NS		
P-soil		1				-0.07	-0.21	NS		
N-soil		1				-0.02	-0.10	NS		
Leaf N:P		1				0.01	1.16	NS		
Soil N:P		1				-0.01	-0.26	NS		
Porewater N:P		1				-0.002	-1.66	NS		
Variable $\delta^{15}N$:	model	9	19.06	4.49	0.0002					0.41
	error	55	4.25							
Independent variables										
DIN		1				-0.099	-4.08	0.0001	0.10	0.16
Salinity		1				0.112	3.41	0.0012	0.16	0.26
Leaf N:P		1				-0.026	-2.63	0.0110	0.15	0.41
SRP		1				0.423	0.07	NS		
Sulfides		1				-0.024	-0.05	NS		
P-soil		1				0.286	0.33	NS		
N-soil		1				-0.273	-0.72	NS		
Soil N:P		1				0.063	0.80	NS		
Porewater N:P		1				0.001	0.47	NS		

Stable Isotopic Values

$\delta^{13}C$ values were significantly different in mangrove leaves among sites and species (Table 2). *A. germinans* and *L. racemosa* had lower $\delta^{13}C$ values

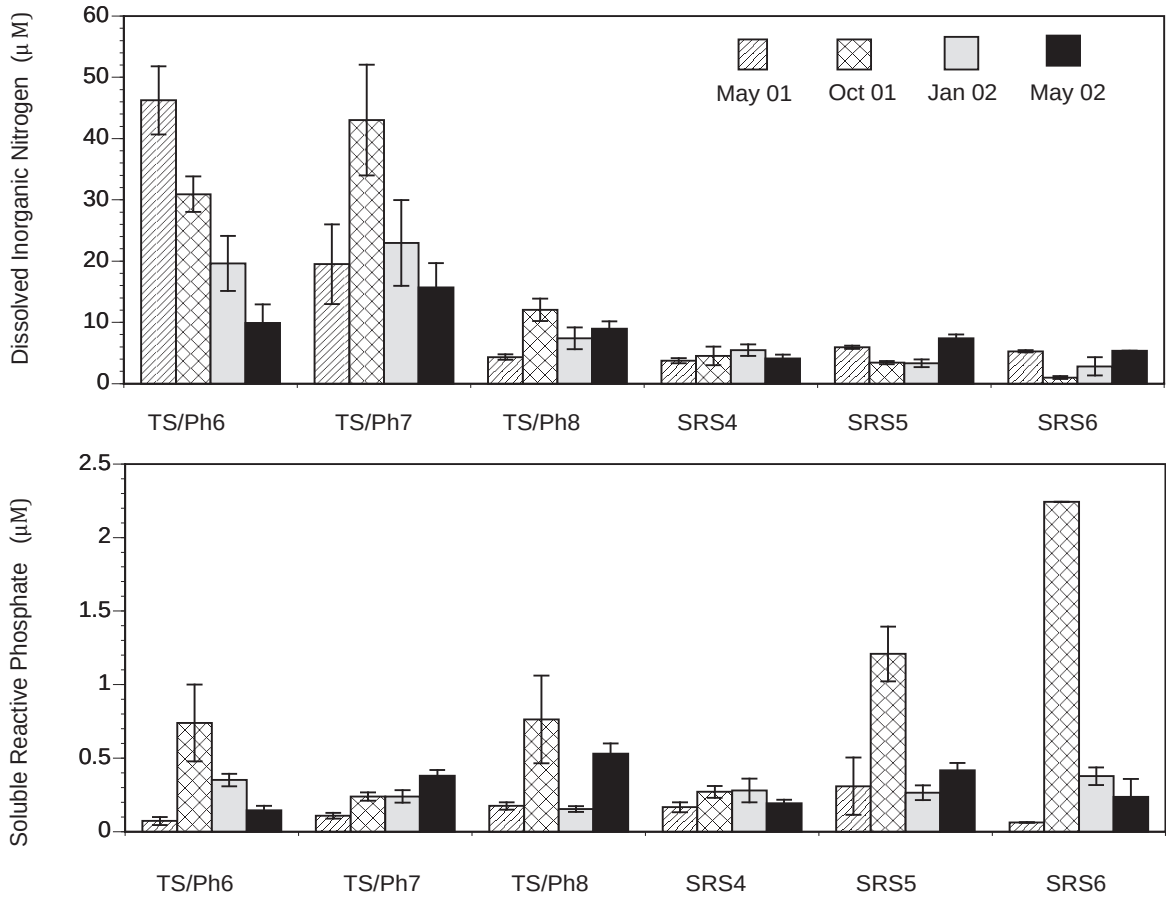

Figure 3. (a) Dissolved inorganic nitrogen (DIN = NO_3^- + NH_4^+) and (b) soluble reactive phosphorus (SRP = PO_4^{-3}) measured in porewaters. Values are the mean ± 1 SE (n = 358 and 310 for DIN and SRP, respectively).

than *C. erectus* and *R. mangle*. *R. mangle* had the highest average $\delta^{13}C$ abundance among the species. Although $\delta^{13}C$ values did not differ among Shark River sites, they were significantly lower than the values for Taylor Slough sites (Fig. 5). Across the Taylor Slough transect values decreased seaward.

Green leaves of *A. germinans* had $\delta^{15}N$ values that were significantly higher than those measured in *R.. mangle*, *L. racemosa* (about 2‰) and *C. erectus* (about 3.7‰). The exception was SRS6, where $\delta^{15}N$ values were similar to those measured in *L. racemosa.* Each of the four species had significantly different $\delta^{15}N$ values among sites, except for *A. germinans* (Table 2). The most striking difference among sites was observed for *L racemosa* $\delta^{15}N$-values, which were significantly higher in Shark River than in Taylor Slough. Site differences between Shark River and Taylor Slough basins for *C. erectus* and *R. mangle* did not show a clear pattern (Fig. 5). Correlation analyses between isotopic composition of green leaves and edaphic factors revealed significant relationships (Table 3). $\delta^{13}C$ values had an inverse correlation with P-soil and SRP; but a positive and stronger correlation with DIN and sulfide concentrations. Yet, these relationships showed an opposite pattern for $\delta^{15}N$ values (Table 3).

Multiple regression analysis among mangrove isotopic composition and edaphic factors showed that 46% of the variability in mangrove $\delta^{13}C$ composition could be explained by the variability of N-soil, P-soil, and sulfide concentrations. Yet, salinity, N-soil, P-soil, and N:P_a ratios leaf, soil, and porewater were not significant in this linear regression model. On the other hand, DIN, salinity, and leaf N:P_a atomic ratios accounted for 41% of the variability in $\delta^{15}N$ composition of mangrove leaves (Table 4).

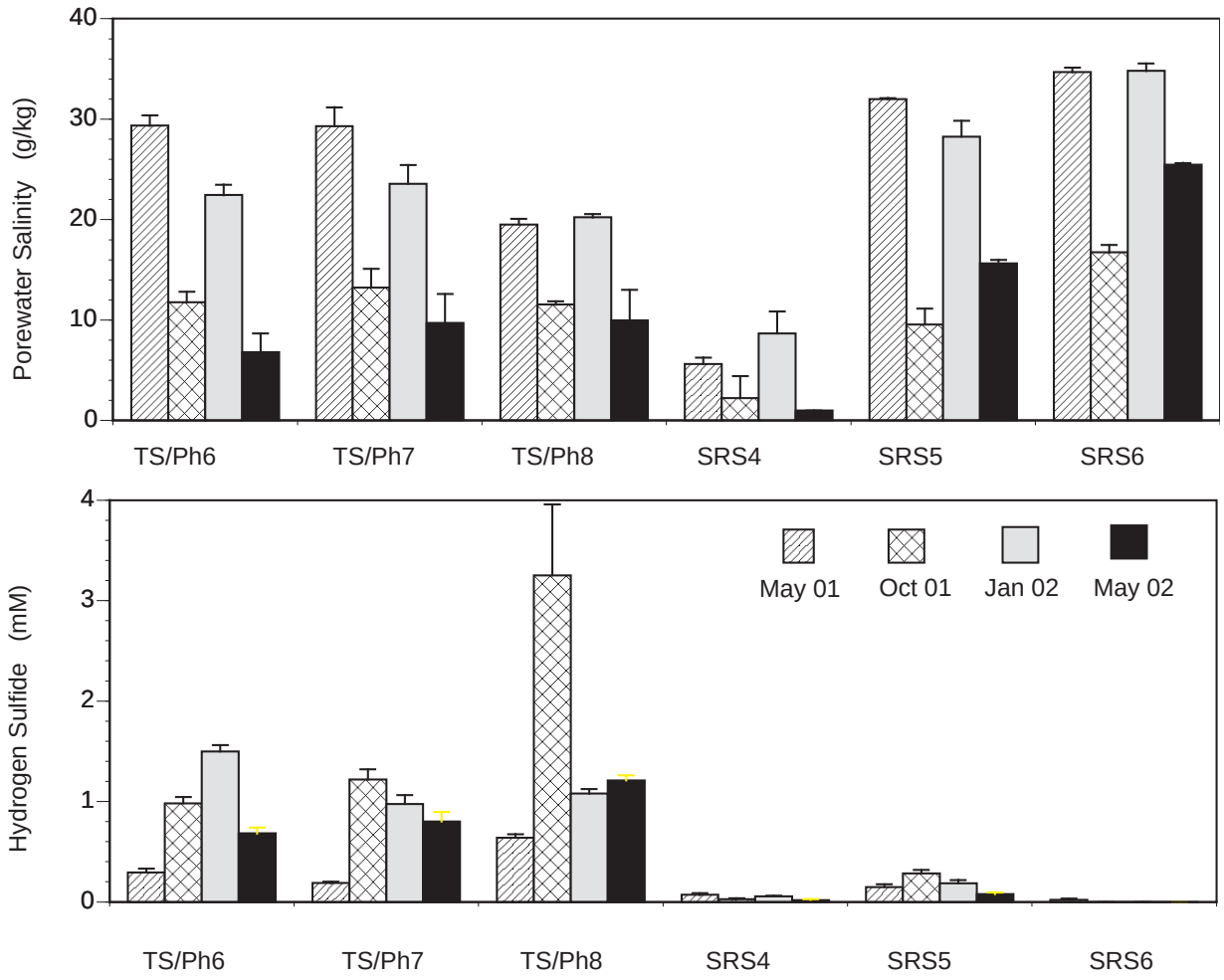

Figure 4. Salinity (a) and sulfide concentrations (b) in porewaters at different sampling sites and seasons. Values are the mean ± 1 SE.

Nutrient Use Efficiency

All mangrove species exhibited higher P than N relative canopy retranslocation (Fig. 6). Among the species measured, *R. mangle* had the highest mean P retranslocation (78%), followed by *L. racemosa* (69%), *A. germinans* (68%), and *C. erectus* (59%). P retranslocation in *R. mangle* was higher in Taylor Slough (77%) than in Shark River (67%), whereas the pattern for *L. racemosa* was opposite this pattern (75% in Shark River and 61% in Taylor Slough). Nitrogen retranslocation was higher in *A. germinans* (64%), followed by *R. mangle* (60%), *L. racemosa* (58%), and *C. erectus* (46%). The trends for each species showed that N retranslocation for *L. racemosa* was higher in Shark River (65%) than in Taylor Slough (46%); yet for *R. mangle* there was very little difference in retranslocation (60%) between the two basins (Fig. 6).

DISCUSSION

The occurrence of scrub mangroves has been attributed to different factors including extreme conditions of salinity (Pool et al. 1977, Cintron et al. 1978), poor aeration of shallow sediments (Davis 1940, Lugo et al. 1981), compacted peat preventing the deep penetration of roots (Craighead 1971), nutrient limitation (Lugo and Snedaker 1974, Feller 1995), lower ground water level (Lin and Sternberg 1992a), and high sulfide concentrations (McKee 1993, 1995). The single or cumulative effect of these factors may increase with distance from the shoreline resulting in poorer conditions for plant growth and eventual scrub tree formations (Fry et al. 2000).

Salinity used as a single factor cannot explain the variation in mangrove structure in the Shark

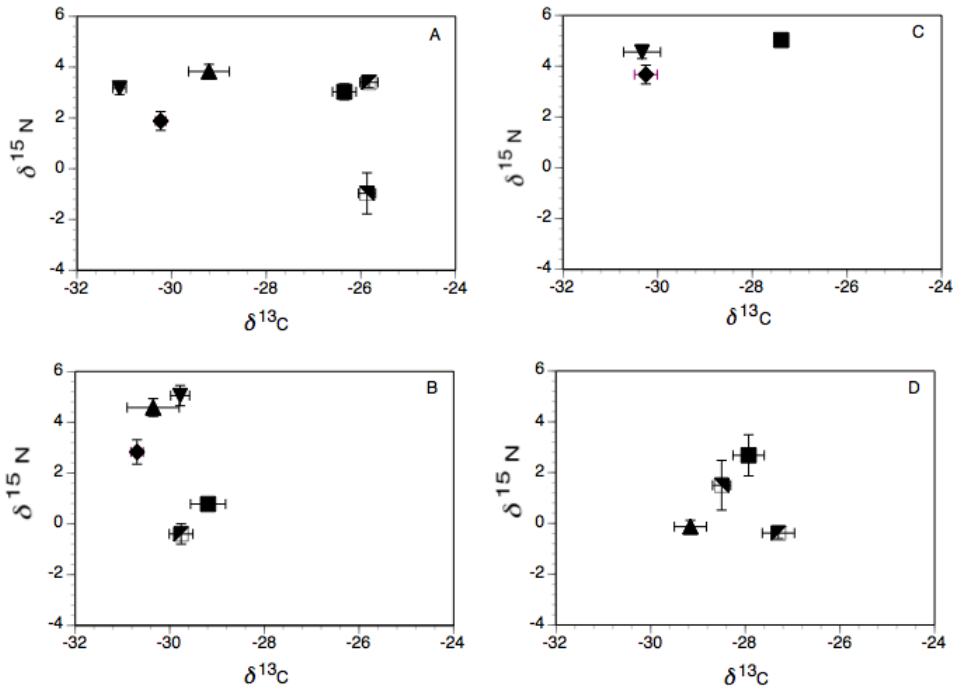

Figure 5. $\delta^{15}N$ and $\delta^{13}C$ (‰) measured in green leaves of (a) *Rhizophora mangle*, (b) *Laguncularia. racemosa*, (c) *Avicennia germinans*, and (d) *Conocarpus erectus*. Values are the mean ± 1 SE. See Figure 11 for symbols.

River and Taylor Slough basins of south Florida. Although mangroves along the Shark River exhibited an inverse relationship between salinity and mangrove height, this pattern was not consistent with sites along Taylor Slough. Along Taylor Slough, neither tree height nor salinity changes with distance from the shoreline. In this transect, temporal changes of salinity were more abrupt, ranging from less than 10 to more than 30. On average the two transects were not significantly different (contrast: $F_{1, 320}$=0.20; *P*=0.65).

The structure of mangrove wetlands along the Shark River and Taylor Slough basins parallels P-soil concentrations and SRP gradients. $N:P_a$ ratios indicated not only that mangroves at Shark River and Taylor Slough are P-limited as has been shown in other studies (Koch 1997, Koch and Snedaker 1997, Davis et al. 2003), but also that this limitation is more significant upstream, as also has been suggested in previous findings for Shark River basin (Chen and Twilley 1999). Although P deficiency has been identified as the main nutritional factor limiting growth and development in other mangrove wetlands (Feller 1995, Koch and Snedaker 1997), N also can limit mangrove growth across tidal gradients (Boto and Wellington 1983, Feller et al. 2003). A strong correlation (r^2=0.99, *P*<0.01) has been observed between $N:P_a$ ratio and tree height along the Shark River estuary (Chen and Twilley 1999). Likewise, lower soil-P concentrations on the Shark River transect are similar to TS/Ph6-8, and trees have a mean height scrub forest indicating resource gradient limitation.

Carbon Isotopes

Stable isotopes can be used to understand ecological principles such as effects of environmental factors on plant development (Farquhar et al. 1982, Ball and Farquhar 1984, Clough and Sim 1989, Lin and Sternberg 1992a,b, Medina and Francisco 1997, Fry et al. 2000, McKee et al. 2002). There were significantly negative correlations between foliar $\delta^{13}C$ abundance and P concentrations (including plant-P, soil –P, and SRP) for all mangrove species along both transects (Table 3). Less negative $\delta^{13}C$ values indicate lower ^{13}C discrimination during photosynthesis due to lower intercellular CO_2 concentrations and higher water-use efficiency (Farquhar et al. 1982, Farquhar et al.1989, Guy et al. 1989, DeLucia and Schlesinger 1995). Since under natural conditions the availability of nutrients cannot be separated from the supply of water (Medina and Francisco 1997), the difference in carbon isotope discrimination among mangroves associated with low soil-P concentrations suggests a connection between P concentration and water-use efficiency (Table 3). Phosphorus deficiency may contribute to drought in plants that are inundated with water by reducing root hydraulic conductivity (Radin and Boyer 1982). This variation in carbon isotope composition could therefore indicate the effect of limit-

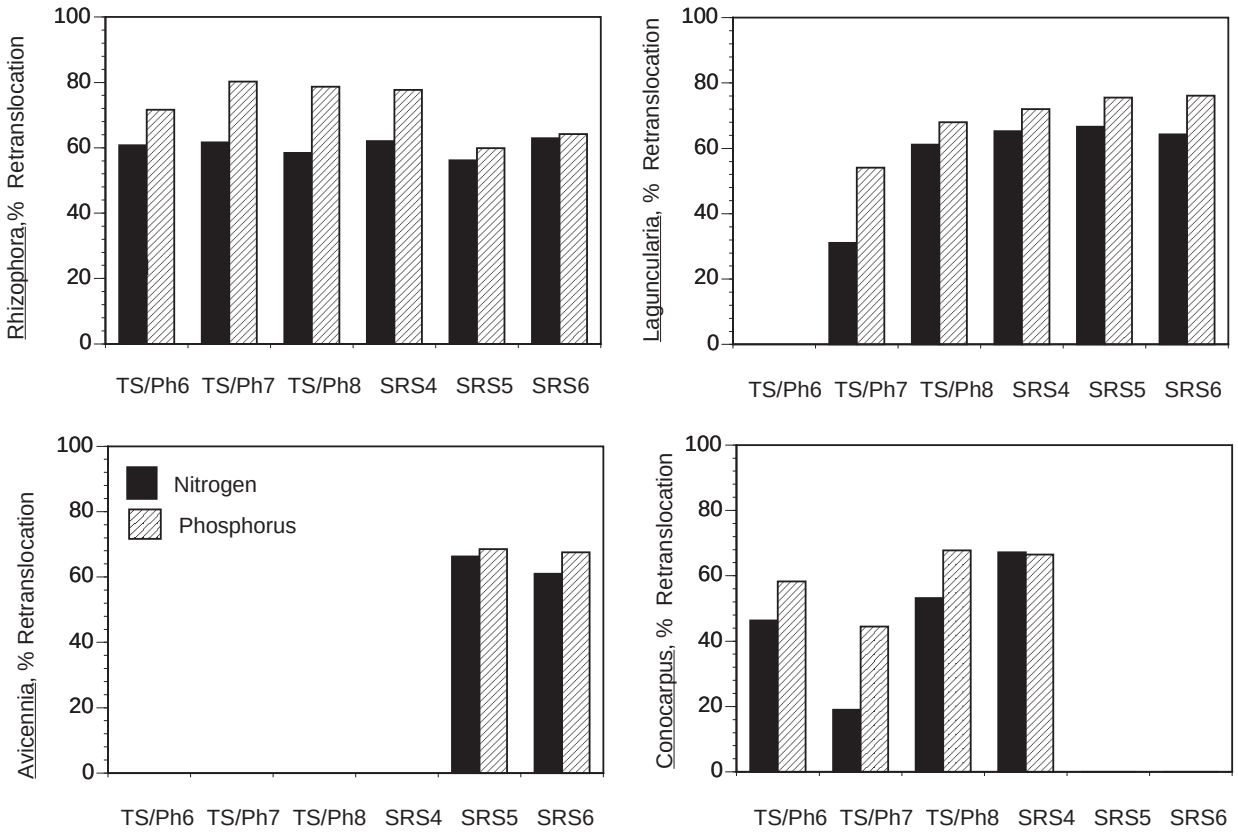

Figure 6. Nitrogen (solid) and phosphorus (stipled) retranslocation from senescent leaves estimated for *Rhizophora mangle*, *Laguncularia*. *racemosa*, *Avicennia germinans*, and *Conocarpus erectus* at all sampling sites.

ing nutrients as an environmental stress in these two river basins of the FCE.

Patterns linking leaf stomatal conductance, carbon isotope discrimination and water-use efficiency under nitrogen deficiency have been presented for maritime pine and oaks (Guehl et al. 1995). However, previous studies carried out on mangroves have found no relationship between nutrients and leaf $\delta^{13}C$ (Lin and Sternberg 1992b, McKee et al. 2002). Our study would find a similar result if $\delta^{13}C$ composition and soil fertility regressions were applied separately to Taylor Slough and Shark River transects. However, when all sites were included in a multiple regression model, porewater nutrients and sulfides were highly significant explaining together 46% of the variability of mangrove $\delta^{13}C$ ratios (Table 4).

Studies of salinity, sulfides and nutrients on *R. mangle* have concluded that lower carbon isotope discrimination or higher transpiration efficiency observed for scrub mangroves in the field is caused only by higher salinity (Lin and Sternberg 1992b,c). However, this conclusion was based on greenhouse experiments where only seedlings were tested. Since these plants were approximately 1 year old, there was a high probability that they were still using maternal resources. This could explain the lack of correlation between leaf $\delta^{13}C$ values and nutrient concentration. Likewise, it has been reported that high sulfide concentrations can decrease root energy status affecting nutrient uptake (Koch et al. 1990). This interference of nutrient acquisition could explain the lack of correlation between leaf $d^{13}C$ composition and sulfide concentration in the greenhouse experiment.

In our study, *R. mangle* collected at TS/Ph8 had $\delta^{13}C$ values 5‰ higher than those measured at SRS6, indicating strong differences in water-use efficiency at similar soil-P concentrations. These contradicting results may explain the lack of significance between soil-P concentrations and $\delta^{13}C$ composition in the multiple regression model (Table 4). Besides phosphorus other variables may play an important role as environmental stressors

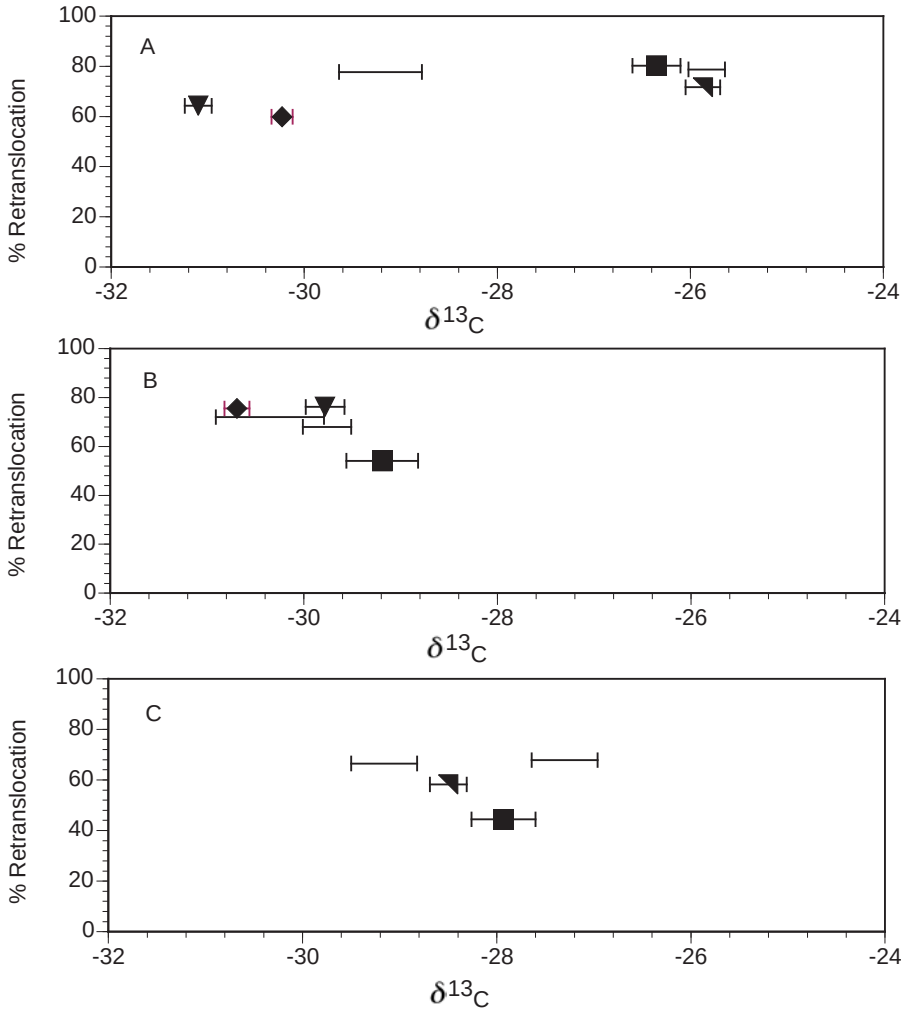

Figure 7. Phosphorus retranslocation (%) from senescent leaves versus leaf $\delta^{13}C$ (‰) in (a) *Rhizophora mangle*, (b) *Laguncularia racemosa*, and (c) *Conocarpus erectus*. Values are means ± 1 SE. See Fugure 11 for symbols.

across this mangrove landscape.

Salinity stress has been frequently associated with reduced mangrove forest structure (Pool et al. 1977, Cintron et al. 1978). Explaining the occurrence of scrub mangroves in south Florida, Lin and Sternberg (1992a, b) found a strong negative correlation (r=0.90, $P<0.01$) between tree height and foliar carbon isotope composition. Since these results were consistent with photosynthetic gas exchange measurements on *R. mangle*, Lin and Sternberg (1992a, b) related tree height and mangrove water-use efficiency. Also, they postulated salinity as the likely edaphic factor accounting for the differences between scrub and fringe forests. However, they did mention nutrient concentrations as a factor that should also be investigated.

Comparing riverine and fringe mangroves using osmolality and leaf $\delta^{13}C$ composition, Medina and Francisco (1997) identified salinity as the factor controlling water-use efficiency, photosynthetic activity, and structural development of fringe mangroves in eastern Venezuela. These results agree with those of Clough and Sim (1989), who found that intrinsic water-use efficiency decreases with interstitial soil salinity for several species of *Rhizophora*. Maximum photosynthetic rates and leaf stomatal conductance seem to be correlated with carbon isotopic values (Andrews et al. 1984). Adaptations of water-use efficiency may explain, at least for *R. mangle*, its dominance in the oligohaline regions of Shark River and Taylor Slough basins, as well as much of the Everglades National Park (about 6000 ha). It is the largest single area of scrub mangrove forest in Florida (Lin and Sternberg 1992a).

In our study, salinity did not show a significant correlation with carbon isotope values (Table 3). This result is expected given the similarity in salinities among the sites on both transects, in contrast to the very different forest structure. Temporal changes in salinity are evident between the wet and dry seasons, and according to some evidence from greenhouse experiments, salinity fluctuations from 18 to 53 can decrease growth of *R. mangle* seedlings (Lin and Sternberg 1993). However, the temporal variation in salinity is similar in magnitude along both Shark River and Taylor Slough transects.

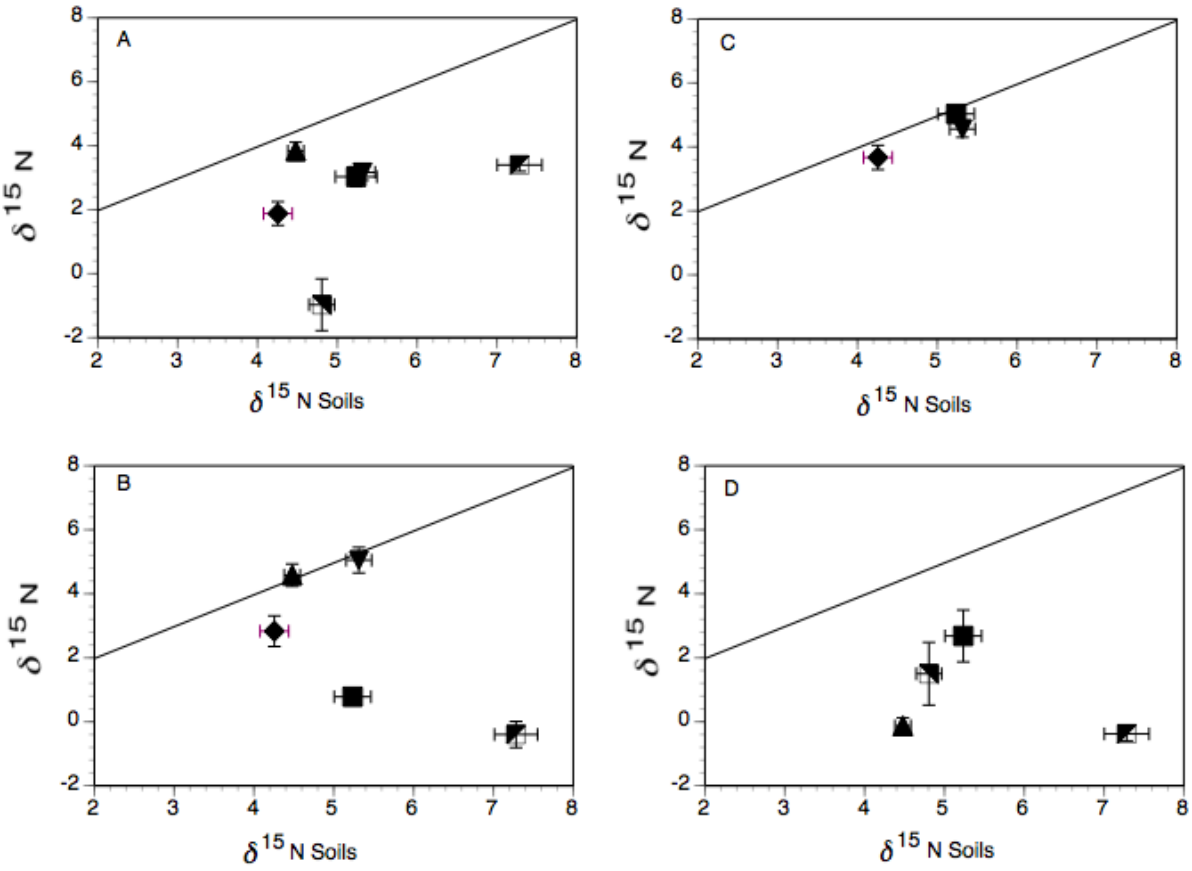

Figure 8. Green leaf d^{15}N composition versus soil δ^{15}N composition (‰) with a line representing the 1:1 relationship. Values below the line indicate species at sites that are ^{15}N-depleted versus soils including (a) *Rhizophora mangle*, (b) *Laguncularia racemosa*, (c) *Avicennia germinans*, and (d) *Conocarpus erectus*. Values are means ± 1 SE. See Figure 11 for symbols.

Mangrove stomatal conductance can be reduced by prolonged waterlogging of saline soils compared to drained non-saline environments under greenhouse conditions (Naidoo 1985, Cardona-Olarte et al. 2006, Krauss et al. 2006,). The fact that leaf δ^{13}C values were less negative at Taylor Slough, where hydroperiod is longer than at SRS5 and SRS6, suggests that hydroperiod is probably an important factor determining carbon isotope fractionation between these two mangrove landscapes.

Sulfide concentrations show a highly significant and positive correlation with δ^{13}C values for all mangrove species combined ($r=0.5$, $P\leq0.01$), indicating that mangroves are more water-use efficient when exposed to this soil phytotoxin. Hydrogen sulfide has been described as a causative factor affecting mangrove plant growth (Nickerson and Thibodeau 1985, McKee 1993). Although the precise mechanism of this stress is not thoroughly understood, evidence from freshwater and salt-marsh species reveals that sulfide concentrations >1.0 mM affect aerobic respiratory enzymes as well as alternate anaerobic pathways. These metabolic inhibitors decrease the root energy status affecting energy-dependent processes such as nutrient uptake in salt marshes (Koch et al. 1990). Concentrations of porewater sulfide $\geq$1.0 mM were found in all Taylor Slough sites (Fig. 4b), where mangrove δ^{13}C signatures were higher than at Shark River sites. Similar direct relationships between green leaf sulfur concentrations and δ^{13}C abundance in mangrove leaves have been observed before in Shark River (Fry and Smith 2002).

An alternative hypothesis to explain the differences in foliar δ^{13}C distribution in south Florida mangrove ecosystems, especially in *R. mangle*, is the increase of carboxylation efficiency as a result of an increase in plant-N concentration of green leaves (Cordell et al. 1999). However, data from our study do not support this hypothesis. δ^{13}C val-

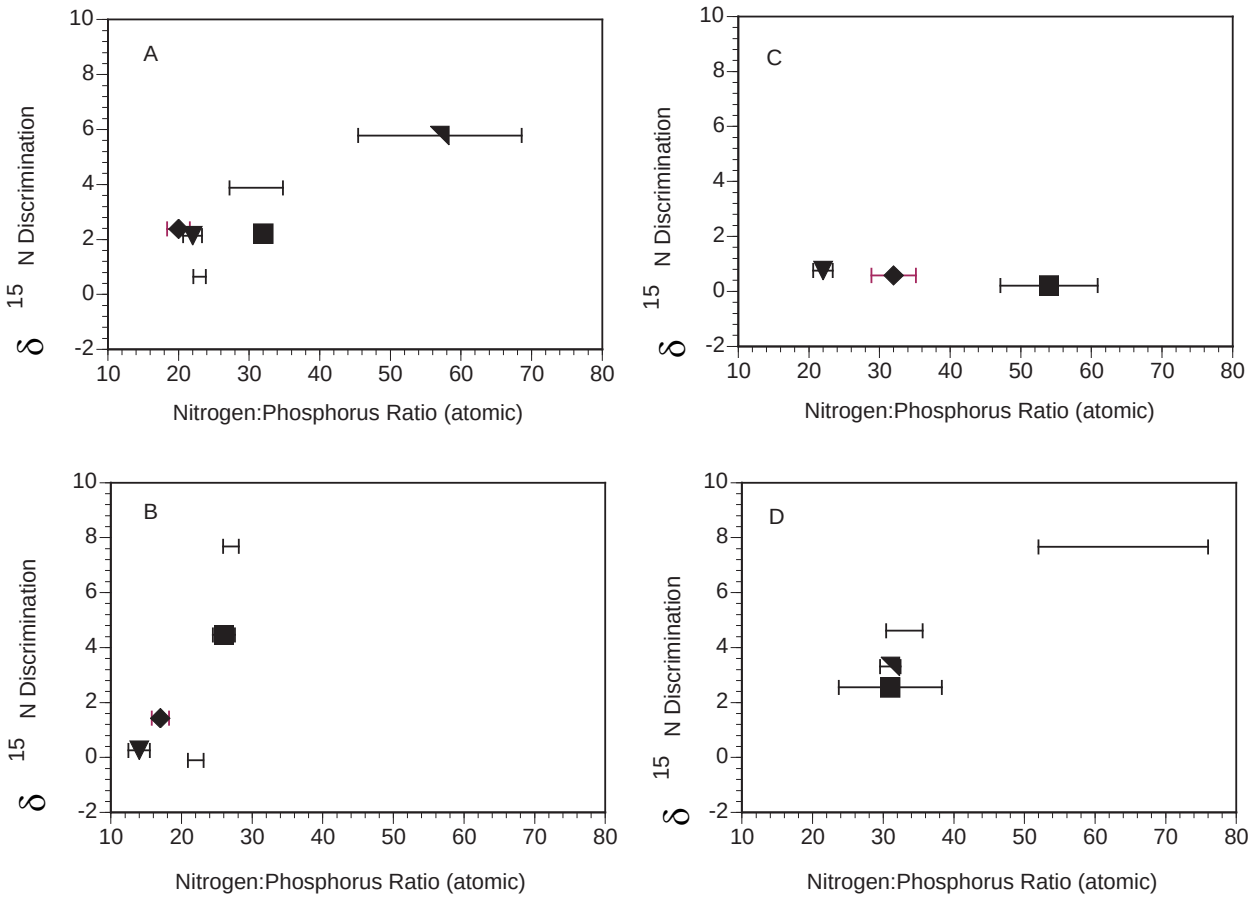

Figure 9. ^{15}N discrimination ($\delta^{15}N$ soil - $\delta^{15}N$ plant, in ‰) and green leaf N:P atomic ratios for the species (a) *Rhizophora mangle*, (b) *Laguncularia. racemosa*, (c) *Avicennia germinans*, and (d) *Conocarpus erectus*. Values are means ± 1SE. See Figure 11 for symbols.

ues and plant-N concentrations for *A. germinans* and *C. erectus* showed no significant correlation, and for *R. mangle* and *L. racemosa* these correlations were negative. Not all mangrove species growing under similar chronic stress exhibit the same reduction in ^{13}C discrimination (Ball and Farquhar 1984, Guy et al. 1989). In our study, there were different patterns between foliar $\delta^{13}C$ and edaphic variables for each species. In *R. mangle* and *A. germinans* significant correlations were negative between $\delta^{13}C$ values and soil-P concentrations, whereas *L. racemosa* and *C. erectus* did not show this relationship. However, $\delta^{13}C$ values for all species were significantly correlated with sulfide concentrations, confirming the role of this variable as a mangrove stress-factor along Taylor Slough.

Leaf $\delta^{13}C$ values were positively correlated with P canopy retranslocation rates, indicating that scrub mangroves in Taylor Slough have greater capability of retaining P than mangroves from Shark River. This pattern was clear for *R. mangle* but not for the other species (Fig. 7). Nutrient resorption from senescing leaves is a significant nutrient-conservation mechanism in plants (Chabot and Hicks 1982). Thus, in nutrient-limited environments, low nutrient loss via litter fall can increase the fitness of plant populations (Chapin 1980, Vitousek 1982, May and Killingbeck 1992). $\delta^{13}C$ values were not correlated with nitrogen retranslocation. $N:P_a$ ratios greater than 15:1 in green mangrove leaves indicate that as phosphorus becomes a limiting factor, nitrogen concentrations are probably enough to carry out the metabolic processes. This coincides with the lower N resorption compared with P observed in the system.

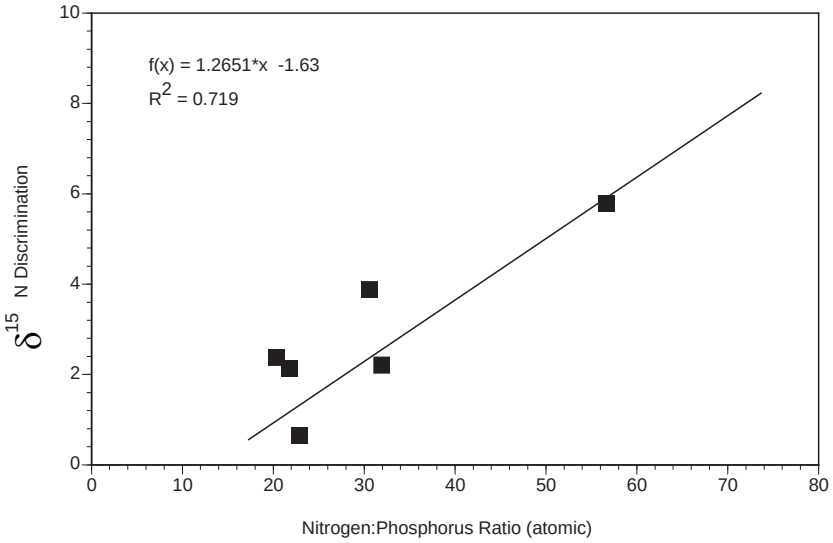

Figure 10. Relationship between ^{15}N discrimination (‰) and leaf N:P atomic ratios in *Rhizophora mangle* based on results of our study (squares) compared to values from Belize (McKee et al. 2002; circles).

lands in these two basins of south Florida.

Different factors such as N demand, inorganic N supply, plant growth, and soil $\delta^{15}N$ composition have been associated with ^{15}N discrimination during plant N uptake (Montoya and McCarthy 1995, Schmidt and Stewart 2003). In our study, leaf N:P_a ratio, salinity and N pore water account for 41% of the variability of foliar $\delta^{15}N$ composition (Table 4). Likewise, leaf N:P_a ratio, porewater nutrients and salinity explained 37% of the variability of ^{15}N discrimination ($\delta^{15}N$ soil - $\delta^{15}N$ plant) in south Florida mangrove wetlands. Under N limited conditions, isotope fractionation is expected to be low, because all N is used regardless of isotopic ratio (Montoya and McCarthy 1995, Evans et al. 1996). This explains the results obtained in the present study, where ^{15}N discrimination and N:P_a ratios of green leaves had significantly positive correlations for all mangrove species, except *A. germinans* (Fig. 9).

Comparing the four mangrove species, *A. germinans* had the lowest ^{15}N discrimination value, near zero, even in sites with relative high N:P_a ratio (>50) (Figs. 9c). This result may indicate that *A. germinans* has higher N requirements than the other mangrove species, which would be supported by the high N concentration measured in green leaves of this species. It also has been found that *A. germinans* responds to fertilization with nitrogen by increasing rates of photosynthesis (Lovelock and Feller 2003). ^{15}N discrimination for the other three species ranged from -0.1 to 7.7‰, with higher values in Taylor Slough sites, especially TS/Ph8, where *L. racemosa* and *C. erectus* showed the highest discrimination values.

In the present study, as mentioned before, a direct relationship between ^{15}N discrimination and leaf N:P_a ratios was found in these two mangrove basins ($r=0.4$, $P<0.0001$). Similar results have been reported in other mangrove systems. Comparing N discrimination in *R. mangle* between Florida and Belize (McKee et al. 2002), it is consistent that leaf

Nitrogen Isotope Ratios

Foliar $\delta^{15}N$ composition integrates long-term processes in which N from different sources is transformed through the interaction of several biotic and abiotic processes (Yoneyama et al. 1991, Garten 1993). N discrimination occurs as a consequence of biological and physico-chemical processes (Schmidt and Stewart 2003). Thus natural variation in foliar ^{15}N is so complex as to prevent successful interpretation of spatial patterns (Garten 1993). To elucidate the variation of leaf $\delta^{15}N$ values observed in south Florida mangroves (*R. mangle*), Fry et al. (2000) proposed two models that explain the interactions of isotope source and processing. In the first model, lower mangrove $\delta^{15}N$ results from the isotopic fractionation during N uptake in a high source $\delta^{15}N$ environment. In the second model, higher mangrove $\delta^{15}N$ values in a lower $\delta^{15}N$ source environment are due to microbial fractionation. The $\delta^{15}N$ patterns (Fig. 7) observed during the present study support the first model proposed by Fry et al. (2000). Average soil $\delta^{15}N$ values (‰ ±SE) for Shark River were higher than observed for mangrove leaves, especially at Taylor Slough sites. Plants were ^{15}N-depleted in reference to soils across most of the sites, indicating that microbial fractionation was not likely an important process in the N cycle of mangrove wet-

$N:P_a$ ratios account for 70% of the variability in ^{15}N discrimination (Fig. 10). These patterns support the hypothesis that foliar $\delta^{15}N$ composition may be related to site N status (Garten 1993).

^{15}N fractionation also is favored when plant growth is limited (Goericke et al. 1994), as at Taylor Slough and other mangrove sites of south Florida (Fry et al. 2000). Likewise, ^{15}N discrimination increases when NH_4^+ is the major source of inorganic nitrogen (Yoneyama et al. 1991). This is the case in south Florida, especially at Taylor Slough sites, where DIN in porewater is dominated by NH_4^+ and reaches values higher than 50 μM (Fig. 3a).

A strong relationship between net ammonification rate of mangrove soils and P availability (r^2 = 0.989, P<0.01) occurs at the Shark River mangrove sites (Chen and Twilley 1999). Likewise, in our study there was a negative slope between foliar $\delta^{15}N$ relative to foliar $N:P_a$ ratio for *R. mangle*, *L. racemosa*, and *C. erectus.* These results are consistent with the previous discussion. Shark River mangroves presented lower $N:P_a$ ratios due to a relative higher P concentration. Since the tall trees at this site likely demand high N concentration to grow, ^{15}N discrimination was lower than that observed in scrub mangroves from Taylor Slough. Similar results were obtained for *Kandelia candel* (Kao and Chang 1998) and *R. mangle* in Belize (McKee et al. 2002).

A phosphorus-limited environment combined with high sulfide concentrations appears to be responsible for environmental stress to the mangrove wetlands of Shark River and Taylor Slough basins. Foliar carbon and nitrogen isotope signatures of mangroves express the intensity of this environmental stress (Fig. 11). Foliar carbon isotope ratios of four mangrove species exhibited a relatively wide range from -24.6 to –32.7‰. These differences are related to biochemical properties of the carboxylation enzymes of photosynthesis that reflect physiological responses of mangroves to environmental stress. Thus, mangrove wetlands in Shark River and Taylor Slough have lower ^{13}C discrimination associated with P-limited soils that select for efficient use of resources such as water and nutrients. ^{15}N discrimination reflects site N status and its range in mangrove species (from –0.1 to 7.7‰) is another response of mangrove vegetation to environmental stress of this coastal Everglades landscape. Since P is a limiting factor in mangrove wetlands of these two basins, the increase of P availability may increases N demand, reducing ^{15}N discrimination (Fig. 10). Nutrient retranslocation analysis revealed that scrub mangroves, living in P-limited conditions, are not only more water-use efficient, as indicated by lower $\delta^{13}C$, but have greater capability of retaining P than do tall

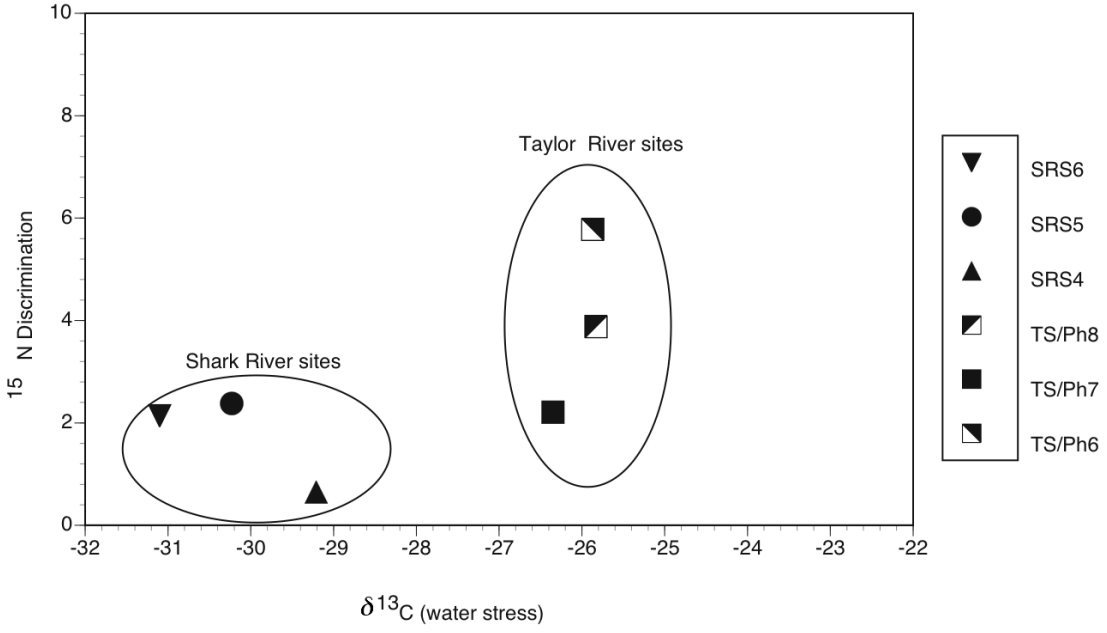

Figure 11. Nitrogen discrimination and carbon isotopic fractionation (‰) in *Rhizophora mangle* across the environmental stress gradient in south Florida mangrove wetlands.

mangroves. These scrub mangroves had higher ^{15}N discrimination most likely because of their low growth rate and high porewater $N:P_a$ ratio.

Our results support the hypothesis that the variability of mangrove $\delta^{13}C$ and $\delta^{15}N$ is potentially high in landscapes with sharp boundaries of environmental stress. These environmental stressors could obscure the linkages between mangrove wetlands and estuarine food webs by introducing mechanisms that discriminate both ^{13}C and ^{15}N metabolism. These stress conditions must be considered in applications of natural isotopes to trace organic matter in coastal ecosystems. Plant nutrient dynamics and mangrove-based food webs should be analyzed following a site-specific analysis of mangrove source material (Odum 1984, Fry and Smith 2002).

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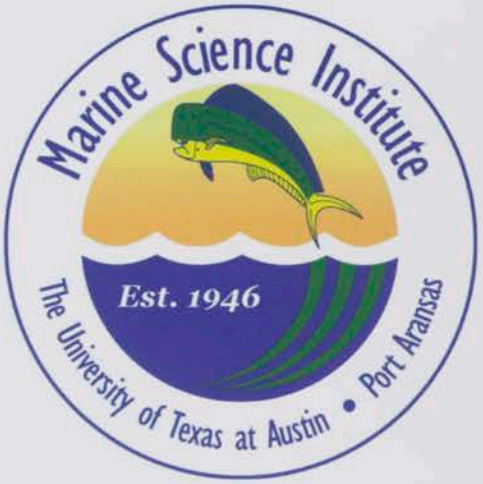
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