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Ectoenzyme kinetics in Florida Bay: Implications for bacterial carbon source and nutrient status

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Abstract

Ectoenzyme kinetics [alkaline phosphatase, aminopeptidase (AM), lipase, α-glucosidase, and β-glucosidase] were determined over a seasonal cycle at four stations, Little Madeira Bay (northeast), Duck Key (east), Bob Allen Key (south-central), and Whipray Basin (north-central), which represent distinct regions of Florida Bay. Spatial and temporal variations in ectoenzyme kinetics were related to biotic and abiotic drivers in order to discern potential ectoenzyme substrate sources. Generally, ectoenzyme activities were higher in the central bay and lowest in the eastern bay. One pronounced exception was AM activity, which was highest at the mangrove fringe along the northeastern bay and reflected increased contribution of organic nitrogen inputs from upstream Everglades wetlands. When ectoenzymes were normalized to bacterial abundances, these trends dissipated and highest cell-specific activities were observed consistently in the south-central region. Relationships between ectoenzyme kinetics and environmental and biological parameters were complex, but three main spatially determined differences were discernable. Ectoenzyme kinetics were controlled by phosphorus availability in the eastern bay, by organic matter availability in the south-central bay, by microbial community composition and organic matter availability in northeastern bay (wetlands transition area), and by microbial community composition in the north-central bay. These differences in enzyme kinetics further support the hypothesis of distinct microbial communities in different regions of Florida Bay and provide insight into biogeochemical cycles and the microbial food web within Florida Bay.

Introduction

The microbial food web is increasingly recognized as an important pathway in organic matter (OM) decomposition and nutrient cycling in marine ecosystems (Azam et al., 1983). Within the microbial food web, phytoplankton exudation and release by microbial grazers are the main sources of OM to the water column, and an estimated average of 50% of autochthonous production is recycled through this pathway (Azam, 1989). In estuarine systems, benthic resuspension, benthic plant/algal exudation, and terrestrial/riverine inputs represent additional sources of nutrients and OM, which can stimulate heterotrophy and enhance the microbial food web and microbial biogeochemical cycles. Most OM inputs into marine systems are not directly utilizable by bacteria, which cannot access organic material >600 Da (Weiss et al., 1991). Therefore, bacteria induce ectoenzymes that hydrolyze polymers and oligomers into labile monomers that can pass through cellular membranes (Hoppe et al., 2002).

Two commonly associated designations of noncytoplasmic enzymes are ecto- and extracellular enzymes, where ectoenzymes are attached to the

outer cell wall of microorganisms or occur inside the periplasmic space of gram-negative bacteria and extracellular enzymes are freely dissolved in the water or associated with particles of non-parent origin (Martinez & Azam, 1993). Here, non-cytoplasmic enzymes are inclusively referred to as ectoenzymes, unless noted otherwise. Ectoenzymes target specific chemical bonds which link subunits in biosynthetic polymers (i.e. sugar phosphates, nucleotides, peptides, amino sugars, lipids, structural polysaccharides, and storage polysaccharides). The direct substrates for ectoenzymes are often unknown, and ectoenzyme activities on natural substrates are difficult to quantify. Therefore, fluorogenic model substrate analogs are used to quantify in situ ectoenzyme rates (Hoppe et al., 2002). While model substrates lack the chemical complexity of natural substrates (Arnosti et al., 2005), they are employed frequently to determine potential ectoenzyme activities over a broad range of aquatic environments (Chróst, 1989; Rath et al., 1993; Hoppe et al., 2002; Sala et al., 2005).

Ectoenzymes respond rapidly, directly or through changes in microbial community composition, to changes in nutrient and substrate availability by manipulating enzyme kinetics to best capitalize on environmental conditions. Reciprocal transplants of natural bacterial communities into marine and brackish (more eutrophic) environments caused a rapid increase in marine and decrease in brackish community aminopeptidase (AM) and β-glucosidase (β-glu) activities (Cunha et al., 2001). When these communities were returned to their natural environments, the marine community returned rapidly (within 2 h) to previous activities. Across broad marine pelagic regimes, variability in extracellular polysaccharide hydrolases reflected the diversity reflected by 16S rDNA bacterial communities but was unrelated to bacterial abundance and production (Arnosti et al., 2005). In the Ria de Aveiro estuary, Portugal, increased β-glu activity near the river mouth suggested that bacterial communities relied on complex, autochthonous carbohydrates, while seaward communities with increased AM activity (AMA) relied on simpler proteinaceous compounds in the polluted lagoon (Cunha et al., 2001). Assessing distinct alkaline phosphatase (AP), AM, lipase, and glucosidase activities for 44 marine bacteria isolates in identical media revealed that no individual bacterium strain was capable to hydrolyze multiple substrates at high rates (Martinez et al., 1996).

Spatial patterns in community ectoenzyme kinetics can elucidate potential OM source and availability in organically rich, oligotrophic systems, but in Florida Bay, ectoenzymes have received limited attention. Florida Bay is a shallow (3 m), seasonally hypersaline estuary and experiences a subtropical climate with distinct wet (May–October) and dry (November–April) seasons. Florida Bay is bordered to the north by the Everglades wetlands, to the west by the Gulf of Mexico and to the southeast by the Florida Keys. A general nutrient gradient exists from severe P-limitation in the east to N-limitation in the west (Fourqurean et al., 1993). Alkaline phosphatase activity (APA) normalized to phytoplankton biomass reflects this nutrient gradient with highest activities in the eastern and southern bay (Cotner et al., 2000; Glibert et al., 2004), although community APA peak in the central bay (Boyer et al., 1999). Highest OM concentrations and phytoplankton biomass are found in the central bay (Boyer et al., 1999), where P advection from the Gulf of Mexico (Fourqurean et al., 1993) and high dissolved organic nitrogen concentrations are suspected to favor predominant cyanobacteria blooms (Glibert et al., 2004). The eastern bay is the most physically isolated region in Florida Bay but does receive freshwater OM imports in the northeast from the Everglades via the Taylor Slough/C-111 canal system (Rudnick et al., 1999). The eastern bay is characterized by low phytoplankton biomass and net heterotrophy (Lavrentyev et al., 1998). Irrespective of area, seagrass are the dominant primary producers in the system (Zieman et al., 1989), though cyanobacterial blooms in the central bay can temporarily contribute significantly to local production (Phlips et al., 1999). Easily resuspended, flocculent sediments can attenuate light significantly across the bay, with the greatest potential for light limitation in the deeper western bay (Phlips et al., 1995; Kelble et al., 2005). Sediment resuspension can also introduce benthic nutrients and seagrass derived OM into the water column (Mead et al., 2005), which may enhance pelagic microbial activities.

This study focuses on potential sources and seasonal availability of OM to heterotrophic

bacteria in Florida Bay by examining the relationships between ectoenzyme kinetics and variations in nutrient concentrations, OM, and microbial abundance. Relationships are addressed in terms of spatial differences between four stations within Florida Bay.

Material and methods

Four stations were sampled weekly from April 27 to June 29, 2004 and monthly from July 20, 2004 to May 18, 2005. Sampling stations corresponded to NSF–Florida Coastal Everglades (FCE)–Long Term Ecological Research (LTER) Taylor Slough/Pan-handle stations Little Madeira Bay mangrove fringe at the Taylor Creek mouth (northeast, Stn 7a), Duck Key (east, Stn 9), and Bob Allen Key (south-central, Stn 10) and Southeast Environmental Research Center (SERC) water-quality network Whipray Basin Stn 13 (north-central; Fig. 1). Surface water samples were collected in acid-washed 5 l polycarbonate bottles and kept at ambient temperature and in the dark until laboratory processing within 6 h. During each sampling period, hydrographic conditions, inorganic and organic nutrients, chlorophyll *a* concentrations, bacterial abundances, and ectoenzyme kinetics were determined at each station.

Temperature and salinity were recorded by a YSI 556 MPS probe. Inorganic N and P were measured on an Alpkem RFA 300 flow injected autoanalyzer from GF/F filtered samples stored frozen until analysis. Dissolved inorganic nitrogen (DIN) was calculated as the sum of NH_4^+, NO_3^-, and NO_2^-. Total organic carbon (TOC) and total nitrogen (TN) were determined by high temperature catalytic combustion (Shimadzu TOC-V/TNM-1 organic carbon/nitrogen analyzer), and total organic nitrogen (TON) was calculated as

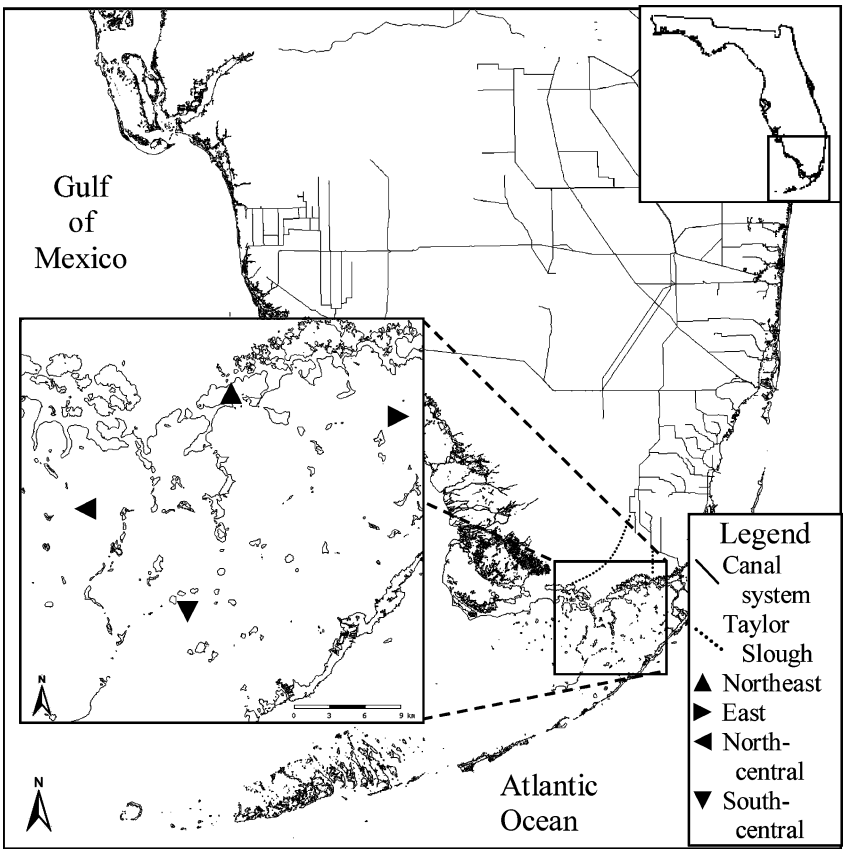

Figure 1. Florida Bay and sampling regions: Northeast (Stn 7a), East (Stn 9), South-central (Stn 10), and North-central (Stn 13).

TN minus DIN. Total phosphorus (TP) was measured by dry ashing and acid hydrolysis (Solorzano & Sharp, 1980).

Chlorophyll *a* concentration (CHL) as a proxy for phytoplankton biomass was determined from 0.25–1.0 l water samples collected on 25 mm GF/F filters stored frozen until extraction. Chlorophyll *a*, extracted with ice-cold 90% acetone from homogenized filters, was measured on a Turner 111 fluorometer calibrated with chlorophyll *a* standards.

Bacteria were quantified on a Becton-Dickinson FACsort flow cytometer. One ml of formalin-fixed sample was incubated for 30 min at 37 °C with 0.1 g l^{-1} RNAse (1:1 mix of RNAse A and B) before staining with SYBR Green I (10^{-5} dilution of commercial stock) in the presence of 30 mM potassium citrate (Marie et al., 1997). Samples analyzed at a flow rate of 0.2 μl s^{-1} were converted to bacteria ml^{-1} from measurement times (60–300 s) based on weight calibration of flow rates (Jochem, 2001).

Ectoenzyme kinetics for alkaline phosphatase (AP), aminopeptidase (AM), lipase (HEP), α-glucosidase (α-glu), and β-glucosidase (β-glu) were determined using fluorogenic model substrate analogs corresponding to classes of natural substrate chemical bonds (Hoppe, 1993). Analogs were 3-*o*-methylfluorescein phosphate, L-leucine-7-amido-4-methylcoumarin, 4-methylumbelliferon (MUF) heptanoate, 4-MUF-α-D-glucopyranoside, and 4-MUF-β-D-glucopyranoside. Triplicate dark incubations (45–180 min) at 21 °C were conducted in 96-well plates, with 180 μl of unfiltered station water and 20 μl of model substrate. For each replicate, four model substrate concentrations of 1–200 μM final conc., depending on substrate, were used to create a unique activity vs. concentration saturation curve. Fluorescence readings were recorded with a BioTek FLx800TB computer-controlled plate reader at T_0 and T_{final} and converted to activity rates using fluorescence end-product standard curves at the corresponding instrument settings. Triplicate saturation curves were analytically pooled and transformed into a Lineweaver–Burk plot to reveal maximum enzyme activity (V_{max}, nM h^{-1}) and substrate affinity (Michaelis–Menten half saturation constant; K_m, μM). Bacterial-specific enzyme activity (amol $cell^{-1}$ h^{-1}) was calculated as V_{max} divided by bacterial abundance and phytoplankton-specific AP (nmol μg Chl^{-1} h^{-1}) as AP V_{max} divided by CHL.

The non-parametric Friedman test, which is equivalent to a repeated measures one-way analysis of variance, was used to determine significant spatial differences ($p \leq 0.05$) between variables. The Wilcoxon signed rank test for repeated measures design was used to discern homogenous station subsets for each variable. Significant relationships ($p \leq 0.05$) between selected variables within each station data set were assessed by Pearson's bivariate correlation analysis.

Results

Hydrology and nutrients

Temperature ranged from 15.5 °C in January to 31.4 °C in July and was similar among stations (data not depicted). Salinity ranged from 10.8 to 50.5, with lowest concentrations at Stn 7a. Temporally, salinity increased during the early, exceptionally dry wet season and decreased during the early dry season for all stations except Stn 9, where no decline in salinity was observed (Fig. 2).

DIN ranged from 0.35 to 18.61 μM and highest concentrations were found at Stn 7a and 9 (Table 1). Seasonally, DIN concentrations were highest during the dry season at Stn 7a and 9, while highest concentrations occurred during the wet season at Stn 10 and 13 (Fig. 2). Soluble reactive phosphorus (SRP) ranged from below detection to 0.13 μM and was significantly higher at Stn 7a and 13 than at Stn 9 and 10 (Table 1). SRP increased seasonally in September at Stn 7a but lacked seasonal trends at other stations (Fig. 3). Redfield ratios (molar DIN:SRP) ranged from 17 to 834, with the highest potential for P-limitation at Stn 9, and generally followed DIN temporal patterns (data not depicted).

TOC ranged from 174.3 to 1685.4 μM and concentrations were significantly higher at Stn 7a and 13 than at Stn 9 and Stn 10 (Table 1). A seasonal TOC trend was only found at Stn 7a, where TOC increased from August to December (Fig. 4). TON ranged from 13.89 to 62.70 μM and was similar across stations (Table 1). Bay-wide TON maximum concentration occurred in

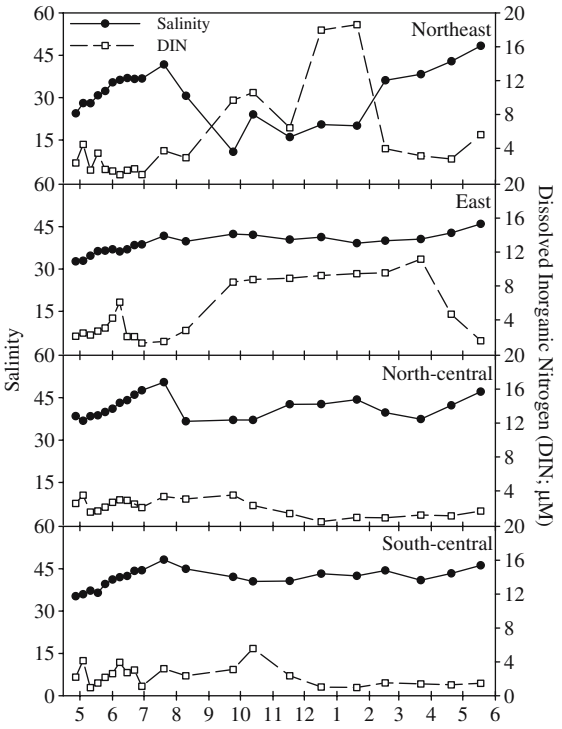

Figure 2. Time-series of salinity (left) and DIN (μM; right).

December at Stn 13 and corresponded to the seasonal TP maximum at this station. TON followed a similar temporal trend at all stations, with increased concentrations in August and December (Fig. 4). TP ranged from 0.04 to 0.57 μM, with significantly higher concentrations at Stn 7a than at Stn 9 and 10 (Table 1), and was increased over the dry season at Stn 7a (Fig. 3).

Chlorophyll a and bacterial abundances

CHL ranged from 0.19 to 8.26 μg l^{-1}, with lowest concentrations occurring at Stn 9 and the maximum in December at Stn 13 (Table 1, Fig. 5). Temporally, CHL was stable at Stn 9, increased in the wet season at Stn 10 and 7a, and was stable at Stn 13 except for a distinct December bloom (Fig. 5). Bacterial abundance (BAC) ranged from 0.27×10^6 to 2.91×10^6 cells ml^{-1}. Abundance was significantly different at all stations, with Stn 7a > Stn 13 > Stn 10 > Stn 9 (Table 1). Temporal trends in BAC were lacking at Stn 9 and 10, but at Stn 7a and 13, BAC was higher during the wet than the dry season, except for the December bloom at Stn 13 (Fig. 5).

Ectoenzyme kinetics

α-Glu affinity, activity, and cell-specific activity ranged 5.7–649.1 μM, 1.9–127.6 nM h^{-1}, and 1.3–74.6 amol $cell^{-1}$ h^{-1}, respectively. α-Glu K_m and activity were positively related at Stn 7a ($r = 0.913$) and 9 ($r = 0.656$) but were unrelated at other stations. α-glu affinity was lowest at Stn 9 and highest at Stn 7a (i.e. enzyme half saturation concentration (K_m) was significantly higher at Stn 9 and lower at Stn 7a, respectively; Table 2) and α-glu activity was significantly lower at Stn 9. A seasonal pattern in α-glu activity was lacking (Fig. 6).

β-Glu affinity, activity, and cell-specific activity ranged 3.7–71.7 μM, 2.3–26.2 nM h^{-1}, and 0.7–23.6 amol $cell^{-1}$ h^{-1}, respectively. No relationship

Table 1. Median values ($n \leq 21$) for dissolved inorganic nitrogen (DIN; μM), soluble reactive phosphorus (SRP; μM), total organic carbon (TOC; μM), total organic nitrogen (TON; μM), total phosphorus (TP; μM), APA:AMA activity ratios, chlorophyll *a* (CHL; μg l^{-1}), and bacterial abundance (BAC; 10^6 cell ml^{-1})

	Northeast	East	North-central	South-central
DIN	3.11^a	3.03^a	2.08^b	2.20^b
SRP	0.04^a	0.03^b	0.04^a	0.03^b
TOC	812.17^a	484.63^b	846.25^a	667.75^c
TON	31.84^a	28.05^a	31.17^a	33.36^a
TP	0.25^a	0.16^b	0.18^{ab}	0.17^b
APA:AMA	0.40^a	0.60^b	1.29^c	1.24^c
CHL	1.19^a	0.34^b	0.76^c	0.64^c
BAC	1.88^a	0.75^b	1.38^c	1.02^d

Note: Non-significantly different station groups indicated by identical superscripts.

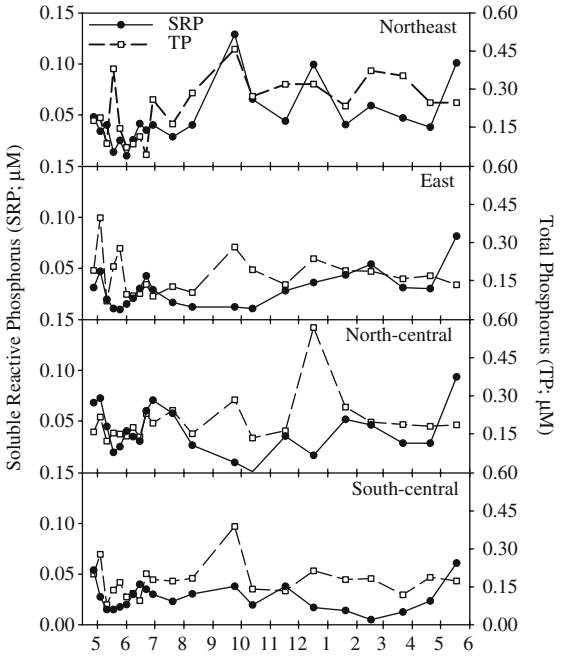

Figure 3. Time-series of SRP (μM; left) and TP (μM; right).

between β-glu affinity and activity was obvious at any station. β-glu K_m and cell-specific activity were lowest at Stn 7a, while β-glu activity was

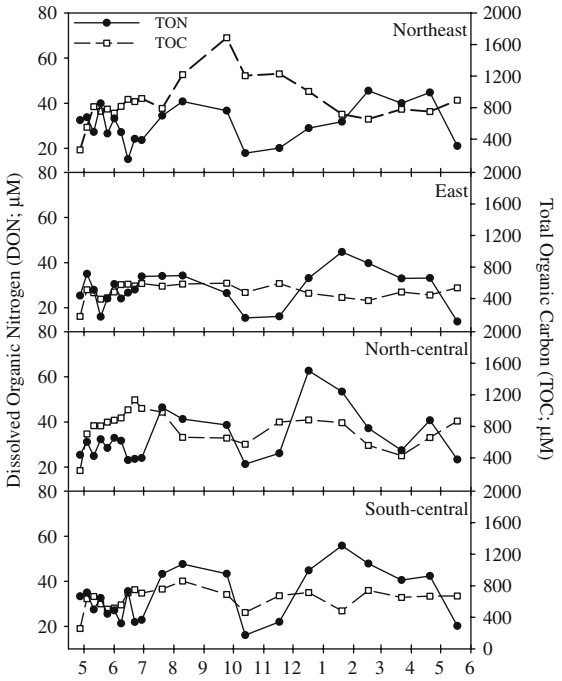

Figure 4. Time-series of TON (μM; left) and TOC (μM; right).

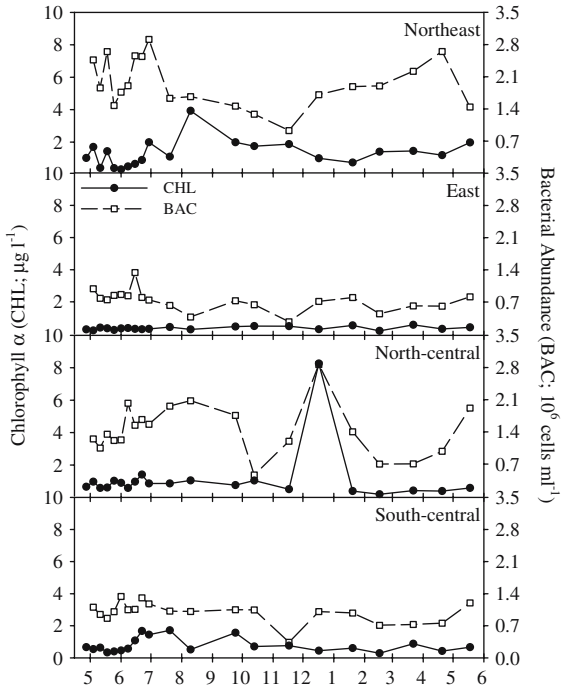

Figure 5. Time-series of CHL ($\mu g\ l^{-1}$; left) and BAC (10^6 cell ml^{-1}; right).

significantly lower at Stn 9 (Table 2). β-Glu activity was variable across stations with no clear temporal trend except at Stn 13, where activity tripled in December concomitant with the phytoplankton bloom (Fig. 6).

HEP affinity, activity, and cell-specific activity ranged 14.7–52.6 μM, 234.7–1089.9 nM h^{-1}, and 169.8–1424.7 amol $cell^{-1}$ h^{-1}, respectively. HEP K_m and activity were only related at Stn 7a (r = 0.815). Although HEP affinity was similar among stations, HEP activity increased significantly from east to central bay but lacked clear seasonal patterns (Table 2, Fig. 6). HEP activities normalized to BAC lacked the regional trend and cell-specific activities were similar at all stations except Stn 7a, where specific activities were significantly lower (Table 2).

AM affinity, activity, and cell-specific activity ranged 8.2–102.8 μM, 69.7–1649.9 nM h^{-1}, and 52.2–1571.7 amol $cell^{-1}$ h^{-1}, respectively. AM K_m and activity correlated positively at all stations. AM affinity was significantly lower at Stn 7a than at other stations (Table 2). AMA was significantly different at all stations, with Stn 7a >

Table 2. Median values ($n \leq 21$) for ectoenzyme affinity (K_m; μM), activity (V_{max}; nM h^{-1}), and bacterial specific activity (bac^{-1}; amol $cell^{-1}$ h^{-1}) for α-glucosidase (α-glu), β-glucosidase (β-glu), heptanase (HEP), aminopeptidase (AM), and alkaline phosphatase (AP) and phytoplankton specific APA (Chl^{-1}; nmol μg chl^{-1} h^{-1})

	Northeast	East	North-central	South-central
α-Glu				
K_m	26.1^{a}	61.4^{b}	40.5^{bc}	32.3^{ac}
V_{max}	8.0^{a}	5.7^{b}	7.2^{a}	6.6^{b}
$Cell^{-1}$	3.7^{a}	8.5^{a}	5.0^{ab}	6.7^{b}
β-Glu				
K_m	11.6^{a}	21.1^{b}	20.8^{b}	17.3^{b}
V_{max}	9.2^{a}	4.9^{b}	9.2^{a}	8.6^{a}
$Cell^{-1}$	4.9^{a}	6.9^{b}	7.1^{bc}	9.0^{c}
HEP				
K_m	21.1^{abc}	24.6^{a}	23.3^{b}	20.2^{c}
V_{max}	471.5^{a}	358.3^{b}	662.0^{c}	576.0^{d}
$Cell^{-1}$	275.9^{a}	493.2^{b}	460.4^{bc}	634.8^{c}
AM				
K_m	65.6^{a}	34.5^{b}	22.0^{b}	28.1^{b}
V_{max}	443.6^{a}	168.2^{b}	202.0^{c}	287.5^{d}
$Cell^{-1}$	255.0^{a}	225.5^{ac}	188.2^{b}	323.7^{c}
AP				
K_m	1.3^{a}	1.9^{b}	1.4^{a}	1.3^{a}
V_{max}	187.6^{a}	103.4^{b}	264.8^{c}	371.6^{d}
$Cell^{-1}$	84.2^{a}	139.4^{b}	212.6^{c}	397.1^{d}
Chl^{-1}	0.15^{a}	0.31^{b}	0.35^{bc}	0.57^{c}

Note: Non-significantly different station groups indicated by identical superscripts.

Stn 10 > Stn 13 > Stn 9. Specific AMA was significantly higher at Stn 10 and lower at Stn 13 than at Stn 7a and 9 (Table 2). Generally, AMA increased during the wet and early dry seasons at Stn 7a, 9, and 10. At Stn 13, AMA followed a temporal trend similar to BAC and peaked in December (Fig. 7).

AP affinity, activity, and BAC- and CHL-specific activities ranged 0.9–3.2 μM, 61.3–1021.8 nM h^{-1}, 44.5–1029.4 amol $cell^{-1}$ h^{-1}, and 0.06–1.27 nmol μg Chl^{-1} h^{-1}, respectively. AP K_m and activity were negatively related at Stn 7a ($r = -0.463$) and 9 ($r = -0.512$), positively related at Stn 10 ($r = 0.481$), and unrelated at Stn 13 ($r = 0.186$). AP affinity was significantly lower at Stn 9 than at the other stations, which did not differ significantly (Table 2). APA differed significantly among stations, with highest activities at Stn 10 and 13. At Stn 10, specific APA (both BAC- and CHL-normalized) was higher than at Stn 13, where it was higher than at Stn 9 and 7a (Table 2). APA decreased during the dry season at Stn 7a, increased from August to December at Stn 9, varied at Stn 10, and was stable with peak activity in December at Stn 13 (Fig. 7). APA:AMA ratios ranged from 0.16 to 3.23, with lower ratios at Stn 7 and 9 than at Stn 10 and 13 (Table 1). APA:AMA ratios were variable at all stations, though they tended to increase during the dry season at Stn 10 and 9 (Fig. 7).

Correlation analysis

Correlations in the northeastern region (Stn 7a) were generally associated with inorganic nutrients, TOC, and CHL, while AM and α-glu activities were significantly related to CHL and inorganic nutrients, respectively (Table 3). In the eastern region (Stn 9), variables were generally unrelated, though APA correlated positively with CHL and negatively with BAC. Correlations were weak in the south-central region, but APA correlated

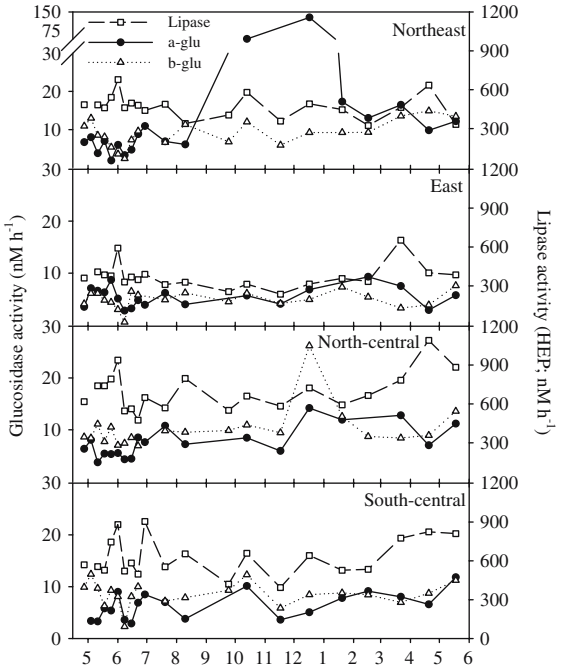

Figure 6. Time-series of α-glu (left), β-glu (left) and HEP (right) activity (nM h^{-1}).

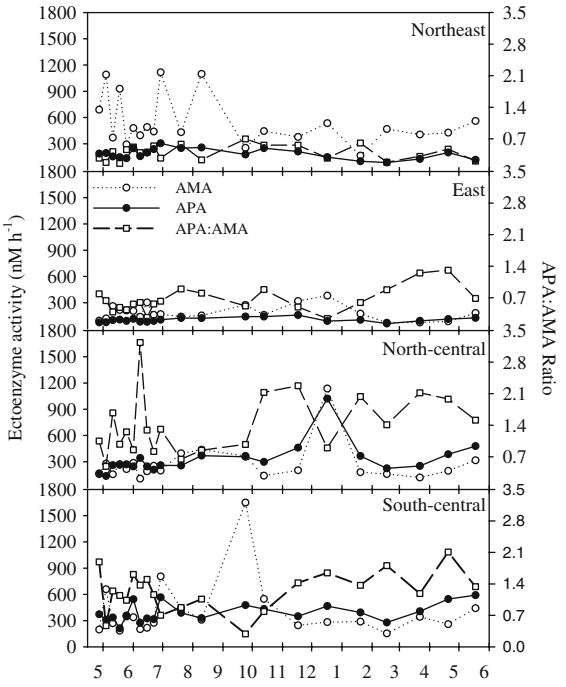

Figure 7. Time-series of APA (left; nM h^{-1}), AMA (left; nM h^{-1}) and APA:AMA ratio (right).

strongly and positively with α-glu and HEP activities and AMA correlated significantly with TP. In the north-central region (Stn 13), all ectoenzymes except HEP activity were interrelated and correlated significantly with organic nutrients and microbial populations (Table 3). Meaningful relationships with correlation coefficients are discussed below in more detail.

Discussion

Ectoenzyme kinetics

Kinetic ectoenzyme assays are infrequently used in marine environmental studies (Chróst & Overbeck, 1987; Rath et al., 1993; Sebastián et al., 2004) because they require time-consuming methodologies. More commonly, ectoenzyme activities are obtained using one model substrate concentration at saturation (Martinez et al., 1996; Cunha et al., 2000; Murrell, 2003; Taylor et al., 2003; Sala et al., 2005). However, only a narrow range of substrate concentrations yielded APA estimates comparable to AP V_{max} determined by kinetic assays in oligotrophic aquatic systems (Sebastián & Niell, 2004). The generality of this concept towards other fluorogenic model substrates is unknown, but the choice of substrate concentration(s) appropriate for the observed system remains an important consideration for determining ectoenzyme kinetics. In the northwestern Mediterranean Sea, α-glu, β-glu, and AM saturation curves revealed two distinct saturation points for low (10 μM) and high (>10 μM) model substrate concentrations, where ectoenzyme activity did not increase with increasing substrate concentrations (i.e. biphasic ectoenzyme kinetics; Unanue et al., 1999). While both concentration ranges and associated activities followed Michaelis–Menten (M–M) kinetics, they resulted in two distinct kinetic patterns: a high-affinity system at low substrate concentrations and a low-affinity system at high substrate concentrations (Unanue et al., 1999). In the present study, biphasic kinetics were observed over a broad range of model substrate concentrations (0.1–1000 μM) for all ectoenzymes except AP. Ectoenzyme kinetics presented here represent the lowest range of substrate concentrations that provided detectable activities using small sample volumes (180 μl),

Table 3. Station comparison matrix of within-station correlations

	Temp	Sal	DIN	SRP	TOC	TON	TP	CHL	BAC	α-Glu	β-Glu	HEP	AMA
DIN	−7a*, −9*, 10, 13*	−7a*	–										
SRP		13	7a	–									
TOC		13*		7a	–								
TON	−10, −13*					–							
TP	−13			7a	7a	13*	–						
CHL	10		9		7a	13*	7a*, 13*	–					
BAC	10				−7a, 13	13	13*	13*	–				
α-Glu	−7a, 13		7a*	7a*		13	9, 13*	13		–			
β-Glu	−13*		13			13*	13*	7a, 13*	13	13*	–		
HEP							−7			10	−7a	–	
AMA						13*	10*, 13*	7a*, 10, 13*	7a, 13*	13	13*		–
APA	−13	9*	13		9	13*	13*	9*, 13*	−9, 13*	10*, 13	13*	10*	13*
APA:AMA			−10		7a		−10	−10					–

Note: Only significant ($p \leq 0.05$) relationships recorded and highly significant ($p \leq 0.01$) relationships indicated by asterisk.

conformed to the M–M model, and were comparable to concentrations applied in other aquatic systems (Chróst, 1989; Hoppe, 1993; Saliot et al., 1996).

Historically, APA has been attributed to both bacteria and phytoplankton, while AM, HEP, α-glu, and β-glu activities were attributed to heterotrophic bacteria alone (Hoppe, 1993; Hoppe et al., 2002). Recently, peptidase activity was also found in photo- and mixotrophic dinoflagellates (Stoecker & Gustafson, 2003) and heterotrophic nanoflagellates (Karner et al., 1994; Mohapatra & Fukami, 2004). Under bloom conditions, phototrophic dinoflagellates were associated with a significant fraction of peptide hydrolysis (Mulholland et al., 2002). In alpine lakes, glucosidase hydrolyses correlated weakly with BAC and CHL, but were strongly related to dry weight of *Daphnia longispina*, and freely released cladoceran digestive enzymes were believed to be an important part of the extracellular glycohydrolase pool (Vrba et al., 2004).

In the present study, all ectoenzyme activities, except APA, were normalized to bacterial abundance, which assumes that most of the activity was associated with bacteria. Due to AP association with phytoplankton and bacteria, APA is commonly normalized to either CHL or BAC, depending on the microbial community under study. Here, APA normalized to CHL and to BAC followed similar spatial patterns, but these patterns were opposite to spatial patterns reported previously from Florida Bay (Cotner et al., 2000; Glibert et al., 2004). The reason for this difference remains unclear but might be related to biomass normalization. Spatial patterns in AP activities were similar to those reported by Cotner et al. (2000), but mean CHL was only ca. 10% of previously reported CHL. Except for a phytoplankton bloom at Rabbit Key, APA reported by Glibert et al. (2004) were spatially comparable, and CHL-normalized activities reflected the spatial decrease in CHL from west to east. In the present study, a general east to west increase in CHL was present, but spatial patterns in CHL-normalized APA were dictated by the spatial east to west increase in community APA (Table 2). These differences highlight the variability in APA and problems associated with attributing ectoenzyme activities to one microbial group when multiple sources potentially co-exist. Bacteria-specific ectoenzymes were within the ranges reported for other aquatic systems (Martinez et al., 1996; Hoppe et al., 2002), and implications of these rates for Florida Bay microbial communities are discussed below.

In order to attribute ectoenzyme activities to specific microbial populations, ectoenzymes are often measured in the filtrate of various functional size fractions. In Florida Bay, size fractionation is

impractical because cyano- and heterotrophic bacteria exhibit overlapping size ranges (Phlips et al., 1999). In addition to imperfect community separation, filters can leach nutrients into the filtrate and disrupt natural enzyme–cell/particle associations (Sala et al., 2001). To avoid problems of filtration and biomass normalization, Sala et al. (2001) proposed APA:AMA ratios to infer community-level inorganic nutrient limitation. Therefore, APA:AMA was expected to be highest in the severely P-limited eastern bay and lowest in the organic nitrogen-rich central bay (Boyer et al., 1999). However, APA:AMA was unrelated to SRP, only weakly, negatively related to DIN ($r = -0.448$) at the south-central station, and did not follow expected temporal and/or spatial patterns (Tables 1 and 3). Three factors might have caused APA:AMA ratios to not reflect ambient nutrient concentrations in Florida Bay: (1) AP kinetics were optimized for low SRP concentrations; (2) organisms used AP and AM primarily for carbon rather than P or N acquisition; (3) changes in microbial community composition affected AP and AM kinetics.

APA was comparable to activities reported previously for Florida Bay (Boyer et al., 1999) and other estuarine systems (Hoppe, 2003). Though reports of AP affinity are rare, observed K_m was consistent with freshwater algal isolates (Healey & Hendzel, 1979), but community AP K_m determined in eutrophic systems were an order of magnitude higher than reported here (Chróst & Overbeck, 1987; Saliot et al., 1996). These differences are not surprising as substrate/enzyme coupling, microbial communities, and environmental conditions, which differed widely between reports, influence ectoenzyme affinities.

AM, HEP, and glucosidase kinetics have not been determined previously in Florida Bay. Though activities are reported frequently from other systems, information on affinities is sparse. The relative magnitude of ectoenzyme activities determined in this study ranked HEP > AM > AP > β-glu > α-glu and agreed well with other aquatic systems (Hoppe et al., 2002), though lipase activities were reported to be both higher (Martinez et al., 1996) and lower (Taylor et al., 2003) than found here. Differences in lipase activity may be related to different MUF-lipid analogs used and differences in OM and microbial community composition (Martinez et al., 1996).

SRP can inhibit APA competitively when the function of the enzyme is to acquire P but SRP does not affect, or can enhance, APA when the enzyme is used to access organic phosphorus to fulfill carbon demands (Chróst & Overbeck, 1987; Nausch & Nausch, 2004; Sebastián et al., 2004). As found previously in Florida Bay, APA was unrelated to SRP but reflected organic components in the bay (Table 3; Boyer et al., 1999). These relationships suggest that APA was mostly associated with bacteria.

Influences on AMA are less clear. AMA correlated negatively with DIN in the Hudson River (Taylor et al., 2003), Antarctic waters (Sala et al., 2005), and in the Pomeranian Bight, Baltic Sea (Nausch & Nausch, 2000). The presence of P enhanced AMA in Baltic Sea mesocosm experiments (Nausch & Nausch, 2000). A general positive relationship between protein hydrolysis rates and dissolved organic nitrogen compounds was described in estuarine/coastal systems (Mulholland et al., 2002, 2003), though this may not always apply (Rath et al., 1993). AMA was unrelated to DIN in Florida Bay (Table 3). AM kinetics and OM in the eastern bay were not related, but AMA correlated positively to TON and TP in the central bay (Table 3).

High HEP activities suggest that lipids were a potentially important bacterial carbon source, but HEP kinetics were unrelated to TOC, CHL, and microbial abundances. Similarly, lipase activity did not correlate with CHL, BAC, or other ectoenzyme activities in the Long Island Sound, but lipids were not considered an important bacterial carbon source (Taylor et al., 2003). In contrast to HEP, α-glu and β-glu activities correlated positively with phytoplankton biomass at northeast and north-central regions. Generally, these enzymes are associated with increased bacterial activity during phytoplankton lysis and bloom breakdown in aquatic systems (Chróst, 1989; Nausch & Kerstan, 2003).

Contributing factors to ectoenzyme kinetics in Florida Bay

Cluster analysis revealed three distinct station clusters, with only the central bay stations (Stn 10

and 13) pairing together (analysis not shown). Previously, the south-central station has been placed in the east-central bay based on benthic plant communities (Zieman et al., 1989), in the eastern bay based on water quality parameters (Boyer et al., 1997), and at the junction of three distinct regions based on light availability (Phlips et al., 1995). Due to these potentially different influences at the south-central station, ectoenzyme kinetics are discussed for each station/region separately.

Northeastern region (Stn 7a)

The northeastern region hosts a transition community between the Everglades wetlands and Florida Bay and receives freshwater inputs directly through Taylor Slough. The study period coincided with a dry early wet season and the northeastern region only received freshwater inputs from Taylor Slough beginning in late July. This freshwater influx (lowered salinity) seemed associated with increased DIN and TOC, an initial spike in SRP and CHL, and decreased BAC (Table 3, Figs. 2–5). Ectoenzyme responses to these changes were variable but suggest that the microbial community was able to adapt rapidly to increased OM inputs and/or changed to better exploit environmental conditions. During the inundation period, α-glu activity increased by over an order of magnitude concomitant with an increase in α-glu affinity, and α-glu activity correlated significantly with SRP ($r = 0.655$) and DIN ($r = 0.729$). These responses suggest that labile polysaccharides imported from the Everglades and to a lesser extent autochthonous, phytoplankton-derived OM were important for bacterial catabolism. The interpretation of these results is temporally limited to one annual cycle and ectoenzyme patterns may or may not be reflective of normal wetlands/bay interactions. In general, α-glu activities were highest in this region, which might explain the previously reported decrease in polysaccharides relative to other organic compounds at this station compared to eastern and south-central regions (Maie et al., 2005). Bacterial specific activities were higher during this period, apparently due to decreases in BAC, but may reflect changes in microbial community composition upon environmental changes, most notably salinity and DIN.

Inverse relationships between APA and K_m enable the microbial community to sequester rapidly dissolved organic phosphorus (DOP) inputs by maximizing enzyme turnover times for high affinity enzyme systems (Unanue et al., 1999). The lowest APA:AMA ratios were observed in this area, which might reflect increased SRP and bacterial dependency on dissolved organic N (DON) at this station. Increased DON loading from mangrove and wetland communities likely caused the highest observed AMA and K_m. Relatively higher β-glu affinities with similar activity supports ectoenzymes designed to access structural polysaccharides such as leaf debris washed into the mangrove fringes with increased Everglades sheet flow and canal discharge. Positive correlations between AM, HEP, and α-glu activities and K_m ($r = 0.473$, $r = 0.815$, and $r = 0.913$, respectively) further indicate that microbial communities were adapted for higher OM concentrations in this area. Given these relationships, it is likely that both substrate availability and changes in microbial community composition induced the observed changes in ectoenzyme kinetics.

Eastern region (Stn 9)

Evidence of external OM inputs from the Everglades was absent at this station, and bacteria relied most likely on autochthonous (seagrass and/or phytoplankton) OM. DIN increased during the dry season and did not appear to affect AMA, which peaked during the dry season and was uncorrelated to all other parameters, suggesting that bacteria utilized AMA for carbon rather than N acquisition. HEP, α-glu, and β-glu kinetics did not show significant relationships to abiotic and biotic parameters, though HEP activities suggest lipids were the most important carbon source.

Characterization of ultrafiltered dissolved organic matter >1 kDa (UDOM) revealed labile neutral sugars and proteinaceous materials in the water column (Maie et al., 2005), which were probably suitable substrates for glucosidases and AM. APA was unrelated to TP or SRP but related to TOC and CHL, with phytoplankton biomass being the dominant influence. A negative relationship between APA and BAC ($r = -0.452$)

suggests that decreases in BAC increased the relative amount of available P and that APA responded rapidly to this vacant resource. These relationships support a predominantly phytoplankton origin of APA. High affinity AP and an inverse relationship between AP V_{max} and K_m indicated that AP was optimized for a low P system. AP kinetic relationships also support a faster enzyme turnover time, which increases the microbial community's ability to sequester DOP. Low BAC and CHL in this area and weak relationships between ectoenzymes and OM with potentially abundant labile substrates suggest that microbial growth was limited. During the study period, top-down control of bacteria and phytoplankton was unlikely, as bacterivory did not balance bacterial growth (M. Rogers unpubl. data) and nutrient addition stimulated phytoplankton growth (F.J. Jochem unpubl. data). P-limitation is likely the underlying cause of these interactions as no evidence of carbon limitation was obvious.

South-central region (Stn 10)

UDOM characterization reflected non-terrestrial origin for this area, where UDOM composition and DOC concentrations were almost identical to the eastern region (Maie et al., 2005). Relationships between ectoenzymes and biological parameters support the importance of autochthonous OM. BAC-specific HEP, AM, and AP activities were highest in this region of the bay, which might indicate a different microbial community composition at the south-central region relative to the eastern bay sites. BAC increased with temperature and generally followed CHL patterns until August, when BAC remained unchanged throughout the remainder of the study period. Positive correlations between APA and HEP ($r = 0.683$) and α-glu ($r = 0.588$), which fluctuated similar to BAC over the wet season, indicate that these activities were most likely of bacterial origin. Furthermore, subtle increases in SRP stimulated by APA might have allowed for increased bacterial utilization of lipids and carbohydrates.

AMA did not increase with the other ectoenzymes, and increased DIN concentrations over the wet season might partly explain this difference, though the relationship between DIN and AMA was not clear. AMA correlated negatively with DIN ($r = -0.448$), although they increased concurrently in September with TP and CHL (Figs. 2, 3, 5 and 7). The cause for this marked quadrupling in AMA remains unresolved, as it was unrelated to variables determined during this sampling period but did not seem to be a measurement artifact. Otherwise, APA was more important than AMA as evident from APA:AMA ratios generally >1 (Fig. 7). AM and AP correlations suggest that this area of Florida Bay is more eutrophic in organic N and P than the eastern region and in organic P compared to the northeastern region. Denser seagrass beds (Zieman et al., 1989), which can release labile materials into the water column (Ziegler & Benner, 1999), might cause higher ectoenzyme activities in this region.

North-central region (Stn 13)

Generally, TOC concentrations were consistently higher in this region of the bay, though ectoenzyme kinetics did not reflect a more eutrophic state over other areas of the bay (Table 2). Other than wet season increases in AMA and BAC ($r = 0.744$), ectoenzyme activities were stable over the time-series until a phytoplankton bloom occurred in December. Based on previous taxonomic description (Phlips & Badylak, 1996), *Synechococcus elongatus* was identified as the dominant phytoplankton species during the bloom. TP, TON, BAC, CHL, and all ectoenzyme activities except for HEP increased significantly during the bloom (Figs. 3–7) and correlated with each other (Table 3). The bloom was short-lived and all parameters, except TON, returned to pre-bloom levels in January. This contrasted the usual increases in heterotrophy and ectoenzyme activity associated with the breakdown of phytoplankton blooms. During the breakdown of a freshwater phytoplankton bloom, β-glu activity increased up to 8 fold along with increased BAC, bacterial production and BAC-specific activity, which was stimulated by microbial grazer release of polysaccharides (Chróst, 1989). Here, cell-specific ectoenzyme activities did not differ before, during, and after the bloom but doublings of AM and β-glu K_m suggest that more proteinaceous material and carbohydrates were available during the bloom. Furthermore, chlorophyll-normalized phytoplankton productivity, cell-specific bacterial pro-

duction, and flow cytometrically determined bacterial community composition (i.e. high- vs. low-DNA bacteria; Jochem, 2001) remained unchanged from November through January (C.J. Williams unpubl. data). These results suggest that ectoenzyme kinetics were relatively fixed (i.e. not substrate-dependent) for the present community, which probably did not change in composition but only increased in abundance. The lack of increased heterotrophy after the bloom might be related to the life-history of *S. elongatus*. As *S. elongatus* became nutrient deplete, cells might have become less buoyant (Phlips et al., 1999) and settled to the benthos, thereby removing incorporated P and autochthonous materials from the pelagic system. *S. elongatus* occur in aggregates surrounded by a thick mucus sheath, which probably hinders microbial grazing that could retain incorporated nutrients in the water column through protistan recycling. Bacteria remaining in the water column, without sufficient autochthonous substrates to sustain their elevated populations, were subsequently grazed back to pre-bloom abundances.

Conclusion

Variations in ectoenzyme kinetics in Florida Bay reflected differences in nutrient, substrate, bacteria, and phytoplankton interactions in different regions of the bay. At the northeast region mangrove fringe (Stn 7a), ectoenzyme kinetics reflected OM contributions from the Everglades wetlands and mangrove communities. At the east region (Stn 9), the most P-limited part of the bay, APA correlated to phytoplankton biomass and negatively to BAC, suggesting that AP was associated predominantly with phytoplankton. Other than AP, ectoenzyme kinetics were uncorrelated with microbial abundances and organic and inorganic nutrients and seemed ultimately linked to low P availability. In the south-central region (Stn 10), bacteria-specific activities were higher than in other regions of the bay, implying suitable N and P substrates were more readily available at this station. Community and cell-specific activities were least variable in the north-central region (Stn 13), which experienced a pronounced bacteria and phytoplankton bloom, dominated by the cyanobacterium *S. elongatus*, in December 2004. Ectoenzyme activities increased during the bloom but bacterial cell-specific activities remained unchanged, suggesting that bacterial community composition did not change and bacteria remained in a similar metabolic state irrespective of the bloom. These differences among stations concur with the hypothesis that distinct basins/regions within Florida Bay harbor distinct microbial communities (Lavrentyev et al., 1998). Evidence for ectoenzyme induction by changes in nutrient and substrate availability was most obvious in east and south-central regions, while evidence for temporal control of ectoenzyme kinetics by microbial community composition appeared in northeast and north-central regions. The degree of ectoenzyme variation caused by variations in microbial community composition as opposed to environmental factors remains unresolved but is currently under investigation. Further elucidating the driving forces for ectoenzyme kinetics might help clarifying the relative importance of phytoplankton production and biogeochemical cycles within the pelagic microbial food web versus benthic/Everglades imports of OM for bacterial carbon demand and the control of bacterial populations and processes in Florida Bay.

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