



Epiphyte loads on seagrasses and microphytobenthos abundance are not reliable indicators of nutrient availability in oligotrophic coastal ecosystems

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ABSTRACT

Despite marked gradients in nutrient availability that control the abundance and species composition of seagrasses in south Florida, and the importance of nutrient availability in controlling abundance and composition of epiphytes on seagrasses in other locations, we did not find that epiphyte load on the dominant seagrass, *Thalassia testudinum*, or that the relative contribution of algal epiphytes to the epiphyte community, was positively correlated with nutrient availability in the water column or the sediment in oligotrophic seagrass beds. Further, the abundance of microphytobenthos, as indicated by Chlorophyll-*a* concentration in the sediments, was not directly correlated with concentrations of nutrients in the sediments. Our results suggest that epiphyte and microphytobenthos abundance are not unambiguous indicators of nutrient availability in relatively pristine seagrass environments, and therefore would make poor candidates for indicators of the status and trends of seagrass ecosystems in relatively low-nutrient environments like the Florida Keys.

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1. Introduction

Increases in resource availability shift the competitive balance from slow-growing to fast-growing organisms. In nutrient-limited aquatic and marine systems, increases in nutrient supply leads predictably from dominance by slow-growing rooted macrophytes, to progressively faster-growing primary producers like free-living and epiphytic macroalgae, culminating in dominance by single-celled algae and light limitation at very high nutrient loads (see Duarte (1995) and Burkholder et al. (2007) for reviews). In seagrass beds, increases in the abundance of epiphytes – the plants and animals growing on the surfaces of the slower-growing seagrass leaves (Borowitzka et al., 2006) – are stimulated by nutrient loading (e.g. Borum, 1985; Silberstein et al., 1986; Neckles et al., 1994; Balata et al., 2008) and these increases in abundance have been implicated as the cause for declines of seagrasses during eutrophication (e.g. Orth and Moore, 1983; Cambridge et al., 1986). Concentration of nutrients in the water column may not increase throughout the eutrophication process until after the slow-growing, rooted macrophytes disappear (Cambridge et al., 1986), so

concentrations of nutrients in the water column are poor indicators of eutrophication in oligotrophic systems. Owing to the relationships between nutrient availability and epiphyte abundance observed in these and other systems, abundance of epiphytes and other fast-growing primary producers may provide a useful indicator of the relative availability of nutrients in macrophyte-dominated coastal systems.

Microphytobenthos are an additional, important primary producer in shallow coastal ecosystems, whose productivity can be substantial in both phytoplankton-dominated (e.g. Schreiber and Pennock, 1995) and macrophyte-dominated systems (e.g. Moncreiff et al., 1992). In areas of high light availability, nutrient addition enhances the biomass of the microphytobenthos (Dizon and Yap, 1999; Hillebrand et al., 2000; Herbert and Fourqurean, 2008), so biomass of microphytobenthos may also be indicative of nutrient availability.

There are few regions where seagrass beds are as extensive as found in south Florida (Fourqurean et al., 2002), and south Florida has a large and rapidly growing coastal human population. Across the shallow coastal marine ecosystems of south Florida, there are pronounced gradients in the relative availability of nitrogen (N) and phosphorus (P), and a qualitative change in the limiting nutrient (Fourqurean and Ziemann, 2002). In general, enclosed estuarine waters in the region are exceedingly P-limited owing to the lack of P with respect to N in freshwater runoff and long water residence

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times (Fourqurean et al., 1992a, 1993), while P availability increases in an offshore direction (Szmant and Forrester, 1996; Fourqurean and Zieman, 2002), leading to N limitation of seagrasses along the back side of the Florida Keys barrier reef. The generally low-nutrient concentrations in costal water of south Florida result in clear water with enough penetration of solar energy to the benthos to support extensive seagrass beds and coral reefs. The growing human population, and the concomitant increases in nutrient discharge, put these oligotrophic marine communities at risk.

In Florida Bay, an enclosed estuarine embayment to the north of the Florida Keys, epiphyte load is positively, but weakly, correlated with measures of P availability in that generally P-limited environment, but near point sources of P supply epiphyte loads are much higher than elsewhere in the ecosystem despite lack of increases in nutrient concentrations in the water column (Frankovich and Fourqurean, 1997). Experimental fertilizations in Florida Bay have shown that the response of the epiphytic and microphytobenthic community to increased nutrient availability are complex, and that these communities sometimes respond to increased nutrient availability with increased abundance and sometimes they do not (Armitage et al., 2006). Reliance on chlorophyll-*a* (Chl-*a*) abundance as an indicator of microalgal biomass may miss important changes in the structure of the community caused by changes in the abundance of algal taxa that contain other pigments in addition to Chl-*a* (Armitage et al., 2006; Frankovich et al., 2009).

Given the observed relationships between nutrient loading and the abundance of epiphytes and microphytobenthos observed in seagrass ecosystems from around the world, and the perceived threat to water quality owing to human population, the abundance of epiphytes and microphytobenthos in seagrass beds may prove to be a valuable metric for assessing both the current status and trends of the seagrass beds. In this paper, we present data on epiphyte and microphytobenthos abundance collected at 30 benthic monitoring sites in the Florida Keys National Marine Sanctuary (FKNMS), and compare these data to nutrient concentration in the water column and to the N and P content of the seagrass leaves which has been shown to be a reliable indicator of nutrient availability in the region (Fourqurean et al., 1992a; Fourqurean and Zieman, 2002), with a goal of assessing the viability of using epiphyte

and microphytobenthos abundance as indicators of nutrient availability in south Florida. Specifically, we test the hypotheses that epiphyte and microphytobenthos abundance is correlated with concentrations of nutrients in the water column, sediments and seagrass tissues. We also examine the time series of epiphyte abundances from these 30 sites for intra-annual trends.

2. Methods

Thirty sites were selected within the 9600 km² of the FKNMS to serve as permanent monitoring locations for benthic habitats (Fig. 1). These sites were a randomly selected subset of a more spatially-intensive water quality monitoring program. The water quality monitoring sites were selected using a stratified-random procedure, where strata were determined by both hydrogeographic province and distance from shore (see Boyer and Jones (2002) for detailed description). Both benthic habitat and water quality monitoring have been conducted at these sites four times per year since 1995, but the water quality and the benthic habitat sampling efforts are not temporally coincident.

Water quality data were collected quarterly at each site beginning in 1995, please see Boyer and Jones (2002) for a complete description of the water quality sampling program's field and laboratory methods. We restrict our analyses in this paper to data collected during the period 2001–2007. At each station, depth profiles of salinity, temperature and available light were obtained using a CTD, and grab samples of surface water were collected approximately 25 cm below the surface. Duplicate unfiltered water samples were collected in 120 ml HDPE bottles for analysis of total nutrient concentrations. Duplicate water samples for dissolved nutrients were filtered (25 cm diameter Whatman GF-F) by hand in the field into 60 ml HDPE bottles. The filters were extracted in 90% acetone/water for Chl-*a* analysis.

Sediment data were collected bi-annually from June 2001 to January 2003 to capture summer and winter conditions. Four sediment cores (1.15 cm diameter, 2.61 cm depth) were collected at each site. Two sediment cores were used to estimate microphytobenthos abundance via determination of Chl-*a* content of the

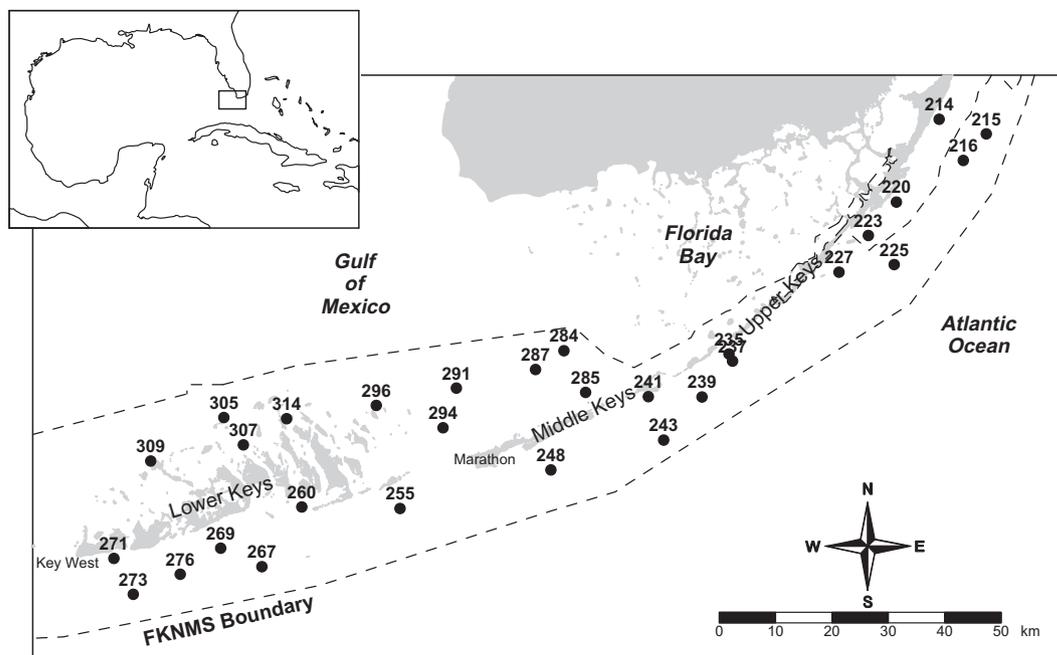


Fig. 1. Map of the study area showing station locations and geographic regions.

sediment [(mg Chl-*a*)(m⁻²)]. Sediment samples were transferred into 20 ml glass scintillation vials and placed on ice in the dark. Ten ml of 100% acetone was added to the wet sample to extract Chl-*a*; concentrations of Chl-*a* were measured fluorometrically (Strickland and Parsons, 1972) on a Guilford Fluoro IV Spectrofluorometer (excitation = 435 nm, emission = 667 nm). Two additional sediment cores were used to analyze sediment porosity, bulk density, organic content and elemental content (C, N, P). Sediment was transferred into pre-weighed 20 ml glass scintillation vials and wet weight was calculated. The sample was dried at 70 °C for 48 h to obtain a dry weight. An aliquot of each sample was ashed at 500 °C for 5 h and organic content was calculated as loss on ignition (LOI). Dry sediment samples were ground using a ceramic mortar and pestle and total nutrients (CNP) were determined. Powdered samples were analyzed in duplicate for carbon and nitrogen content using a CHN analyzer (Fisons NA1500). Ashed sediments were also analyzed for total inorganic carbon (IC) and the percentage of organic carbon was calculated as the difference between IC and total carbon (TC). Phosphorus content was determined by a dry-oxidation, acid hydrolysis extraction followed by a colorimetric analysis of phosphate concentration of the extract (Fourqurean et al., 1992b). Elemental content was calculated on a dry weight basis; elemental ratios were calculated on a mole:mole basis.

At each sampling site, six intact short shoots of the dominant seagrass *Thalassia testudinum* were haphazardly collected from a 10 m² area. These short shoots were returned to the lab, where all attached green leaves were cut from the short shoots, measured for length and width, and cleaned of adhering epiphytes by gently scraping with a razor blade. Leaf area was calculated by multiplying leaf length by leaf width, corresponding to a one-sided leaf area calculation. All leaves from a site were pooled and dried at 80 °C. Because the mass of seagrass leaves per area of seagrass leaves has been suggested as an indicator of nutrient availability and eutrophication (Lee et al., 2004), we calculated this based on the one-sided leaf area and the dry mass of the collected seagrass short shoots. Dried leaves were ground to a fine powder using a ceramic mortar and pestle, and analyzed for C, N and P content following the procedures outlined above.

Epiphyte data were collected 2–4 times a year from June 2001 to March 2009. From the six short shoots of *T. testudinum* collected for elemental analysis, we formed three replicate sets of two shoots. The epiphytic material that was scraped from the shoots was placed into pre-weighed 20 ml glass scintillation vials and stored at –20 °C. Epiphytes were freeze-dried to obtain a dry weight. Twenty ml of 90% acetone were added to each vial, shaken and stored at –20 °C for a minimum of 72 h. Chl-*a* content of the acetone extracts was measured fluorometrically as described above. We calculated epiphyte load as the dry mass of epiphytes per cm² of seagrass leaf area, epiphyte Chl-*a* load as the mass of Chl-*a* in epiphyte extracts per cm² of seagrass leaf area, epiphyte and epiphyte Chl-*a* abundances as mass per seagrass short shoot, and epiphyte autotrophic index (AI) as the mass of Chl-*a* (μg) per dry mass of epiphytes in mg (Frankovich and Fourqurean, 1997).

3. Statistical methods

We calculated median values for water quality parameters at each of the 30 monitoring sites because medians are a better than means as representations of central tendencies in water quality from this region because of infrequent high values in some of the parameters. For seagrass morphology, nutrient content and epiphyte values, we calculated site means based on the period of record of each parameter for examination of spatial pattern. We calculated the mean for each sampling period across all 30 stations

for analysis of temporal pattern. We tested for cyclical annual patterns in the data by fitting a sine function to the data of the form $Y = Y_{\text{bar}} + \alpha \sin(\text{DOY} + \Phi)$, where α is the amplitude, DOY is the day of the year expressed in radians ($365d = 2\pi$ radians), and Φ is a phase angle that sets the timing of the sine wave, using a non-linear least-squares regression procedure. This model has been shown to describe seasonality in many aspects of seagrass community dynamics in south Florida (Fourqurean et al., 2001). The analyzed data were deemed seasonal if the parameter estimate for α was significantly different than 0. We analyzed data for correlations using bivariate Pearson's correlation; we also further analyzed the relationships between some variables with linear regression.

4. Results

In general, the waters in the Florida Keys National Marine Sanctuary were clear and warm with relatively low concentrations of dissolved nutrients and phytoplankton (Table 1). The median water temperature was 27.28 °C, with winter lows of 16.00 °C and summer highs of 33.16 °C. The median salinity was 36.26, but salinity fluctuations did occur in the shallow waters of the Gulf of Mexico side of the Florida Keys; the lowest salinity we measured was 28.12 and the highest was 39.50. Most of the nutrients in the water column were in organic forms. Total nitrogen concentrations had a median value of 12.21 μM, while dissolved inorganic nitrogen (DIN) concentrations were two orders of magnitude lower (median = 0.54 μM). DIN was generally dominated by NH₄⁺, which had a median value of 0.33 μM, compared to 0.12 μM for NO₃⁻ and 0.05 μM for NO₂⁻. Total phosphorus (TP) concentrations had a median of 0.20 μM, and the median TN:TP ratio was 56. A very small portion of the phosphorus in the water column was in dissolved inorganic form, with median soluble reactive P values of 0.03 μM. Perhaps as a consequence of the low-nutrient concentrations, phytoplankton biomass was quite low, with a median value for the entire data set of 0.15 μg Chl-*a* l⁻¹.

The median phytoplankton biomass at a site was correlated with the median concentrations of TOC, TN and TP in the water column, but not the concentrations of dissolved inorganic forms of N and P (Table 2). There were marked spatial patterns in water quality across the sampling area, with lower median concentrations of TN and TP offshore on the Atlantic side of the Florida Keys, with increasing values of TN and TP towards the Gulf of Mexico side of the Lower Keys (Fig. 2a and b). Median phytoplankton biomass,

Table 1

Water quality summary for quarterly sampling at the 30 monitoring sites, 2001–2007.

	Median	Min	Max	n
Chl- <i>a</i> (μg l ⁻¹)	0.15	0.00	9.01	838
NO ₃ ⁻ (μM)	0.12	0.00	4.16	840
NO ₂ ⁻ (μM)	0.05	0.00	0.37	840
NH ₄ ⁺ (μM)	0.33	0.00	6.07	840
Dissolved inorganic nitrogen (μM)	0.54	0.03	6.35	840
Total organic nitrogen (μM)	11.06	0.00	212.89	839
Total nitrogen (μM)	12.21	0.34	213.21	839
Soluble reactive phosphorus (μM)	0.03	0.00	0.22	840
Total phosphorus (μM)	0.20	0.01	1.78	834
Total organic carbon (μM)	154.46	28.81	1310.83	836
Si(OH) ₄ (μM)	0.86	0.00	125.03	831
Turbidity (NTU)	0.75	0.00	7.38	833
Salinity (PSU)	36.26	28.12	39.50	830
Temperature (°C)	27.28	16.00	33.16	830
Dissolved oxygen (mg l ⁻¹)	5.51	1.56	13.81	830
K _d (m ⁻¹)	0.13	0.00	2.89	474
DIN:SRP	19	1	1445	806
TN:TP	56	1	2591	833

Table 2

Pearson's correlations (r) among the median water quality values at the 30 stations. Correlation coefficients are shown above the diagonal; the p value for the correlations are below the diagonal.

	Chl- <i>a</i>	TOC	DIN	TN	SRP	TP
Chl- <i>a</i>		0.497**	0.321	0.545**	0.074	0.680**
TOC	0.005		0.379*	0.799**	-0.109	0.615**
DIN	0.084	0.039		0.398*	-0.013	0.321
TN	0.002	<0.001	0.030		-0.204	0.638**
SRP	0.697	0.565	0.945	0.279		0.026
TP	<0.001	<0.001	0.084	<0.001	.891	

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

as evidenced by Chl-*a*, followed a similar pattern, with the lowest values on the Atlantic side of the Upper Keys and highest values towards the Middle Keys and the Gulf of Mexico (Fig. 2d). Taken together, these data indicate that the fertility of the water column is generally lowest in the Upper Florida Keys on the Atlantic Ocean side of the Keys, with increasing fertility closer to the islands. Fertility was generally higher on the ocean side of the Middle and Lower Keys compared to the upper Keys, with the highest fertility in the waters on the Gulf of Mexico side of the Middle and Lower Keys. Across the whole system, none of the water quality parameters exhibited a regular sinusoidal seasonality; when the water quality data for all sites were averaged by time period our sine function model did not describe a significant portion of the seasonal variability (i.e. the parameter estimates for the amplitude component of the sine model were not significantly different from zero for any of the water quality parameters).

The sediments of our study area are predominantly biogenic calcium carbonates. Site means of TC content of the sediments varied from a low of 11.7% of dry weight at site 269 to a high of 12.8% at site 296. The mean average TC content of the sediments from all sites was $12.0 \pm 0.2\%$ (± 1 SD). Porosity of the sediments ranged from a site mean of 0.51 to a maximum of 0.88; in general porosity decreased from inshore towards the reef tract (Fig. 3a). The LOI of the sediments varied with the porosity (Pearson's $r = 0.849$, $p < 0.001$); the lowest organic content was 1.74% at site 309 and the highest was 12.06% at site 296 (Fig. 3b). Both the nitrogen content ($r = 0.997$, $p < 0.001$) and phosphorus content ($r = 0.839$, $p < 0.001$) of the sediments were highly correlated with the organic

carbon content. The ratio of organic carbon to nitrogen in sediments averaged 10.5 ± 1.4 , with site means from 7.4 to 14.1. In general, OC:N was lower in the central portion of our study area (Fig. 3c). The organic carbon to phosphorus ratio was more variable; the site means varied from 37.4 to 405.1, with a mean of 193.1 ± 117.9 and a clear spatial pattern of low OC:P in the back-reef sediments with increases shoreward (Fig. 3d). The ratio of N to P in the sediments mirrored the spatial pattern seen in OC:P, with low N:P along the reef tract and increased values shoreward (Fig. 3e).

Microphytobenthos abundance, as indicated by the concentrations of benthic Chl-*a* in surficial sediments, ranged from a site average of 17.2 mg m^{-2} at site 216 to a maximum of 65.0 mg m^{-2} at site 294 (Fig. 4). The mean benthic Chl-*a* abundance was $39.8 \pm 11.9 \text{ mg m}^{-2}$. Summer and winter sampling revealed no biologically significant seasonal patterns in benthic microalgal abundance. During winter sampling, Chl-*a* abundance averaged $42.4 \pm 17.6 \text{ mg m}^{-2}$ compared to summer averages of $37.4 \pm 14.0 \text{ mg m}^{-2}$.

There were no significant correlations between benthic Chl-*a* abundance and sediment nutrient concentrations, but there was a tendency to find higher benthic Chl-*a* at sites with higher porosity ($r = 0.41$, $p = 0.025$). The stations with higher mean benthic Chl-*a* concentrations had higher median concentrations of TN ($r = 0.70$, $p < 0.001$) and TOC ($r = 0.56$, $p = 0.001$) in the water column.

The morphology of the seagrass *T. testudinum* varied substantially among the 30 sites. Mean values over the period of record at each of the 30 sites indicated that some sites were characterized by leaves with widths of as little as 6.6 mm and as wide as 10.6 mm; similarly, there were wide ranges in other measures of the robustness of seagrass short shoot, including mean leaf length, the number of leaves per short shoot, the total leaf area per short shoot and the mass of leaves per short shoot (Table 3). The mass per area of seagrass leaves, on the other hand, was less variable than the other measures of morphology, from a low of 3.8 mg cm^{-2} to a high of 5.2 mg cm^{-2} . Most of the measures of the size of seagrass short shoots were positively and significantly correlated (Table 4), so one measure of short shoot size captures most of the pattern in short shoot morphology across the spatial extent of our sampling stations. Short shoots of *T. testudinum* tended to be smaller (less than 100 mg SS^{-1}) along the seaward margin of our study area inside of the barrier reef, and increased in size shore-

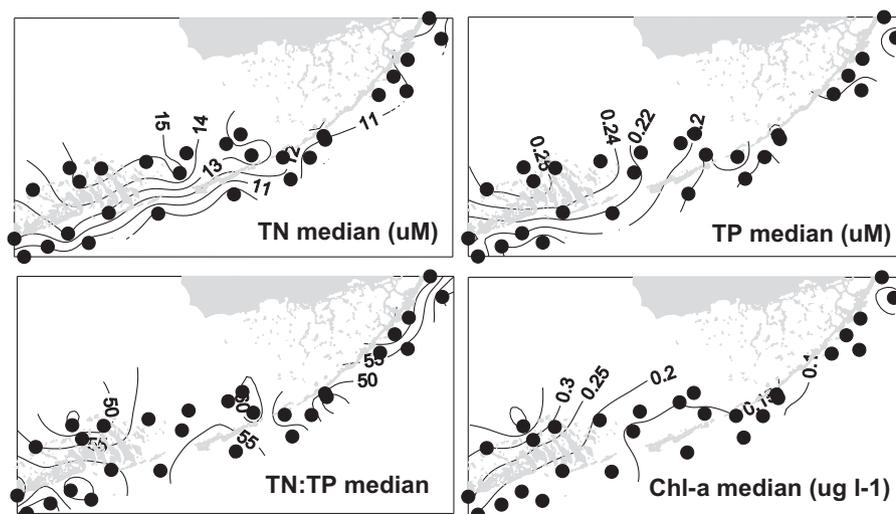


Fig. 2. Isopleth maps of the median values of total nitrogen (TN), total phosphorus (TP), TN:TP ratio and phytoplankton biomass as chlorophyll-*a* (Chl-*a*) in the water column for the period of record (2001–2007) at the thirty stations. Ratios calculated on a mole:mole basis.

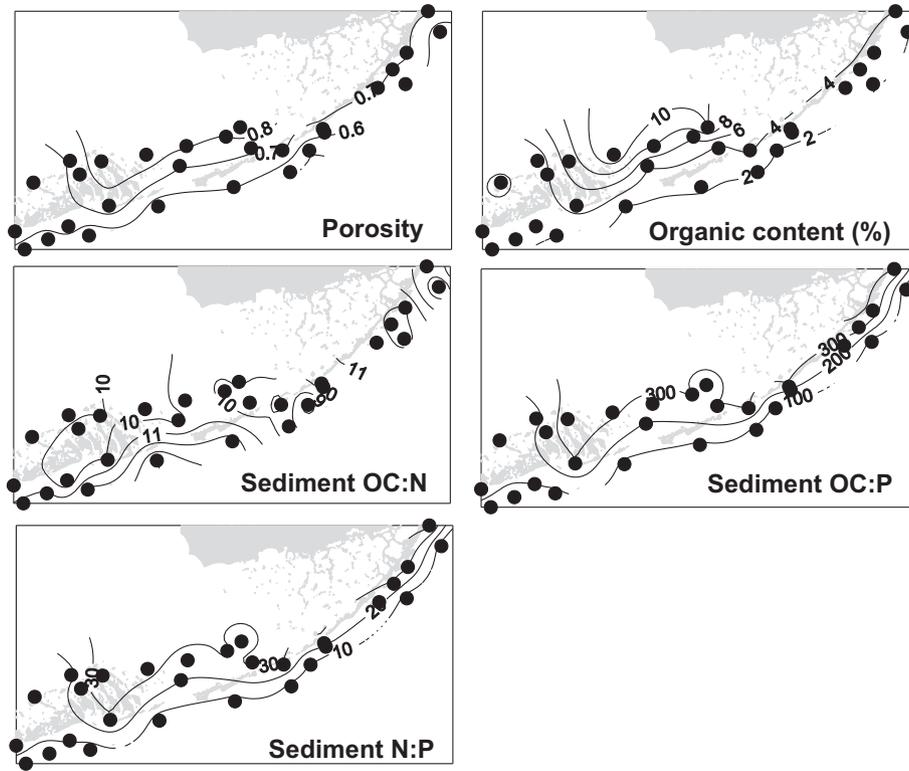


Fig. 3. Isoleth maps of the mean values of porosity, organic content, organic carbon to nitrogen (OC:N), organic carbon to phosphorus (OC:P) and N:P of the sediments from the thirty stations. Ratios calculated on a mole:mole basis.

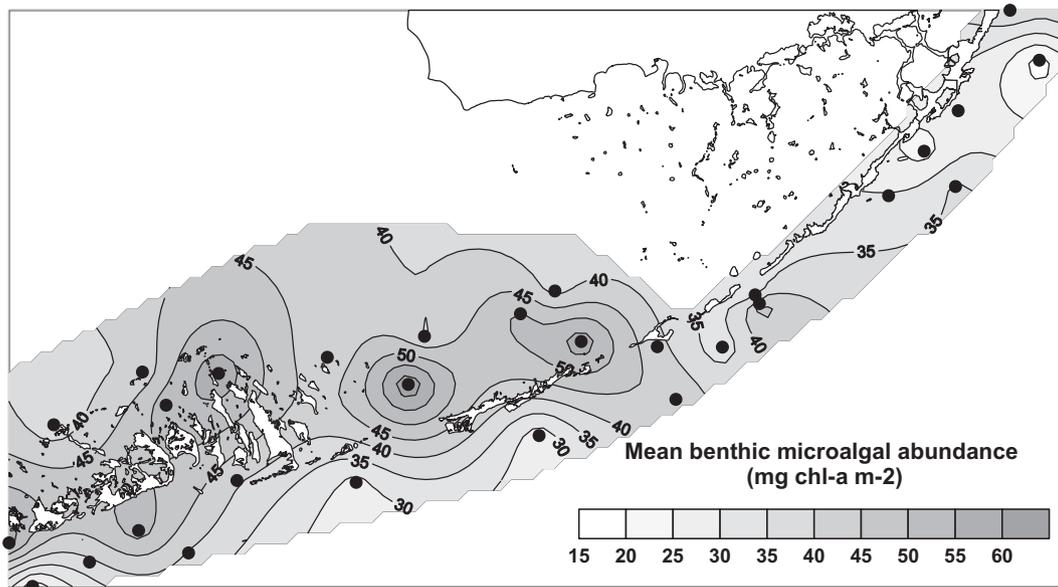


Fig. 4. Station mean microphytobenthos abundance, as mg chlorophyll-*a* m⁻², at the thirty stations.

ward towards the Florida Keys, reaching sizes of 150–200 mg SS⁻¹ along the Atlantic shore of the main Florida Keys (Fig. 5). Seagrass short shoots were larger still along the Gulf of Mexico side of the Florida Keys, reaching average short shoot sizes of over 400 mg SS⁻¹. Short shoot size was inversely related to the density of seagrass short shoots (Fig. 6): sort shoots from dense seagrass beds were smaller than shoots from more sparse beds. The relationship between size and density followed a power relationship of the form $y = ax^b$ with the power parameter b equal to

-1.14 ± 0.22 . The spatial pattern in the mass of seagrass leaves per area of seagrass leaves (Fig. 7) did not coincide completely with the spatial pattern in the mass of the short shoots.

Seagrass leaf elemental content varied substantially in both time and space. For a detailed description of these patterns, see Fourqurean et al. (2005). The station mean C content of leaves of *T. testudinum* from the 30 sites had a narrow range, from 34.8% to 38% of dry weight. N and P contents were more variable, with N ranging from 1.50% to 2.33% of dry weigh (mean $1.95\% \pm 0.04\%$

Table 3
Thalassia testudinum morphology. Calculations were based on the means for the period of record at each of the 30 sites shown in Fig. 1.

	Mean	SD	Min	Max
<i>Thalassia testudinum</i> morphology				
Leaf width (mm)	8.5	0.9	6.6	10.6
Leaf length (mm)	145.3	46.9	86.4	291.7
# Leaves/short shoot	3.0	0.4	2.4	3.6
Leaf area/short shoot (cm ²)	39.1	18.8	16.2	90.8
Leaf mass/short shoot (mg)	179.8	93.7	70.4	443.5
Leaf mass/leaf area (mg/cm ²)	4.6	0.4	3.8	5.2
<i>Epiphyte community</i>				
Epiphyte load (mg cm ² of seagrass leaf)	2.2	1.1	0.6	6.1
Epiphyte Chl- <i>a</i> load (μg cm ² of seagrass leaf)	0.3	0.1	0.1	0.6
Epiphyte autotrophic index (μg Chl- <i>a</i> (mg epiphyte) ⁻¹)	0.21	0.08	0.09	0.43
Epiphyte abundance (mg SS ⁻¹)	84.2	36.9	42.4	37.0
Epiphyte Chl- <i>a</i> abundance (μg SS ⁻¹)	11.2	5.0	4.7	26.1

Table 4
Correlations among the station mean morphology measures for *Thalassia testudinum*.

	Width	Length	Leaves/SS	Area/SS	Mass/SS	Leaf mass/area
Leaf width (mm)		0.352	0.394*	0.508**	0.505**	0.334
Leaf length (mm)	0.056		0.640**	0.963**	0.942**	0.324
# leaves/short shoot	0.031	>0.001		0.777**	0.801**	0.580**
Leaf area/short shoot (cm ²)	0.004	>0.001	>0.001		0.988**	0.426
Leaf mass/short shoot (mg)	0.004	>0.001	>0.001	>0.001		0.547**
Leaf mass/leaf area (mg/cm ²)	0.071	0.081	0.001	0.019	0.002	

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

(±SE) and P ranging from 0.080% to 0.166% of dry weight with a mean of 0.125% ± 0.004%. Because of the variability in N and P content, C:N, C:P and N:P ratios were variable, with C:N ranging from 17.6 to 28.5 (mean 22.7 ± 0.5), C:P ranging from 550.4 to 1253.0

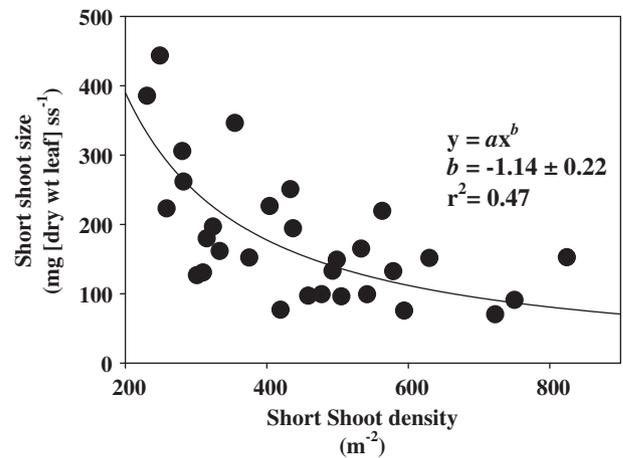


Fig. 6. Relationship between the mean size of short shoots of *Thalassia testudinum* at a station and the density of the short shoots. Solid line is the least-squares fit of the model to the data.

(mean 818.0 ± 32.6) and N:P ranging from 24.1 to 56.2 (mean 36.5 ± 1.5).

The median concentration of TN in the water column, and the mean concentrations of N in both sediments and seagrass leaf tissue at the 30 sites were all significantly and positively correlated (Pearson's $r > 0.655$, $p < 0.001$ for all comparisons). Similarly, sediment and seagrass leaf tissue P concentrations were correlated, but neither measure of P availability in the benthic system was significantly correlated to the median concentration of P in the water column (Fig. 8).

Site mean seagrass morphology was significantly related to the N content of seagrass leaf tissue (linear regression, $p < 0.001$, Fig. 9), but not the P content ($p = 0.233$). Larger, more robust short shoots of *T. testudinum* had higher N content than smaller short shoots. On the other had, the thickness of seagrass leaves, as assessed by the mass of seagrass tissue per area of seagrass tissue, was not significantly related to N content (linear regression, $p = 0.269$, and was negatively related to the P content of seagrass leaf tissue ($p = 0.050$, Fig. 9).

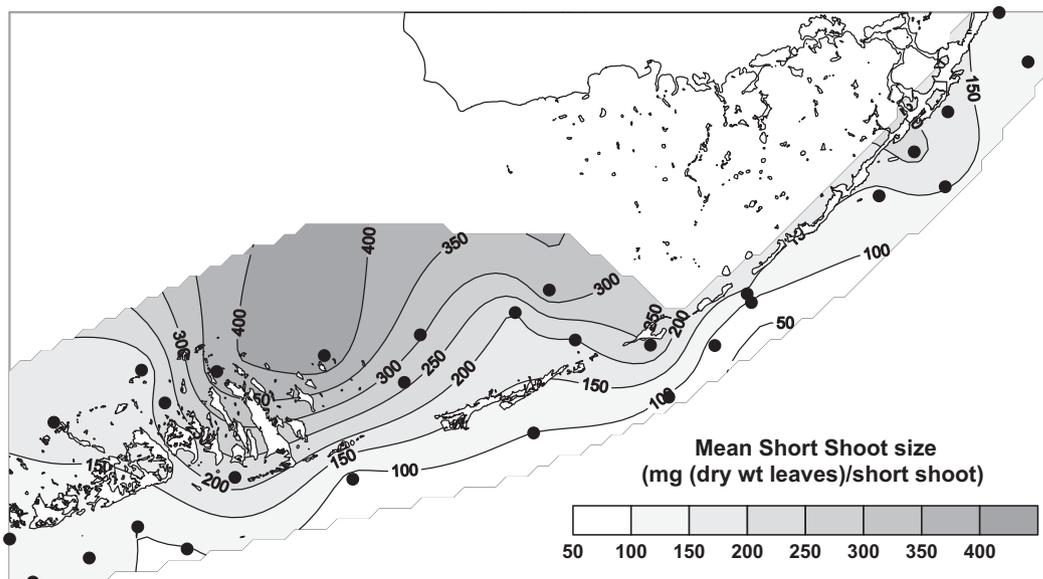


Fig. 5. Station mean size of short shoots of the seagrass *Thalassia testudinum*, at the thirty stations.

The mean epiphyte load on seagrass blades varied by an order of magnitude across the 30 sites (Table 3, Fig. 10). The lowest mean load was 0.6 mg (dry weight of epiphytes) cm⁻² of seagrass leaf, and the highest was 6.1 mg cm⁻²; the mean of the station means was 2.2 ± 1.1 mg cm⁻² (±1 SD). Epiphyte loads were higher on the Atlantic Ocean side of the Florida Keys than on the Gulf of Mexico

side (Fig. 10a). Because of a significant correlation between station-mean epiphyte load and epiphyte Chl-a load (Table 5), the spatial pattern in the mean load of epiphytic Chl-a was similar (Fig. 10b). However, because seagrass shoot size was largest in the gulf of Mexico and smallest in the back-reef environment, the pattern in epiphyte abundance (i.e. the mass of epiphytes per

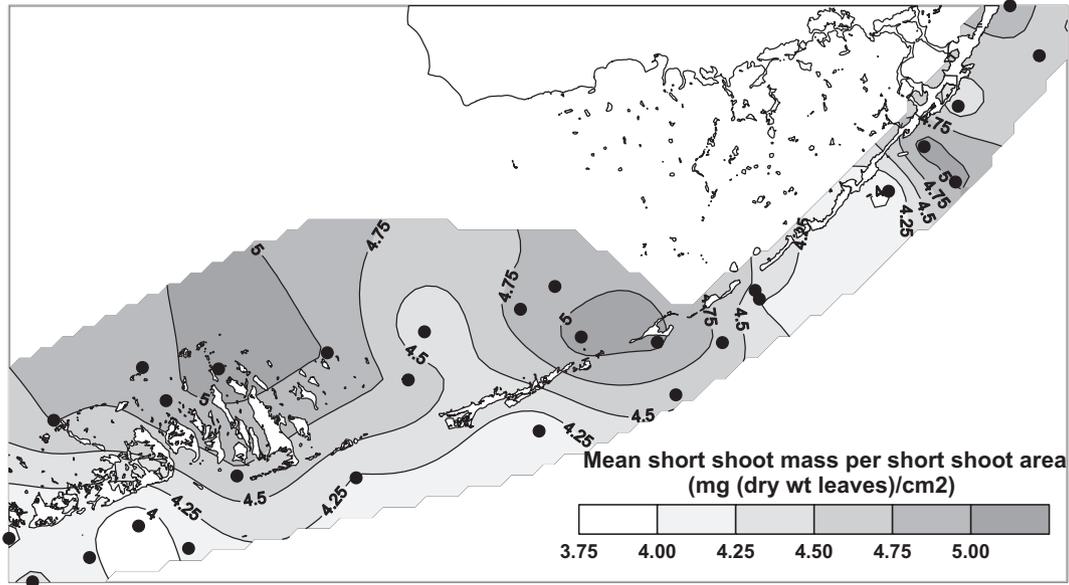


Fig. 7. Station mean mass of seagrass leaves per unit area of seagrass leaf for the seagrass *Thalassia testudinum*, at the thirty stations.

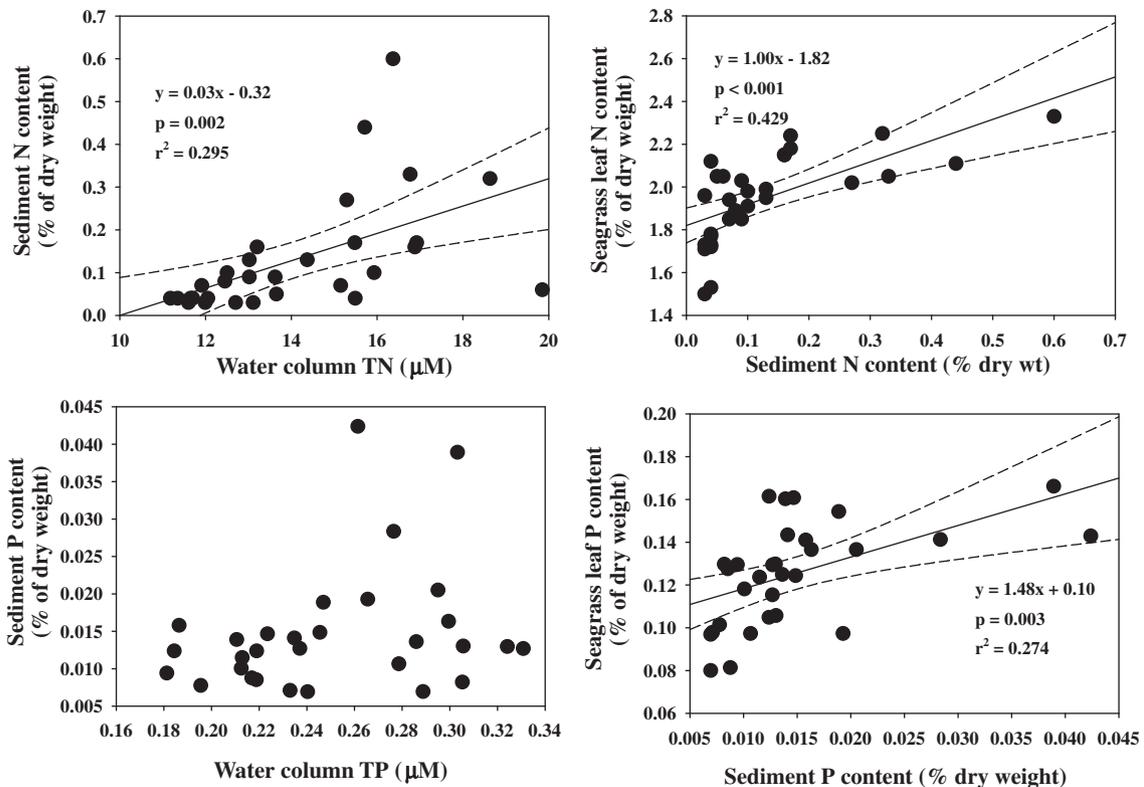


Fig. 8. Relationships between measures of nutrient availability. Points are the means of the period of record at each of the 30 sites. Lines are significant linear regressions with 95% confidence intervals. Upper left: Sediment N concentrations are positively correlated with median water column TN concentrations. Lower left: Sediment P concentrations are not significantly correlated with median water column TP concentrations. Upper right: The N content of *Thalassia testudinum* leaves is positively correlated with sediment N content. Lower right: The P content of *Thalassia testudinum* leaves is positively correlated with sediment P content.

seagrass short shoot) showed a maximum in the protected waters of the lower Florida Keys and in the back-reef environment of the upper Florida Keys (Fig. 10c). Interestingly, the AI tended to be lowest in the offshore sites with the highest mean epiphyte load, and highest in the Gulf of Mexico side of the keys where total and epiphyte Chl-a loads were lowest (Table 5, Fig. 10d).

The mean epiphyte load across stations varied seasonally (Fig. 11). A seasonal sine model fit the data of the mean epiphyte load (mg (dry weight of epiphytes) cm⁻²) at each sampling interval fairly well, describing 32% of the variance in the data. The mean load for the entire dataset was 2.02 mg cm⁻², with a 95% confidence interval of 1.72–2.32. The amplitude of the sine wave was 0.67 mg cm⁻², indicating that, on average, there was 1.34 mg cm⁻² difference between seasonal minima and maxima. The timing of the sine wave indicated that peak epiphyte loads occurred at 0.04 + π/2, or on the 91st day of the year (April 2) and minimum epiphyte loads occur on the 273rd day of the year (October 4). In contrast, we detected no seasonality in either the epiphyte Chl-a

Table 5

Correlations among the station mean measures of epiphyte community abundance.

	Epiphyte load	Chl-a load	Epiphyte abundance	Chl-a abundance	AI
Epiphyte load		0.684**	0.606**	0.176	–.525**
Epiphyte Chl-a load	<0.001		0.459*	0.565**	–0.057
Epiphyte abundance	<0.001	0.011		0.726	–0.104
Epiphyte Chl-a abundance	0.352	0.001	<0.001		0.433*
Epiphyte autotrophic index	0.003	0.767	0.583	0.017	

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

load or the epiphyte autotrophic index (the amplitude of the sine function fit was not significantly different than zero in each case).

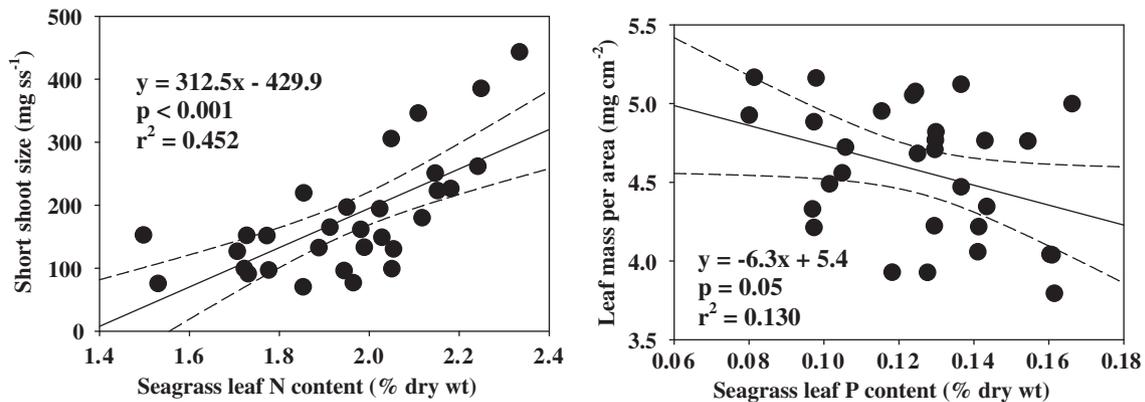


Fig. 9. Left: Mean shoot size is positively correlated with the N content of leaves of *Thalassia testudinum* at the 30 sites. Right: Mean mass per unit area of leaves of *Thalassia testudinum* is negatively correlated with leaf P content of at the 30 sites. Lines are significant linear regressions with 95% confidence intervals.

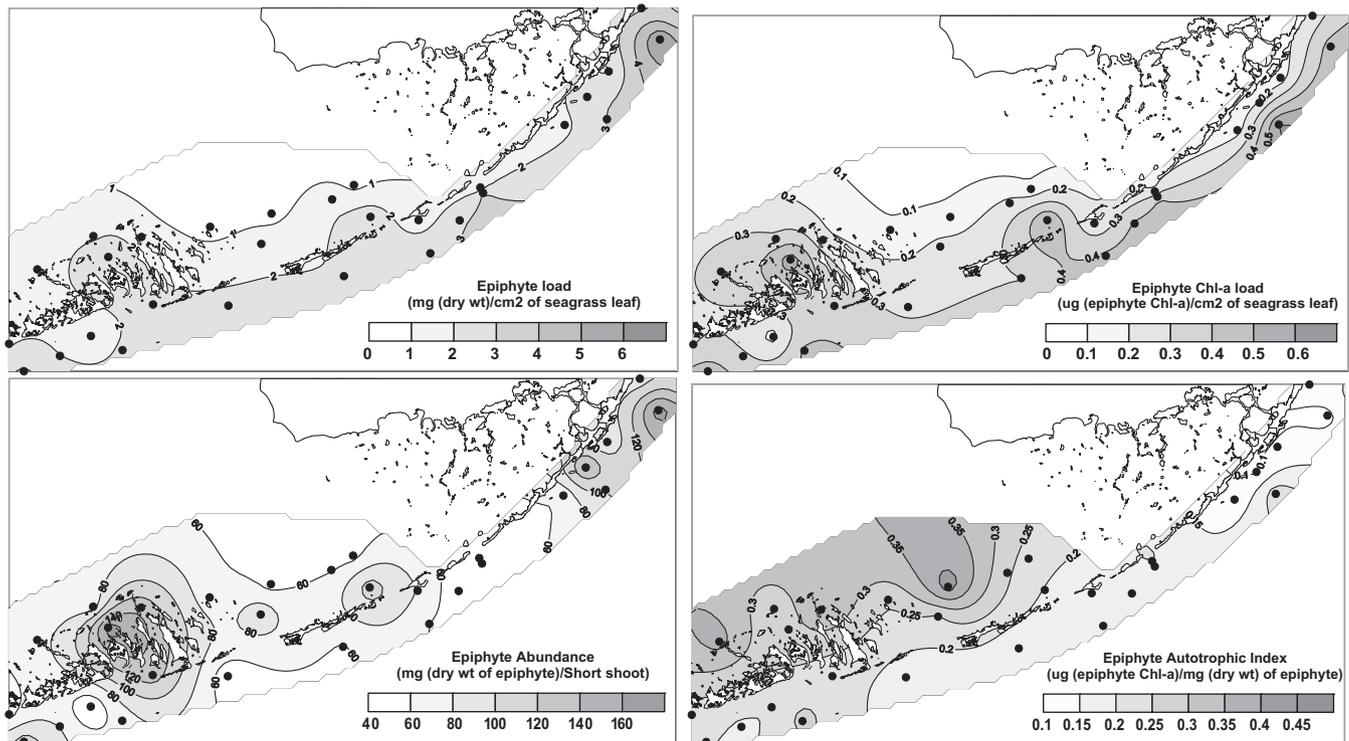


Fig. 10. Isopleth maps of the mean values of epiphyte load, epiphyte chlorophyll-a (Chl-a) load, epiphyte abundance and epiphyte Chl-a abundance at the thirty stations.

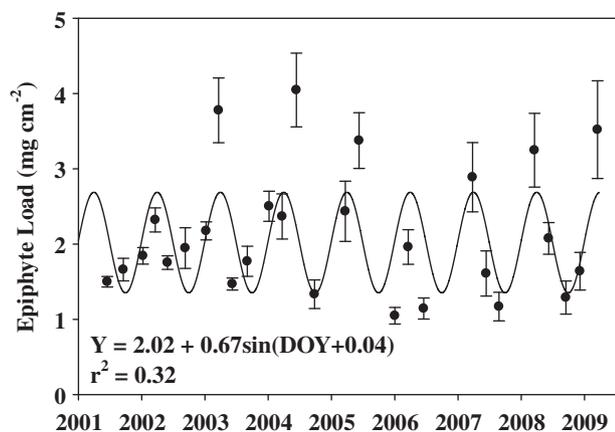


Fig. 11. Seasonality of the epiphyte load on leaves of the seagrass *Thalassia testudinum* in the study area. Points are the mean (\pm SE) of all stations for each sampling period. The line represents the least-squares fit of the seasonal sine model.

Because station-average epiphyte load, epiphyte Chl-*a* load and epiphyte abundance were all significantly correlated (Table 5), we used the epiphyte load as a proxy for these variables when examining the relationships between water column, sediment, seagrass morphology and seagrass nutrient content indicators of nutrient availability across the study area. We separately examined the relationship of the epiphyte autotrophic index to these other nutrient availability indicators. The mean epiphyte load at a station was significantly, but negatively, related to the median water column TN concentration, the mean sediment N content, and the mean N content of seagrass leaves (Fig. 12), contrary to the positive relationships expected if N availability were controlling the epiphyte load. In contrast, mean epiphyte load was not related to the median water column TP concentration nor the mean P content of seagrass leaves. There was, however, a significant but inverse relationship between sediment P content and epiphyte load (Fig. 12). We found no significant relationships between the mass per area of seagrass leaf and water column, sediment or seagrass leaf N and P concentrations (linear regression, $p > 0.05$ in all cases). Further, we found that larger seagrass shoots had lower epiphyte loads than smaller shoots (Fig. 13). And, despite the lack of significant correlation between epiphyte autotrophic index and epiphyte load (Table 5), the same significant linear relationships, with the same sign for the slopes, found between nutrient availability and epiphyte load were also found between autotrophic index and nutrient availability (relationships not shown).

5. Discussion

Water column, sediment and seagrass indicators of nutrient availability generally told the same story, of relatively low N availability offshore and low P availability nearshore across our study area, as has been documented previously for the water column (Boyer and Jones, 2002), sediments (Szmant and Forrester, 1996) and seagrass nutrient content (Fourqurean et al., 2005). Despite these well documented gradients in nutrient availability that control the abundance and species composition of seagrasses in our study area (Fourqurean et al., 1995; Fourqurean and Zieman 2002; Ferdie and Fourqurean 2004), and the importance of nutrient availability in controlling abundance and composition of epiphytes on seagrasses in other locations (e.g. Borum, 1985; Cambridge et al., 2007; Balata et al., 2008), we did not find that epiphyte load on the dominant seagrass, *T. testudinum*, or the relative contribution of algal epiphytes to the epiphyte community at our study

sites, was positively correlated with nutrient concentrations in the water column or the sediment. This suggests that, at least in the relatively low-nutrient waters surrounding the Florida Keys, epiphyte load is a poor indicator of nutrient availability. This lack of a relationship may mean that the gradient we observed in nutrient availability was all below some threshold value for stimulating epiphyte communities, or that controls other than nutrient availability are important determinants of epiphyte load in this area. In fact, the intra-annual differences in epiphyte load at a site (Fig. 11) are of the same order of magnitude as the variation in the mean epiphyte load across our study area. Further, the abundance of microphytobenthos, as indicated by Chl-*a* concentration in the sediments, was not directly correlated with concentrations of nutrients in the sediments. Our results suggest that epiphyte and microphytobenthos abundance are not unambiguous indicators of nutrient availability in relatively pristine seagrass environments, and therefore would make poor candidates for indicators of the status and trends of seagrass ecosystems in relatively low-nutrient environments like the Florida Keys.

The lack of a positive relationship between epiphyte loads and nutrient availability we found is not unprecedented, despite the observations that epiphyte loads are often elevated in very eutrophic environments (e.g. Tomasko and Lapointe, 1991). Within 15 m of a point source of nutrients in Florida Bay, epiphyte loads are much higher than found across the rest of the bay, but across the bay as a whole the relationship between epiphyte loads and nutrient availability explains only 14% of the variation in epiphyte loads (Frankovich and Fourqurean, 1997). Similarly, epiphyte loads on *Posidonia oceanica* in the Mediterranean are not clear indicators of nutrient availability. Neither Terrados and Pons (2008) nor Piazzzi et al. (2004) found differences in epiphyte loads between control and anthropogenically eutrophied *P. oceanica* meadows. In contrast, other authors do report increased epiphyte loads in areas near urban shores (e.g. Balata et al., 2008). Moreover, decreases, rather than the expected increases, in epiphyte loads have been documented in areas affected by fish farm nutrient loading (Ruiz et al., 2001). The lack of response of epiphytes to gradients in nutrient concentration, as we have shown here, or to experimental nutrient loading, may be an expected consequence of the relative growth rate of epiphytes relative to seagrasses. Because macroalgal and microalgal relative growth rates are so much faster than those of seagrasses, only increases in availability above some threshold defined by the inability of slow-growing seagrasses to utilize the supply would likely stimulate increased biomass of the faster-growing algal competitors. Clearly, epiphyte loads are not effective indicators of nutrient availability in oligotrophic seagrass meadows; factors other than nutrient availability must also play large roles in controlling epiphyte abundance.

Given that epiphyte loads are determined by both bottom-up forces that affect rates of production and top-down forces that control rates of removal of epiphyte biomass, it is likely that, at least for the range of nutrient availability that we encountered in the FKNMS, top-down forces may obscure any influence of nutrient availability on the epiphyte community. Grazing of epiphytes has been shown many times to be as important, or more important, than nutrient supply in determining epiphyte loads (e.g. Hootsman and Vermaat, 1985; Neckles et al., 1993; Williams and Ruckelshaus, 1993; Short et al., 1995; Heck et al., 2000). However, in oligotrophic environments, epiphyte grazers may have a limited impact on epiphyte biomass but a major impact on the community composition of the epiphyte community (Keuskamp, 2004).

The ranges in nutrient concentrations in the water column and the spatial patterns in water quality we report are similar to the patterns described previously by Szmant and Forrester (1996) and Boyer and Jones (2002). In the upper Florida Keys, P is supplied to coastal habitats largely by upwelling of deep P-rich water from

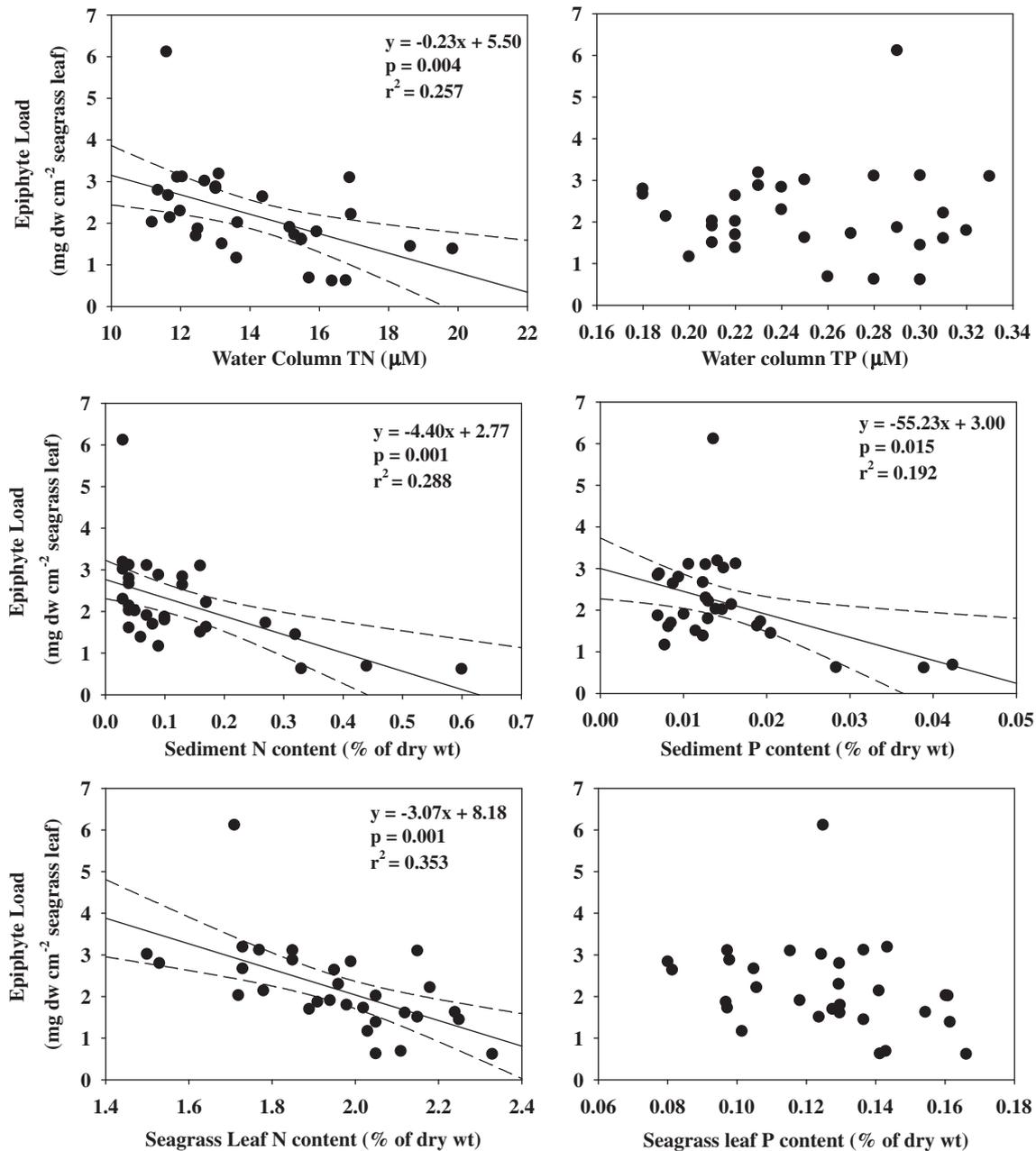


Fig. 12. Station mean epiphyte load as a function of station median water column concentrations of N and P; station mean sediment concentrations of N and P; and station mean N and P content of leaves of *Thalassia testudinum*. Lines are significant linear regressions with 95% confidence intervals.

the Florida Straits (Leichter et al., 1996), and in the lower Keys, P supply comes from relatively high-P water from the Gulf of Mexico (Boyer and Jones, 2002). In contrast, N is likely supplied by terrestrial runoff, and is found in higher concentrations in nearshore than offshore waters. Hence, N:P ratios in the water column tend to be relatively high close to shore and on the Gulf of Mexico side of the Florida Keys, and lower offshore. This pattern is also present in sediments of the region: N concentrations tend to decrease from onshore to offshore, while P concentrations increase from onshore to offshore; hence the N:P of sediments has been shown to decrease with distance offshore (Szmant and Forrester, 1996; Fig. 3). Seagrass leaf N:P follows this same regional pattern, prompting Fourqurean and Zieman (2002) to hypothesize that seagrass primary productivity in the region is limited by P in Florida Bay and close to shore in the Florida Keys, and limited by N further offshore. Experimental tests of this hypothesis have largely borne

out these stoichiometric predictions (Ferdie and Fourqurean, 2004; Armitage et al., 2005). Further, these experiments have shown that epiphyte abundance increases in Florida Bay in response to P addition (Armitage et al., 2005) and that the autotrophic index of the epiphyte community increases in response to N addition offshore (Ferdie and Fourqurean, 2004).

Epiphyte loads reported here are very similar to the values reported elsewhere for oligotrophic areas in the Gulf of Mexico and the Caribbean. In Florida Bay, to the north and east of our study site, Frankovich and Fourqurean (1997) found a median epiphyte load of 376 mg (dry weight of epiphytes) g⁻¹ (dry weight of seagrass leaves), with a range of 23–1569 mg g⁻¹. Our median epiphyte load in these units was 393.7 mg g⁻¹. Across the Veracruz Reef System in Mexico, epiphyte loads are 310–560 mg g⁻¹ and increase as distance from shore increases (Terrados et al., 2008). In more eutrophic locations, epiphyte abundances can be much higher.

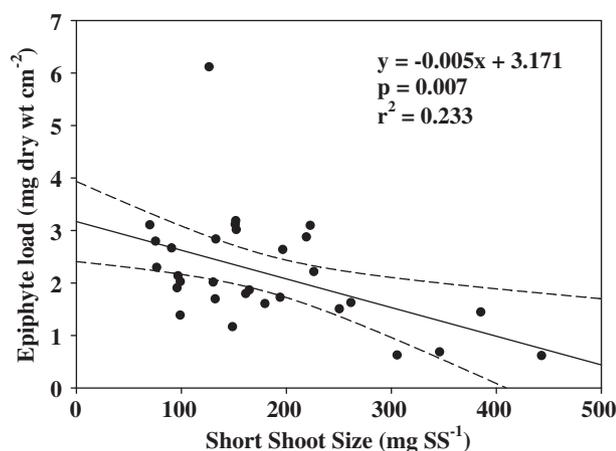


Fig. 13. Station mean epiphyte load decreases as the size of short shoots of the seagrass *Thalassia testudinum* increase. Lines are significant linear regression with 95% confidence intervals.

Tomasko et al. (1996) report values as high as 3500 mg g⁻¹ from Sarasota Bay, Florida, which receives high N loading from the watershed. At one site in the lower Florida Keys deemed to have been impacted by sewage effluent from nearby septic tanks, total epiphyte loads were ca. 1000 mg g⁻¹ (Tomasko and Lapointe, 1991). There can also be episodic blooms of epiphytes in the FKNMS; Lapointe et al. (2004) report a summertime 1996 (before our period of record) peak in epiphyte load of greater than 3500 mg g⁻¹ at Looe Key, and offshore reef near our site 267 (Fig. 1). This bloom was dominated by the brown algae *Cladosiphon occidentalis* and was attributed to a transient spike in ammonium concentrations (Lapointe et al., 2004). It seems clear that epiphyte loads do respond to nutrient availability, but the lack of positive relationships between epiphyte load and water quality in the generally oligotrophic conditions normal in the FKNMS (Fig. 12), and the weak relationship found for Florida Bay (Frankovich and Fourqurean, 1997) suggest that at low-nutrient availability, epiphyte load is insensitive to relatively small changes in nutrient availability.

We did not assess the relative dominance of different components of the epiphyte community and whether the community changed in response to nutrient availability. The epiphyte community in south Florida is composed of representatives from many algal groups, cyanobacteria, bacteria, and sessile and motile animals, including Annelids, Bryozoans, Chordates, Hydrozoans, and Mollusks (Frankovich and Fourqurean, 1997). One might expect autotrophs to increase in relative importance in areas with relatively high nutrient availability. Some fertilization experiments have shown shifts in relative dominance among algal species, with a shift towards green macroalgae (Armitage et al., 2006; Cambridge et al., 2007) and cyanobacteria (Armitage et al., 2006) under nutrient enrichment. Within algal groups, shifts in species also follow nutrient addition: Frankovich et al. (2009) documented shifts in the relative abundance of diatoms with nutrient enrichment. In one experimental fertilization, the AI of the epiphyte community (the mass of Chl-*a* (µg) per dry mass of epiphytes in mg) increased in response to fertilization (Ferdie and Fourqurean, 2004), but AI is not related to nutrient availability in nearby Florida Bay (Frankovich and Fourqurean, 1997). Similarly, we did not find AI to be positively correlated with concentrations of nutrients in the water column or sediment; in fact, as N availability increased, the AI decreased. Given that relative abundance of different components of the epiphyte community shift with eutrophication, and that seagrass loss often follows eutrophication, changes in relative dominance of different macroalgal and microalgal groups may prove to be an indicator of nutrient status of seagrasses. However, Cam-

bridge et al. (2007) found that shifts in epiphyte communities so closely preceded seagrass loss as to make the shift in species abundance an impractical early warning sign for eutrophication of seagrass beds.

Unlike for *P. oceanica* meadows in the Mediterranean, which have maximum epiphyte loads in late summer (Romero, 1988; Terados and Pons, 2008), the epiphyte load on *T. testudinum* in south Florida peaked in late winter/early spring. The growth rate of leaves of *T. testudinum* in the study area is markedly seasonal, with maximal leaf growth in August and minimal growth in February, with a difference in growth rates from about 12 mg g⁻¹d⁻¹ in winter to 22 mg g⁻¹d⁻¹ in August (Fourqurean et al., 2001). This difference in growth rates translates to a difference in the turnover of leaf tissue, which is the substrate for the mostly sessile epiphyte community. As leaf turnover increases, old blades are shed more quickly, reducing the epiphyte load on the seagrass shoot. It has been hypothesized that shedding of old leaves is an evolutionary adaptation to limit the shading effects of epiphytes on submerged macrophytes (Littler and Littler, 1999). We did observe that epiphyte loads in spring, following the period of lowest leaf growth rate and turnover, were roughly two times higher than the loads in fall, following the season of more rapid leaf growth (Fig. 11). However, recent experimental manipulation of the turnover rate of artificial seagrass blades shows that a doubling of seagrass blade turnover results in a reduction of only 20% in the epiphyte load (Peterson et al., 2007), suggesting that factors other than turnover of substrate contribute to the seasonality in epiphyte load. Differences in leaf turnover rate have been found to be a relatively minor contribution to differences in epiphyte load on seagrasses compared to other factors like herbivory (Borum, 1987; Frankovich and Zieman, 2005a) or nutrient availability (Tomasko and Lapointe, 1991; Frankovich and Zieman, 2005a). We found no regular seasonality in N or P concentrations in the water column across the FKNMS, suggesting that seasonal variability in epiphyte loads were not being driven by seasonality in nutrient availability. Perhaps there are significant seasonal patterns in the grazing rates of epiphytes or in the relative growth rates of seagrass substrate and epiphytes that lead to our observed seasonality.

The epiphyte loads we measured in the FKNMS have a quantifiable, but relatively minor, impact on the amount of light reaching the seagrass leaves. Applying the relationships between epiphyte load and transmission of light through the epiphyte community presented in Frankovich and Zieman (2005b) to the station-mean epiphyte loads we measured, we estimate that as little as 61% of the light striking the epiphytized leaves penetrates the epiphyte community at site with the highest epiphyte load (site 216), and as much as 95% of the light penetrates the epiphyte community at our sites with the lowest average load (sites 284, 291). Owing to the seasonality in the mean epiphyte loads (Fig. 11), there is also seasonality to the mean attenuation of light by the epiphyte community, with an average of 75% of the light penetrating the epiphyte community during the wintertime peaks in epiphyte load and ca. 90% of light penetrating the epiphyte community in the low-load summer period. The coincidence of high epiphyte loads, and therefore increased attenuation of light by the epiphyte community, with wintertime lows in solar radiation indicates that light stress in the winter may be more severe than in summer for seagrasses in our region.

In addition to their role in attenuating light, epiphytes can also negatively affect their seagrass hosts by altering flow regimes and thereby influencing the diffusion of CO₂ and nutrients to seagrass leaves. They also increase the drag forces on seagrass blades and therefore the vulnerability of seagrasses to uprooting, and changing the seagrasses' susceptibility to grazing (see review by Michael et al. (2008)). Our documentation of both spatial (Fig. 10) and temporal (Fig. 11) variability in epiphyte load would suggest that these

influences on seagrass health will also vary in space and time. During winter, we expect that epiphyte influences on their seagrass hosts are maximal, so seagrasses should be more susceptible to CO₂ limitation, blocked foliar nutrient uptake, and dislodgement by hydrodynamic forces in areas that support high epiphyte loads.

We found sediment porosity alone among our measured parameters significantly, positively correlated with microphytobenthos biomass. Work along natural nutrient gradients and experimental fertilizations have shown biomass of microphytobenthos is often positively correlated with nutrient availability, both locally in south Florida (Ferdie and Fourqurean, 2004; Armitage et al., 2006; Herbert and Fourqurean, 2008) and elsewhere (e.g. Dizon and Yap, 1999; Hillebrand et al., 2000). The lack of relationship between microphytobenthos abundance and measures of nutrient availability suggest that factors such as sediment stability, bioturbation, grazing by infauna and deposit feeders, and light penetration through the seagrass canopy have as much influence on the biomass of microphytobenthos as nutrient availability at the regional scale.

Unlike measures of epiphyte load and microphytobenthos abundance, seagrass morphology and nutrient content were strongly related to measures of nutrient availability, suggesting these parameters could be sensitive indicators of nutrient status and trends in seagrass beds. It is well-established that the nutrient content of seagrass leaves reflects the availability of nutrients in the environment (Duarte, 1990; Fourqurean et al., 1992b). We found most measures of the size of short shoots of *T. testudinum* were correlated (Table 4), and that shoot size was positively correlated with N content, and negatively correlated with P content, of seagrass leaves (Fig. 9). It is interesting to note that epiphyte load decreased as a function of short shoot size (Fig. 13). Larger short shoots come from regions with higher N availability, yet they have smaller epiphyte loads than smaller shoots from areas with lower N availability. Perhaps this is a function of greater growth rates, and therefore faster turnover of seagrass leaf surface, of seagrass shoots from higher nutrient areas, as documented in Florida Bay (Herbert and Fourqurean, 2009).

The relationship between the shoot density and shoot size for *T. testudinum* from our 30 permanent sites (Fig. 6) approximates the 3/2 power law of self thinning for plants (Yoda et al., 1963; White and Harper, 1970), suggesting strong intraspecific competition for resources among seagrass short shoots. Given the variability in the amount of light reaching the bottom at these sites, and the marked gradients in the availability of N and P across the study area (Fourqurean and Zieman, 2002), it is likely that resource availability varied substantially among sites. Shoot size was positively correlated with N availability and negatively correlated with P availability (Fig. 9), so large, widely spaced shoots were found in areas of high N availability and smaller, more closely placed shoots were found in areas of high P availability. Whether the relationship in Fig. 6 is truly an example of self thinning of a population of intensely competing shoots, or is driven by other environmental factors, remains to be determined.

It has been proposed that the mass per unit area of seagrasses is an indicator of nutrient pollution (Lee et al., 2004). Nutrient-replete, fast-growing seagrass shoots have less mass per unit area than slow growing, nutrient-depleted shoots. The mass per unit area of leaves of *Zostera marina* in New England, USA varies between ca. 2 and 7 mg cm⁻², and this value was negatively correlated with N content of leaves in this N-limited ecosystem. While we found no relationship between seagrass leaf mass per area and N content, we did find a significant, albeit weak, relationship between mass per unit area and P content of *T. testudinum* leaves, with mass per area decreasing as P content increased (Fig. 9). Given the important role that P plays in limiting plant biomass over much of our study area (Fourqurean and Zieman, 2002; Fourqurean et al.,

2005), it is not surprising that we found mass per unit area responded to P availability. The relationship we found explained only 13% of the variation in the mass per unit, but our results support Lee et al.'s (2004) suggestion that mass per unit area may be an indicator of nutrient availability for seagrasses.

Despite the importance of nutrient availability in controlling the biomass of epiphytes and microphytobenthos in seagrass ecosystems demonstrated by field and experimental studies, we found that biomass of these groups of organisms to be insensitive to differences in nutrient availability in the FKNMS, a relatively oligotrophic coastal region. Because of this, it does not seem that epiphyte or microphytobenthos abundance would make good monitoring parameters in a program designed to assess the status and trends of seagrass ecosystems in response to nutrient loading in oligotrophic systems like the Florida Keys. Observations have been made of very high epiphyte loads in very eutrophic sites, but we contend that epiphyte and microphytobenthos abundance is insensitive to the changes that would occur at the beginning of nutrient loading. Similarly, Cambridge et al. (2007) concluded that changes in epiphyte community structure occur too late in the eutrophication process to provide adequate warning of impending collapse of seagrass ecosystems while there is still time to act to prevent the collapse. Conversely, we found that sediment nutrient content is related to water column nutrient concentration, seagrass leaf nutrient content is an indicator of concentrations of nutrients in the water column and sediments, seagrass shoot morphology changes in response to nutrient availability, and the mass per unit area of seagrass leaves decreases as nutrient availability increases. Each of these parameters shows promise as an indicator of ecosystem nutrient status in oligotrophic seagrass meadows. We propose that nutrient content, biomass and morphology of species with low relative growth rates, like seagrasses, are sensitive to changes in nutrient availability in oligotrophic conditions, and not until some tipping point, defined by a nutrient availability high enough to satiate the needs of slow-growing species, will faster-growing species like epiphytes and microphytobenthos respond to increases in nutrient availability.

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References

- Armitage, A.R., Frankovich, T.A., Fourqurean, J.W., 2006. Variable responses within epiphytic and benthic microalgal communities to nutrient enrichment. *Hydrobiologia* 569, 423–435.
- Armitage, A.R., Frankovich, T.A., Heck, K.L.J., Fourqurean, J.W., 2005. Experimental nutrient enrichment causes complex changes in seagrass, microalgae, and macroalgae community structure in Florida Bay. *Estuaries* 28, 422–434.

- Balata, D., Bertocci, I., Piazzini, L., Nesti, U., 2008. Comparison between epiphyte assemblages of leaves and rhizomes of the seagrass *Posidonia oceanica* subjected to different levels of anthropogenic eutrophication. *Estuarine, Coastal and Shelf Science* 79, 533–540.
- Borowitzka, M.A., Lavery, P.S., van Keulen, M., 2006. Epiphytes of seagrasses. In: Larkum, A.W.D., Orth, R.J., Duarte, C. (Eds.), *Seagrasses: Biology, Ecology and Conservation*. Springer, Dordrecht, pp. 441–461.
- Borum, J., 1985. Development of epiphytic communities on eelgrass (*Zostera marina*) along a nutrient gradient in a Danish estuary. *Marine Biology* 87, 211–218.
- Borum, J., 1987. Dynamics of epiphyton on eelgrass (*Zostera marina* L.) leaves: relative roles of algal growth, herbivory, and substratum turnover. *Limnology and Oceanography* 32, 869–992.
- Boyer, J.N., Jones, R.D., 2002. A view from the bridge: external and internal forces affecting the ambient water quality of the Florida Keys National Marine Sanctuary. In: Porter, J.W., Porter, K.G. (Eds.), *The Everglades, Florida Bay, and Coral Reefs of the Florida Keys*. CRC Press, Boca Raton, pp. 609–628.
- Burkholder, J.M., Tomasko, D.A., Touchette, B.W., 2007. Seagrasses and eutrophication. *Journal of Experimental Marine Biology and Ecology* 350, 46–72.
- Cambridge, M.L., Chiffings, A.W., Brittan, C., Moore, L., McComb, A.J., 1986. The loss of seagrass in Cockburn Sound, Western Australia. II. Possible causes of seagrass decline. *Aquatic Botany* 24, 269–285.
- Cambridge, M.L., How, J.R., Lavery, P.S., Vanderklift, M.A., 2007. Retrospective analysis of epiphyte assemblages in relation to seagrass loss in a eutrophic coastal embayment. *Marine Ecology Progress Series* 346, 97–107.
- Dizon, R.M., Yap, H.T., 1999. Short-term responses of coral reef microphytobenthic communities to inorganic nutrient loading. *Limnology and Oceanography* 44, 1259–1267.
- Duarte, C.M., 1990. Seagrass nutrient content. *Marine Ecology Progress Series* 67, 201–207.
- Duarte, C.M., 1995. Submerged aquatic vegetation in relation to different nutrient regimes. *Ophelia* 41, 87–112.
- Ferdie, M., Fourqurean, J.W., 2004. Responses of seagrass communities to fertilization along a gradient of relative availability of nitrogen and phosphorus in a carbonate environment. *Limnology and Oceanography* 49, 2082–2094.
- Fourqurean, J.W., Durako, M.J., Hall, M.O., Hefty, L.N., 2002. Seagrass distribution in south Florida: a multi-agency coordinated monitoring program. In: Porter, J.W., Porter, K.G. (Eds.), *The Everglades, Florida Bay, and the Coral Reefs of the Florida Keys*. CRC Press, Boca Raton, pp. 497–522.
- Fourqurean, J.W., Escorcia, S.P., Anderson, W.T., Zieman, J.C., 2005. Spatial and seasonal variability in elemental content, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ of *Thalassia testudinum* from south Florida and its implications for ecosystem studies. *Estuaries* 28, 447–461.
- Fourqurean, J.W., Jones, R.D., Zieman, J.C., 1993. Processes influencing water column nutrient characteristics and phosphorus limitation of phytoplankton biomass in Florida Bay, FL, USA: inferences from spatial distributions. *Estuarine, Coastal and Shelf Science* 36, 295–314.
- Fourqurean, J.W., Powell, G.V.N., Kenworthy, W.J., Zieman, J.C., 1995. The effects of long-term manipulation of nutrient supply on competition between the seagrasses *Thalassia testudinum* and *Halodule wrightii* in Florida Bay. *Oikos* 72, 349–358.
- Fourqurean, J.W., Willsie, A.W., Rose, C.D., Rutten, L.M., 2001. Spatial and temporal pattern in seagrass community composition and productivity in south Florida. *Marine Biology* 138, 341–354.
- Fourqurean, J.W., Zieman, J.C., 2002. Seagrass nutrient content reveals regional patterns of relative availability of nitrogen and phosphorus in the Florida Keys, USA. *Biogeochemistry* 61, 229–245.
- Fourqurean, J.W., Zieman, J.C., Powell, G.V.N., 1992a. Phosphorus limitation of primary production in Florida Bay: evidence from the C:N:P ratios of the dominant seagrass *Thalassia testudinum*. *Limnology and Oceanography* 37, 162–171.
- Fourqurean, J.W., Zieman, J.C., Powell, G.V.N., 1992b. Relationships between porewater nutrients and seagrasses in a subtropical carbonate environment. *Marine Biology* 114, 57–65.
- Frankovich, T.A., Armitage, A.R., Wachnicka, A., Gaiser, E.E., Fourqurean, J.W., 2009. Nutrient effects on seagrass epiphyte community structure in Florida Bay. *Journal of Phycology* 45, 1010–1020.
- Frankovich, T.A., Fourqurean, J.W., 1997. Seagrass epiphyte loads along a nutrient availability gradient, Florida Bay, USA. *Marine Ecology Progress Series* 159, 37–50.
- Frankovich, T.A., Zieman, J.C., 2005a. A temporal investigation of grazer dynamics, nutrients, seagrass leaf productivity, and epiphyte standing stock. *Estuaries* 28, 41–52.
- Frankovich, T.A., Zieman, J.C., 2005b. Periphyton light transmission relationships in Florida Bay and the Florida Keys, USA. *Aquatic Botany* 83, 14–30.
- Heck Jr., K.L., Pennock, J.R., Valentine, J.F., Coen, L.D., Sklenar, S.A., 2000. Effects of nutrient enrichment and small predator density on seagrass ecosystems: an experimental assessment. *Limnology and Oceanography* 45, 1041–1057.
- Herbert, D.A., Fourqurean, J.W., 2008. Ecosystem structure and function still altered two decades after short-term fertilization of a seagrass meadow. *Ecosystems* 11, 688–700.
- Herbert, D.A., Fourqurean, J.W., 2009. Phosphorus availability and salinity control productivity and demography of the seagrass *Thalassia testudinum* in Florida Bay. *Estuaries and Coasts* 32, 188–201.
- Hillebrand, H., Worm, B., Lotze, H.K., 2000. Marine microbenthic community structure regulated by nitrogen loading and grazing pressure. *Marine Ecology Progress Series* 204, 27–38.
- Hootsman, M.J.M., Vermaat, J.E., 1985. The effects of periphyton-grazing by three epifaunal species on the growth of *Zostera marina* L. under experimental conditions. *Aquatic Botany* 22, 83–88.
- Keuskamp, D., 2004. Limited effects of grazer exclusion on the epiphytes of *Posidonia sinuosa* in South Australia. *Aquatic Botany* 78, 3–14.
- Lapointe, B.E., Barile, P.J., Matzie, W.R., 2004. Anthropogenic nutrient enrichment of seagrass and coral reef communities in the lower Florida Keys: discrimination of local versus regional nitrogen sources. *Journal of Experimental Marine Biology and Ecology* 308, 23–58.
- Lee, K.S., Short, F.T., Burdick, D.M., 2004. Development of a nutrient pollution indicator using the seagrass, *Zostera marina*, along nutrient gradients in three New England estuaries. *Aquatic Botany* 78, 197–216.
- Leichter, J.J., Wing, S.R., Miller, S.L., Denny, M.W., 1996. Pulsed delivery of subthermocline water to Conch Reef (Florida Keys) by internal tidal bores. *Limnology and Oceanography* 41, 1490–1501.
- Littler, M.M., Littler, D.S., 1999. Blade abandonment/proliferation: a novel mechanism for rapid epiphyte control in marine macrophytes. *Ecology* 80, 1736–1746.
- Michael, T.S., Shin, H.W., Hanna, R., Spafford, D.C., 2008. A review of epiphyte community development: surface interactions and settlement on seagrass. *Journal of Environmental Biology* 29, 629–638.
- Moncreiff, C.A., Sullivan, M.J., Daehnick, A.E., 1992. Primary production dynamics in seagrass beds of Mississippi Sound: the contributions of seagrass, epiphytic algae, sand microflora, and phytoplankton. *Marine Ecology Progress Series* 87, 161–171.
- Neckles, H.A., Koepfler, E.T., Haas, L.W., Wetzel, R.L., Orth, R.J., 1994. Dynamics of epiphytic photoautotrophs and heterotrophs in *Zostera marina* (Eelgrass) microcosms: responses to nutrient enrichment and grazing. *Estuaries* 17, 597–605.
- Neckles, H.A., Wetzel, R.L., Orth, R.J., 1993. Relative effects of nutrient enrichment and grazing on epiphyte-macrophyte (*Zostera marina* L.) dynamics. *Oecologia* 93, 285–295.
- Orth, R.J., Moore, K.A., 1983. Chesapeake Bay: an unprecedented decline in submerged aquatic vegetation. *Science* 222, 51–53.
- Peterson, B.J., Frankovich, T.A., Zieman, J.C., 2007. Response of seagrass epiphyte loading to field manipulations of fertilization, gastropod grazing and leaf turnover rates. *Journal of Experimental Marine Biology and Ecology* 349, 61–72.
- Piazzini, L., Balata, D., Cinelli, F., Benedetti-Cecchi, L., 2004. Patterns of spatial variability in epiphytes of *Posidonia oceanica*: differences between a disturbed and two reference locations. *Aquatic Botany* 79, 345–356.
- Romero, J., 1988. Epifitos de las hojas de *Posidonia oceanica* – variaciones estacionales y batimétricas de biomasa en la pradera de las Islas Medes (Girona). *Oecologia Aquatica* 9, 19–25.
- Ruiz, J.M., Pérez, M., Romero, J., 2001. Effects of fish farm loadings on seagrass (*Posidonia oceanica*) growth. *Marine Pollution Bulletin* 42, 749–760.
- Schreiber, R.A., Pennock, J.R., 1995. The relative contribution of benthic microalgae to total microalgal production in a shallow sub-tidal estuarine environment. *Ophelia* 42, 335–352.
- Short, F.T., Burdick, D.M., Kaldy III, J.E., 1995. Mesocosm experiments quantify the effects of eutrophication on eelgrass, *Zostera marina*. *Limnology and Oceanography* 40, 740–749.
- Silberstein, K., Chiffings, A.W., McComb, A.J., 1986. The loss of seagrass in Cockburn Sound, Western Australia. III. The effect of epiphytes on the productivity of *Posidonia australis* Hook. F. *Aquatic Botany* 24, 355–371.
- Strickland, J.D.H., Parsons, T.R., 1972. *A Practical Handbook of Seawater Analysis*, second ed. Bulletin 167. Fisheries Research Board of Canada, Ottawa, 310p.
- Szmant, A.M., Forrester, A., 1996. Water column and sediment nitrogen and phosphorus distribution patterns in the Florida Keys, USA. *Coral Reefs* 15, 21–41.
- Terrados, J., Pons, F.J.M., 2008. Epiphyte load on the seagrass *Posidonia oceanica* (L.) Delile does not indicate anthropogenic nutrient loading in Cabrera Archipelago National Park (Balearic Islands, Western Mediterranean). *Scientia Marina* 72, 503–510.
- Terrados, J., Ramírez-García, P., Hernández-Martínez, O., Pedraza, K., Quiroz, A., 2008. State of *Thalassia testudinum* Banks ex König meadows in the Veracruz Reef System, Veracruz, México. *Aquatic Botany* 88, 17–26.
- Tomasko, D.A., Dawes, C.J., Hall, M.O., 1996. The effects of anthropogenic nutrient enrichment on turtle grass (*Thalassia testudinum*) in Sarasota Bay, Florida. *Estuaries* 19, 448–456.
- Tomasko, D.A., Lapointe, B.E., 1991. Productivity and biomass of *Thalassia testudinum* as related to water column nutrient availability and epiphyte levels: field observations and experimental studies. *Marine Ecology Progress Series* 75, 9–17.
- White, J., Harper, J.L., 1970. Correlated changes in plant size and number in plant populations. *Journal of Ecology* 58, 467–468.
- Williams, S.L., Ruckelshaus, M.H., 1993. Effects of nitrogen availability and herbivory on eelgrass (*Zostera marina*) and epiphytes. *Ecology* 74, 904–918.
- Yoda, K., Kira, T., Ogawa, H., Hozumi, H., 1963. Self-thinning in overcrowded pure stands under cultivated and natural conditions. *Journal of Biology, Osaka City University* 14, 107–129.