Spatial and Seasonal Variability in Elemental Content, δ^{13}C, and δ^{15}N of *Thalassia testudinum* from South Florida and Its Implications for Ecosystem Studies

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ABSTRACT: Elemental and isotopic composition of leaves of the seagrass *Thalassia testudinum* was highly variable across the 10,000 km² and 8 years of this study. The data reported herein expand the reported range in carbon:nitrogen (C:N) and carbon:phosphorus (C:P) ratios and δ^{13}C and δ^{15}N values reported for this species worldwide; 13.2–38.6 for C:N and 411–2,041 for C:P. The 981 determinations in this study generated a range of −13.5‰ to −5.2‰ for δ^{13}C and −4.3‰ to 9.4‰ for δ^{15}N. The elemental and isotope ratios displayed marked seasonality, and the seasonal patterns could be described with a simple sine wave model. C:N, C:P, δ^{13}C, and δ^{15}N values all had maxima in the summer and minima in the winter. Spatial patterns in the summer maxima of these quantities suggest there are large differences in the relative availability of N and P across the study area and that there are differences in the processing and the isotopic composition of C and N. This work calls into question the interpretation of studies about nutrient cycling and food webs in estuaries based on few samples collected at one time, since we document natural variability greater than the signal often used to imply changes in the structure or function of ecosystems. The data and patterns presented in this paper make it clear that there is no threshold δ^{15}N value for marine plants that can be used as an unambiguous indicator of human sewage pollution without a thorough understanding of local temporal and spatial variability.

Introduction

Elemental and isotopic composition of macrophytes are increasingly being used as indicators of elemental supply and processing in marine ecosystems (McClelland and Valiela 1998b; Miller and Sluka 1999; Fourqurean and Zieman 2002). Macrophytes are fixed in place and continuously sample the environment around them; their chemical composition is to a large part determined by environmental conditions. The ratios of elements in macrophyte tissues can be used much like the Redfield ratio for particulate organic matter, with departures from taxon-specific means as indicators of nutrient limitation (Atkinson and Smith 1983; Duarte 1990). Spatial patterns in elemental stoichiometry can be used to identify sources and sinks of nutrients in aquatic ecosystems (Fourqurean et al. 1992, 1997; Miller and Sluka 1999; Fourqurean and Zieman 2002). Paleoecological records of elemental ratios of organic matter in sediment cores have been used to infer temporal change in nutrient sources in coastal and lake ecosystems (e.g., Meyers 1997; Orem et al. 1999; Teranes and Bernasconi 2000).

In addition to elemental stoichiometry, ratios of the stable isotopes of carbon (C) and nitrogen (N) have proven useful indicators of supply and processing of nutrients. Stable isotope ratios in macrophytes and consumers have proven valuable in tracing the flow of energy in marine food webs (Peterson et al. 1985; Peterson 1999). Stable isotope ratios can also be used to identify nutrient sources and processing in ecosystems. ^{13}C:^{12}C ratios in macrophytes have been used to identify the importance of allochthonous C to marine ecosystems (Zieman et al. 1984; Lin et al. 1991; Hemminga et al. 1994). Bacterially-mediated processing of N can strongly influence stable N isotope ratios, and as a consequence, spatial pattern in ^{15}N:^{14}N ratios in macrophytes can be used to infer ecosystem-scale processing of organic matter (Fourqurean et al. 1997). C and N isotopes have been used in both paleoceanography and paleolimnology to infer changes in water column nutrient cycles (Schelske and Hodell 1991; Haug et al. 1998; Teranes and

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Bernasconi 2000). Owing to the isotopically heavy N associated with many anthropogenic nutrient sources, stable isotopes of N in macrophytes are potentially invaluable tools for gauging the effect of man on coastal water bodies (McClelland et al. 1997; McClelland and Valiela 1998b). This tool is potentially of primary importance because of the magnitude of the effect man is having on coastal water bodies through anthropogenically-increased N loading (Paerl 1997; Vitousek et al. 1997; Tilman et al. 2001).

Despite the promise of these stoichiometric and isotopic tools for understanding elemental cycles in the coastal ocean, there are still many problems with their routine application. Chief among these problems is that unambiguous application of these tools requires an understanding of the natural variability in elemental and isotopic composition of organisms in both time and space. Relatively small-scale surveys of marine macrophytes have documented spatial variability in elemental and isotopic composition (Stephenson et al. 1984; Fourqurean et al. 1992; Boon and Bunn 1994; Boyce et al. 2001), and meta-analyses have brought to light differences between macrophyte species (Atkinson and Smith 1983; Hemminga and Mateo 1996). Less attention has been given to analysis of temporal patterns in elemental and isotopic composition of macrophytes, which could be important in interpreting food web structure from isotopic evidence, since different consumers integrate isotopic signals of their food sources over different time scales (O'Reilly et al. 2002). It has recently been shown that the stable isotopic composition of the marine macrophytes that form the base of many coastal food webs can exhibit marked seasonality (Cloern et al. 2002; Anderson and Fourqurean 2003). There have been few, if any, detailed investigations of the interrelationships between elemental and isotopic composition of marine macrophytes despite evidence that elemental composition and isotopic composition can be related in salt marsh plants (Cloern et al. 2002) and terrestrial old-field grasses and herbs (Handley and Scrimgeour 1997).

Before the variability in elemental and isotopic content of primary producers can be used to explore the nature of elemental cycling in coastal ecosystems, the nature and causes of the variability must be fully understood. A large monitoring program designed to assess the status and trends of seagrass beds in south Florida allowed for us to determine both spatial and temporal patterns in the elemental and isotopic composition of *Thalassia testudinum,* the dominant marine macrophyte in this region (Fourqurean et al. 2001; Fourqurean and Rutten 2003). Coupled with knowledge of wa-
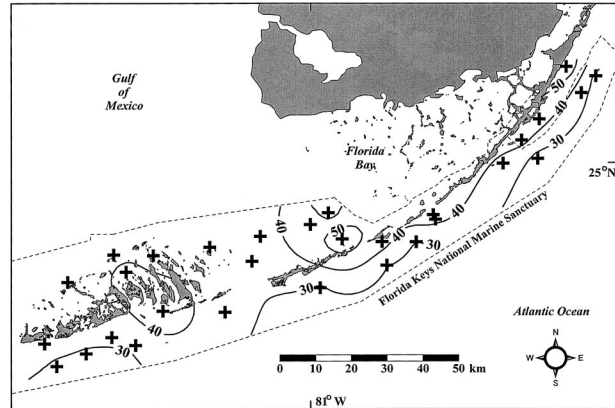

Fig. 1. Distribution of the 30 seasonal monitoring stations in the Florida Keys National Marine Sanctuary (heavy crosses denote monitoring sites). Isopleths of mean N:P of *Thalassia testudinum* leaves for quarterly samples collected during the period 1995–2001 are shown.

ter chemistry and primary productivity of the region, this data set allows us to explore the relationships between environment, nutrient concentrations, nutrient demand, time of year, and the elemental and isotopic composition of this widespread marine macrophyte.

Methods

SITE SELECTION

The seagrass *Thalassia testudinum* Banks ex König is widespread in tropical and subtropical coastal marine areas of the Atlantic and Caribbean, and it is the dominant species in the seagrass beds that are common in south Florida. In order to assess seasonality and interannual patterns in elemental and isotopic composition of *T. testudinum,* data were gathered from the network of 30 permanent seagrass monitoring sites in the Florida Keys National Marine Sanctuary, a subtropical-tropical, shallow-water, marine ecosystem of seagrass beds, coral reefs, and mangroves (Fig. 1, see Fourqurean et al. 2001; Fourqurean and Rutten 2003 for a description of the monitoring program). Extensive water quality monitoring is done at these sites; data from that program (Boyer and Jones 2002) were used to compare elemental composition with nutrients in the overlying water column. These sites were originally located using a stratified-random approach, with 2 random locations being chosen within three strata (inshore, offshore, and intermediate) in each of the defined segments of the Sanctuary (Klein and Orlando 1994). Data on elemental and isotopic composition of seagrasses at the permanent sites were collected 4 times per year during the period December 1995–March 2003. A preliminary examination of 2 yr of seasonal pat-

tern in stable isotopic composition from 4 sites was published previously (Anderson and Fourqurean 2003); those data are analyzed in a larger context in this paper.

To describe the spatial patterns in elemental and isotopic composition of *T. testudinum,* data were collected from an additional 425 randomly selected sites in the Sanctuary. These stations were sampled during the summer months of 1996–2000; during each year approximately 100 samples were collected across the spatial extent of the Sanctuary. Data from all years were pooled to produce a synoptic view of the spatial pattern. Owing to the cost of stable isotopic analyses, $\delta^{13}C$ and $\delta^{15}N$ data were determined only for data from sites sampled in 1999. A limited set of the elemental composition data has been published previously (about 50 of the present data points were included in the analyses presented in Fourqurean and Zieman [2002]). In order to more fully describe the regional variation in elemental and isotopic composition of *T. testudinum,* literature values on the range and distribution of C, N, and phosphorus (P) content from Florida Bay (Fourqurean et al. 1992; Frankovich and Fourqurean 1997) were incorporated into our analyses for this paper. Some previously-published $\delta^{15}N$ data from Florida Bay (15 points from Corbett et al. [1999]) and previously unpublished $\delta^{13}C$ and $\delta^{15}N$ data from Florida Bay collected in 1994 were also incorporated in maps and summary figures.

At each sampling site, 5 intact short shoots of *T. testudinum* were haphazardly collected from a 10-m^2 area. These short shoots were returned to the lab, where all attached green leaves were cut from the short shoots and cleaned of adhering epiphytes by gently scraping with a razor blade. Samples were not acid washed to remove the epiphytes because of the known artifacts in stable isotope determinations of acid washed samples (Bunn et al. 1995); we have found thorough scraping of fresh blades to be efficient at removing adhering carbonate material. All leaves from a site were pooled and dried at 80°C. Dried leaves were ground to a fine powder using a ceramic mortar and pestle. Powdered samples were analyzed in duplicate for C and N content using a CHN analyzer (Fisons NA1500). P content was determined by a dry-oxidation, acid hydrolysis extraction followed by a colorimetric analysis of phosphate concentration of the extract (Fourqurean et al. 1992). Elemental content was calculated on a dry weight basis (i.e., mass of element/dry weight of sample $\times$ 100%); elemental ratios were calculated on a mole:mole basis.

All isotopic analyses were measured at the Southeast Environmental Research Center (SERC) Stable Isotope Laboratory using standard elemental analyzer isotope ratio mass spectrometer (EA-IRMS) procedures. The EA was used to combust the organic material and to reduce the formed gases into N_2 and CO_2, which were measured on a Finnigan MAT Delta C IRMS in a continuous flow mode. The samples' isotopic ratios (R) are reported in the standard delta notation (‰): δ (‰) = $[(R_{sample}/R_{standard}) - 1] \times 1000$. These results are presented with respect to the international standards of atmospheric N (AIR, N_2) and Vienna Pee Dee belemnite (V-PDB) for C. Analytical reproducibility of the reported δ values, based on sample replicates, was better than $\pm$ 0.2‰ for $\delta^{15}N$ and $\pm$ 0.08‰ for $\delta^{13}C$.

Net aboveground productivity of *T. testudinum* was measured on a quarterly basis at each permanent site using a modified leaf marking technique (Fourqurean et al. 2001). Six 10×20 cm quadrats were haphazardly distributed within 10 m of a permanent steel rod that marked the site. Within each quadrat, all short shoots of the seagrass *T. testudinum* were marked near the base of the leaves by driving an 18-gauge hypodermic needle through all of the leaves on a short shoot. Care was taken not to disturb other plant and animal taxa in the quadrats. The marked short shoots were allowed to grow for 10–14 d, after which all aboveground seagrass material in the quadrats was harvested. The number of short shoots was counted, and leaves were separated into newly produced (below the marks) and older leaf material.

Data were analyzed using a variety of parametric and nonparametric methods. Pearson's correlation coefficients and standard linear regression were used to assess the strength of relationships between normally distributed variables. The nonparametric Spearman's ρ was used to assess correlation where parametric tests were not appropriate. To describe the seasonal pattern in elemental and isotopic content, we fit a sine model of the form y = mean + amp $\times$ sin(time + Φ), where amp is the amplitude of a sine wave and Φ is a phase angle in radians (2π radians = 365 d), to time series of data using an iterative least-squares nonlinear curve fitting routine. Analysis of covariance (ANCOVA) was used to test for relationships between variables at the seasonal monitoring sites, e.g., the effect of seagrass growth rate on stable isotopic composition was assessed using ANCOVA with sites as a main effect and seagrass growth rate as a covariate within sites. To aid in the visualization of spatial pattern in the elemental and isotopic data, a kriging algorithm (point kriging using a linear variogram and no nugget) was used to interpolate between data points and contour maps were generated from these interpolated fields.
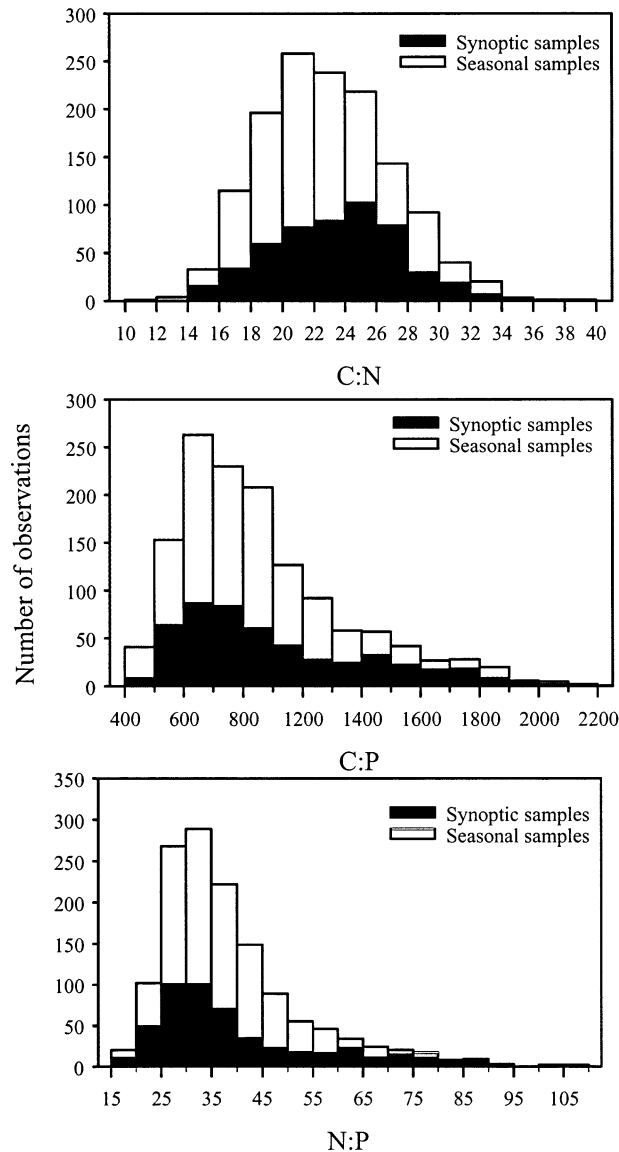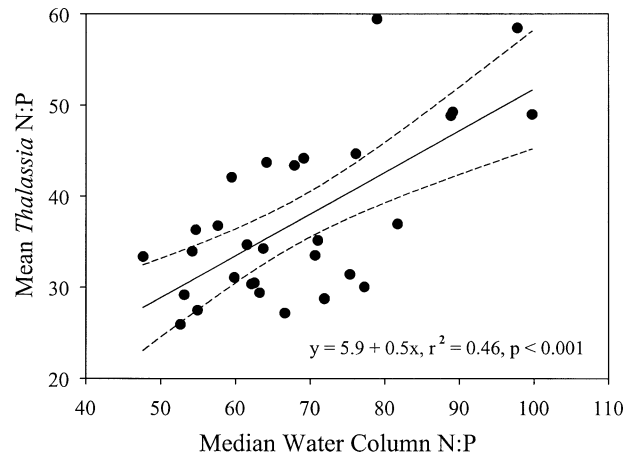
Fig. 3.   Relationship between the mean N:P of *Thalassia testudinum* leaves measured over the period 1995–2001 and the median total Nitrogen: total Phosphorus (N:P) in the water column at the 30 seasonal monitoring. Water column data are the medians of quarterly observations from these sites over the same period, the data were provided by J. N. Boyer (unpublished data), Florida International University. Standard parametric linear regression statistics are shown. 95% confidence interval of the regression is indicated.

Fig. 2.   Frequency distribution of the C:N, C:P, and N:P ratios of green leaves of *Thalassia testudinum* from south Florida. Data from Fourqurean et al. (1992) and Frankovich and Fourqurean (1997) have been added to the 1,015 data points collected for this paper to give a broader view of the distribution of these ratios in the region.

Results

ELEMENTAL CONTENT OF *T. TESTUDINUM* IN SOUTH FLORIDA

There was a broad range in the N and P content of *T. testudinum* collected across south Florida. The N content ranged from 1.15% to 3.15% and P content ranged from 0.049% to 0.241%, while C content was much less variable in the 1,065 samples. For the samples collected from the seasonal monitoring stations over the period 1995–2003, the mean C:N was 22.6, compared to a slightly higher mean of 24.4 for the synoptic mapping sites. C:N was normally distributed about the mean (Fig. 2). The C:P distribution was skewed, with a substantial number of high values; the median C:P was similar between the synoptic mapping samples and the seasonal monitoring samples (780.1 versus 780.7). The distribution of N:P was also skewed, with a tail towards the higher values. The median N:P was similar between the two sets of samples (35.4 for the seasonal monitoring stations compared to 33.5 for the synoptic monitoring sites).

NUTRIENT RATIOS AT THE SEASONAL MONITORING STATIONS

There was a spatial pattern in the relative N and P content of *T. testudinum* leaves across the 30 seasonal monitoring stations in the Florida Keys National Marine Sanctuary, with generally lower mean N:P offshore of the Florida Keys on the Atlantic Ocean side of the island chain, and higher N:P close to shore and on the Gulf of Mexico side of the island chain (Fig. 1). The N:P of seagrass leaves was significantly correlated with the median ratio of total N to total P of the overlying water column (Fig. 3), but the slope of the relationship is less than 1, suggesting that processes act in the benthos to either remove N or store P relative to the water column.

There were repeatable, seasonal patterns in the nutrient content of seagrass. C:N and C:P were low in winter months and high in summer months, but
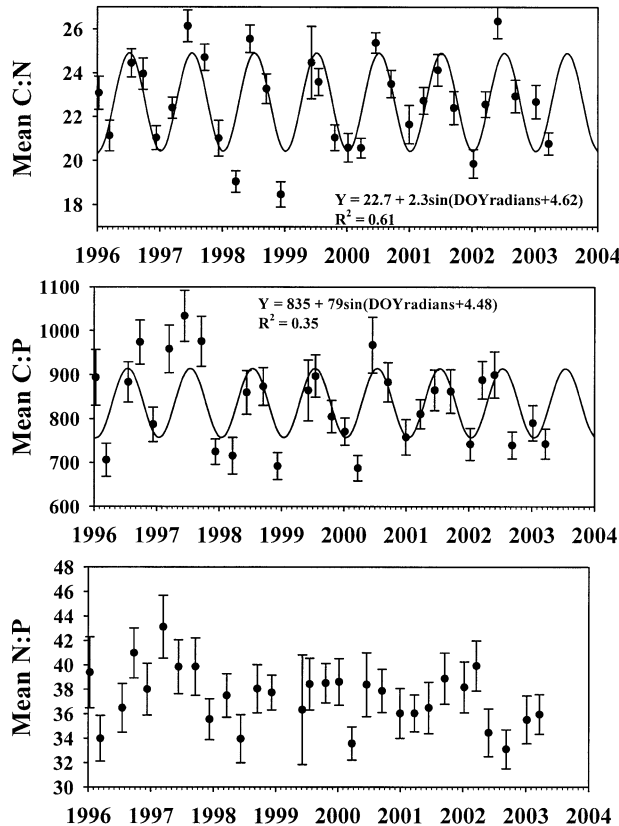
Fig. 4.   Seasonal pattern in C:N, C:P, and N:P of green leaves of *Thalassia testudinum* in south Florida. Each point is the mean of the observations from the 30 seasonal monitoring sites shown in Fig. 2, error bars indicate ± 1 standard error. The best fit sine model of the form y = mean + amp × sin(time + φ), where amp is the amplitude of a sine wave and φ is a phase angle in radians (2π radians = 365 d), is shown for C:N and C:P; estimates of the model parameters for the N:P data were not significantly different than 0.

the degree of seasonality was not constant among stations. The sine model was fit to the mean C:N and C:P ratios for all 30 sites at each sampling event, the model explained 61% of the variance in C:N (Fig. 4). A $\Phi = 4.6 \pm 0.2$ indicated that the peak in C:N occurred on average on the 185th day of the year (July 4), ± 9 d. The sine model described 35% of the variance in C:P, with a maximum on July 14 ± 15 d. The timing of the peaks were not significantly different for C:N and C:P. Note that the average annual excursion in the data is 2 × amp. The sine model was not appropriate to describe the time series of N:P, as the estimates of neither amp or Φ were significantly different from 0.

The sine model was fit to the time series of C:N and C:P from each of the 30 seasonal monitoring stations. The amplitude of the seasonal variation in elemental content at a station varied as a function

of the mean annual nutrient content. Sites with low mean C:N or C:P had low amplitude, while sites with high ratios had high amplitudes (Fig. 5). Seagrass growth rates at these sites have also been shown to follow the sine model (Fourqurean et al. 2001). Within sites, there were strong relationships between seagrass growth rate and elemental content; as growth rate increased, so did the C:N and C:P. Across all sites, there were significant relationships between growth rate and N and P content of *T. testudinum.* The mean slope of the relationship for C:N was 2.4 ± 0.3 (g m^{-2} d^{-1})$^{-1}$ (ANCOVA, F = 72.0, p < 0.001), while for C:P the mean slope was 88.6 ± 18.2 (g m^{-2} d^{-1})$^{-1}$ (ANCOVA, F = 23.8, p < 0.001).

C and N Stable Isotope Ratios of *T. testudinum* in South Florida

In addition to the broad range in the elemental content of leaves of *T. testudinum,* there was also a broad range in the δ^{13}C and δ^{15}N values. δ^{13}C ranged from a minimum of −13.54‰ to a maximum of −5.23‰, and δ^{15}N ranged from −4.31‰ to 9.40‰ in the 981 samples. The mean values of δ^{13}C were similar for samples collected at the seasonal monitoring stations and the synoptic mapping stations (−8.68‰ compared to −8.75‰), but the values from the seasonal monitoring stations had a more peaked distribution than the data from the synoptic mapping stations, which had heavier tails (Fig. 6). The mean values of δ^{15}N were also similar between the two sets of samples (2.28‰ versus 2.34‰). Both the seasonal monitoring data and synoptic station data had long left-hand tails to their distributions, but the synoptic data also had a long right-hand tail, with relatively infrequent heavy values of δ^{15}N.

C and N Stable Isotope Ratios at the Seasonal Monitoring Stations

The average δ^{13}C and δ^{15}N values of *T. testudinum* leaves at the seasonal monitoring sites were not significantly correlated (r = −0.07, p = 0.72), but the average isotopic values were related to the elemental content. Sites with relatively low N:P had more depleted δ^{13}C signatures, while sites with high N:P were more enriched (Fig. 7). Average δ^{15}N increased as the mean C:N at a site increased.

There was a marked seasonal variation in both δ^{13}C and δ^{15}N values of *T. testudinum* at the seasonal monitoring stations, but the magnitude of the seasonal change varied among stations and timing of the peaks varied somewhat between years and stations. In general, values for both δ^{13}C and δ^{15}N were heavier in summer months and lighter in winter months. To describe the average seasonal pattern in the isotope ratios, we fit the sine model to
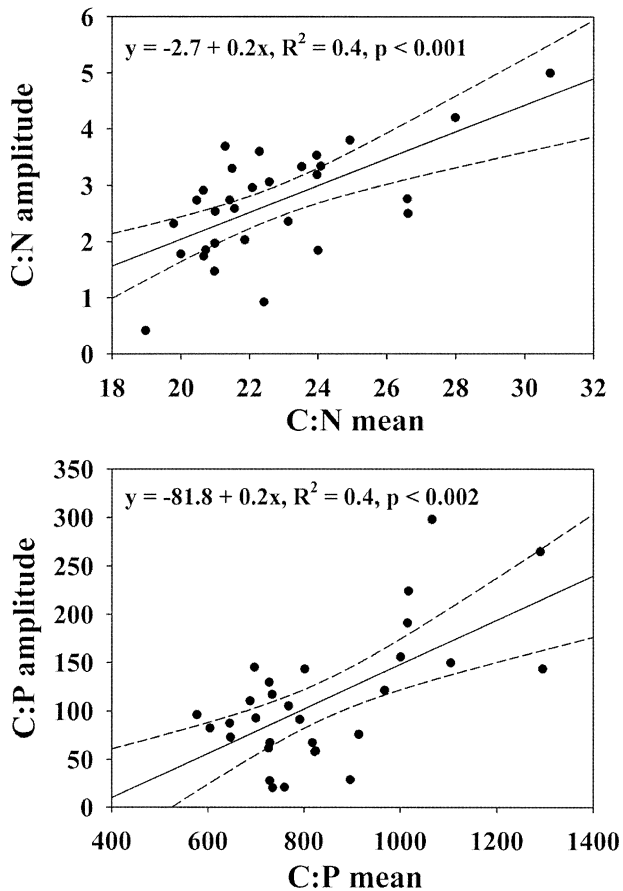
Fig. 5.   The seasonal amplitude in the C:N and C:P of *Thalassia testudinum* leaves increased as a function of the mean C:N or C:P in south Florida. Amplitude and mean were determined for each of the 30 seasonal monitoring sites by fitting the model y = mean + amp × sin(time + φ), where amp is the amplitude of a sine wave and φ is a phase angle in radians (2π radians = 365 d). Standard parametric linear regression statistics are shown. 95% confidence interval of the regressions are indicated.

Fig. 6.   Frequency distribution of the $\delta^{13}C$ and $\delta^{15}N$ of green leaves of *Thalassia testudinum* from south Florida. Data from Corbett et al. (1999), as well as unpublished data from Florida Bay, have been added to the 744 data points collected for this paper to give a broader view of the regional distribution.

the mean values for all 30 sites at each sampling event (Fig. 8). The sine model described 63% of the variance in the $\delta^{13}C$ data, with a mean of $-8.63‰ \pm 0.09$, and an amp of $0.70‰ \pm 0.13$ with a peak on August 11 ± 12 d. The sine model described 40% of the variance in the $\delta^{15}N$ pattern, with a mean of $2.3‰ \pm 0.1$ and an amp of $0.4‰ \pm 0.1$. The yearly peak occurred on July 26 ± 16 d, statistically the same as the peak in the $\delta^{13}C$ data. Averaging across all sites and years, the average seasonal range (2 × amp) was $1.40‰$ in $\delta^{13}C$ and $0.8‰$ in $\delta^{15}N$. Calculated in this fashion, the amp parameter underestimates the magnitude of the true average seasonal variation in the isotope values because the stations are slightly out of phase with one another. Calculating the range of values encountered within each year between 1996–2003
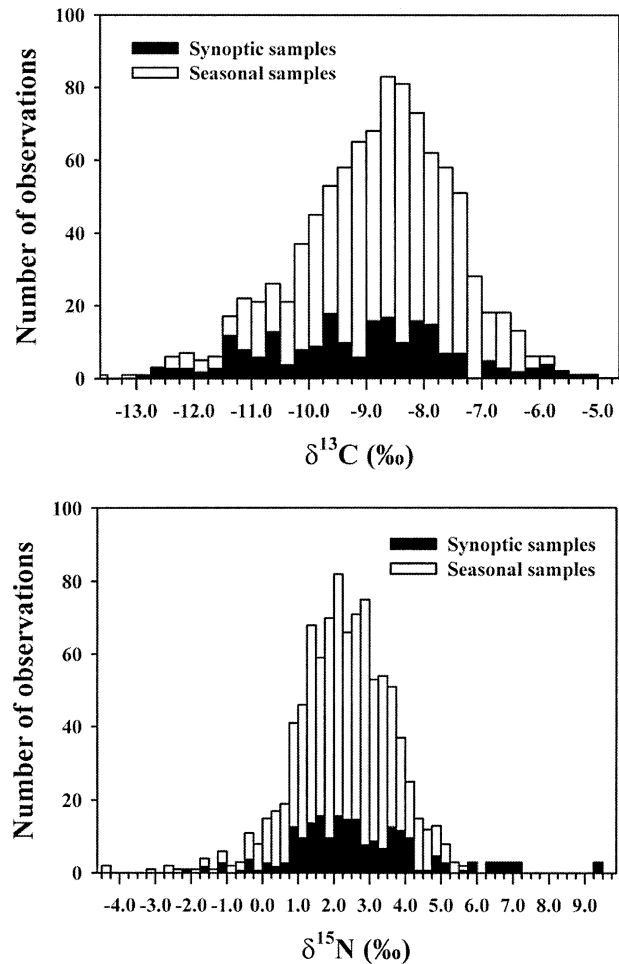
at each site, and then calculating the mean of these yearly ranges at each site, gives a better estimate of the average annual variation in the isotope values at a site. Average annual variation in $\delta^{13}C$ was $1.90‰ \pm 0.11$ for all sites, with minimum of $1.14‰$ and a maximum of $3.58‰$. Average annual variation in $\delta^{15}N$ was $1.7‰ \pm 0.1$, with a minimum of $0.8‰$ and a maximum $3.4‰$.

Seagrass growth rate (Fourqurean et al. 2001), C:N and C:P ratios (Fig. 4), and C and N stable isotope ratios (Fig. 8) all showed peaks during the summer months. Across sites, there was a significant relationship between the seagrass growth rate at a site and $\delta^{13}C$ values; $\delta^{13}C$ increased by $0.30‰$ ± 0.09 for every 1 g m^{-2} d^{-1} of productivity increase (ANCOVA, F = 10.7, p = 0.001). There was no significant relationship between growth rate and $\delta^{15}N$ across all sites (slope of relationship in
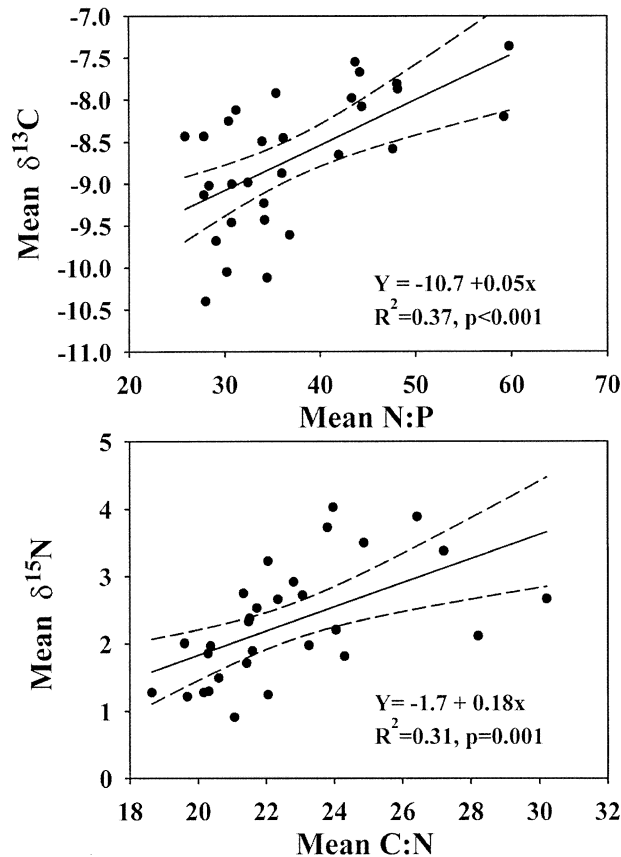
Fig. 7. Relationships between stable isotopic composition and the elemental ratios of green leaves of *Thalassia testudinum*. Each point represents the mean of all values collected between 1995–2001 at each of the 30 seasonal monitoring stations.

Fig. 8. Seasonal pattern in $\delta^{13}C$ and $\delta^{15}N$ of green leaves of *Thalassia testudinum* in south Florida. Each point is the mean of the observations from the 30 seasonal monitoring sites shown in Fig. 2, error bars indicate $\pm$ 1 standard error. The best fit sine model of the form y = mean + amp $\times$ sin(time + ϕ), where amp is the amplitude of a sine wave and ϕ is a phase angle in radians (2π radians = 365 d), is shown.

ANCOVA not significantly different from 0). There was a significant relationship between $\delta^{15}N$ and C:N, with an increase in $\delta^{15}N$ by 0.03‰ $\pm$ 0.01 for each unit increase in C:N (ANCOVA, F = 4.8, p = 0.03).

SPATIAL PATTERN IN ELEMENTAL RATIOS

Samples collected across the study area during the summer months (to coincide with peaks in C:N and C:P ratios, Fig. 4) indicated there was a strong spatial pattern to the nutrient content of leaves of *T. testudinum* (Fig. 9). C:N was lowest in the interior of Florida Bay, with a general trend of increasing C:N as distance from the Florida Keys increased both towards the Atlantic and Gulf of Mexico. C:P ratios were highest in the interior of Florida Bay, with a general decrease in C:P with distance offshore of the Florida Keys. These two patterns together lead to a clear pattern in N:P, with maximum N:P in the interior of Florida Bay, with decreases in N:P with distance offshore of the Florida Keys. Using an N:P of 30 as a rough indi-
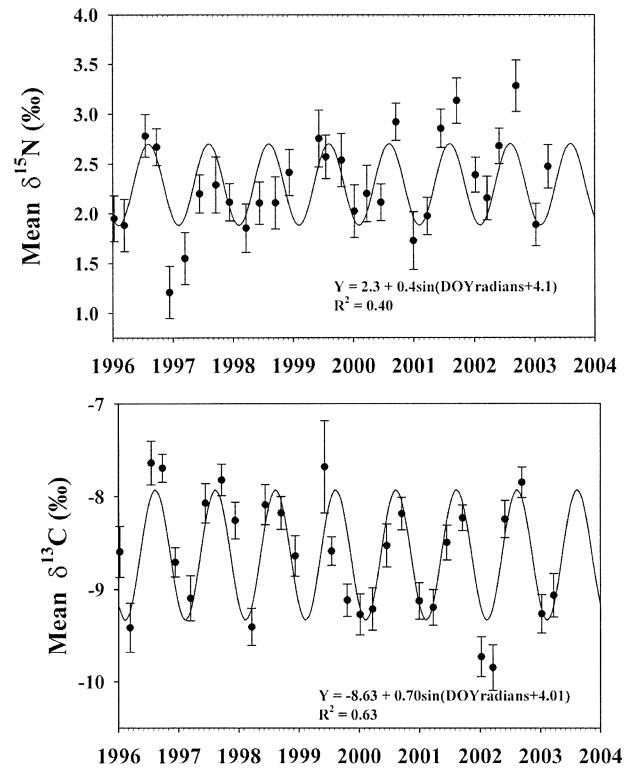
cation of the optimum relative availability of N and P for *T. testudinum* (Atkinson and Smith 1983; Duarte 1990; Fourqurean and Zieman 2002), this trend in N:P indicated that, for the most part, the seagrasses of south Florida were P limited in Florida Bay and near shore in the Florida Keys, with a tendency towards N limitation in the more offshore seagrass beds.

SPATIAL PATTERN IN ISOTOPE RATIOS

As with the elemental ratios, there were strong spatial patterns in the C and N stable isotopic composition of *T. testudinum* (Fig. 10). C isotope ratios were more depleted in the northwest corner of Florida Bay ($-12‰$ to $-11‰$), and more enriched ($-8‰$ to $-6‰$) in areas close to the Florida Keys. A much different spatial pattern was evident for $^{15}N:^{14}N$ ratios. The heaviest $\delta^{15}N$ values ($> 8‰$) were found in Florida Bay, while the lightest $\delta^{15}N$ values ($< 2‰$) were found in the central region of our study area. In contrast to the elemental ratios (Fig. 9) and $\delta^{13}C$, there was no pat-
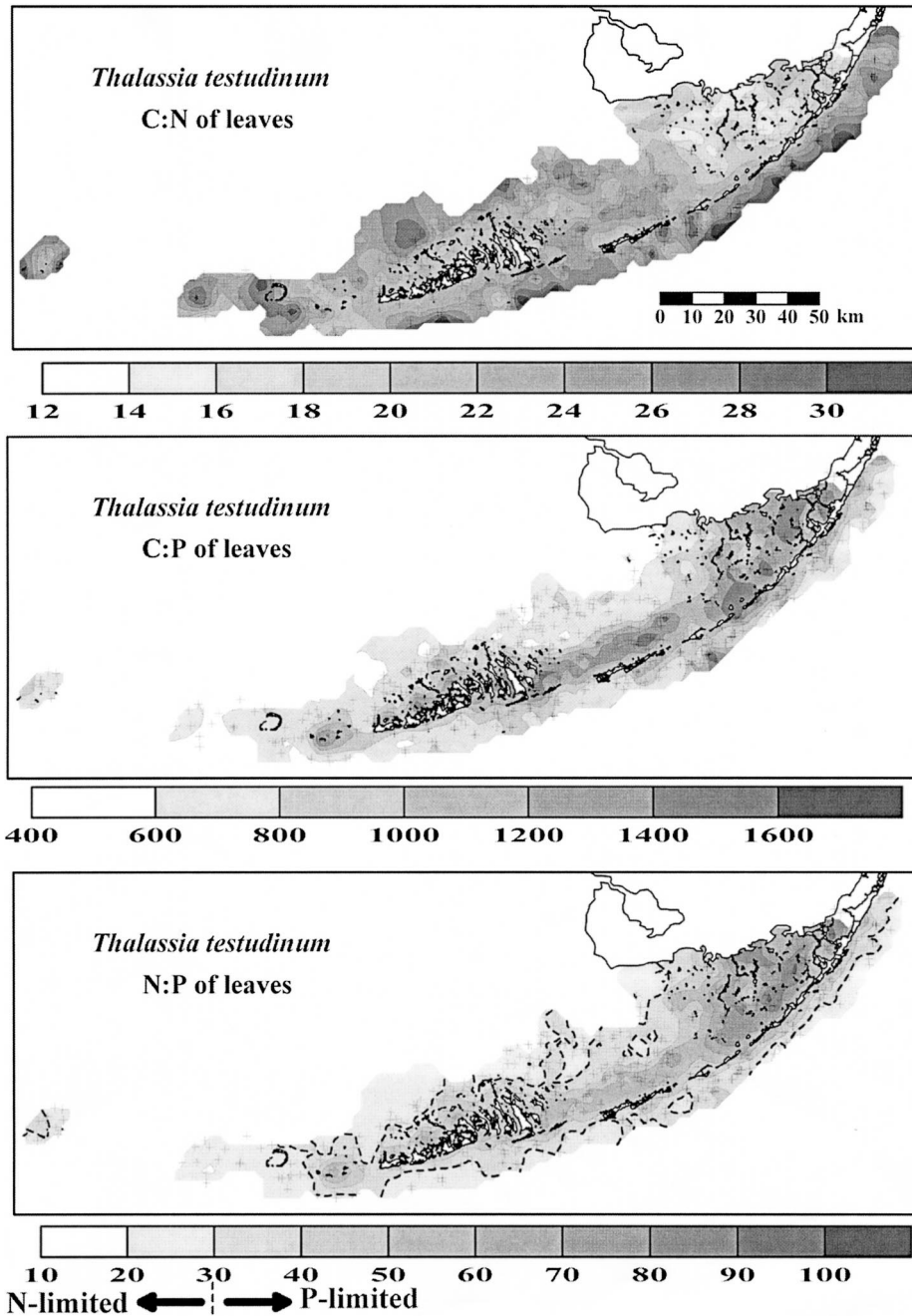
Fig. 9.   Spatial pattern of the C:N, C:P, and N:P of green leaves of *Thalassia testudinum* in south Florida. All data was collected in the summer months to control for the seasonal pattern. Bottom panel: an N:P ratio of 30 was used to delimit N-limited and P-limited areas.

tern in $\delta^{15}N$ attributable to the distance offshore from the Florida Keys.

RELATIONSHIPS BETWEEN ELEMENTAL RATIOS AND ISOTOPE RATIOS AT THE SYNOPTIC SCALE

The spatial patterns in elemental composition of *T. testudinum* (Fig. 9) were based on 836 samples for which C, N, and P content were known. The C:N and C:P ratios of these samples were both correlated with N:P, but C:N and C:P were not correlated with each other (Table 1), indicating that N and P content of *T. testudinum* were independent of each other on this spatial scale. For 350 of these samples, the C and N stable isotopic composition
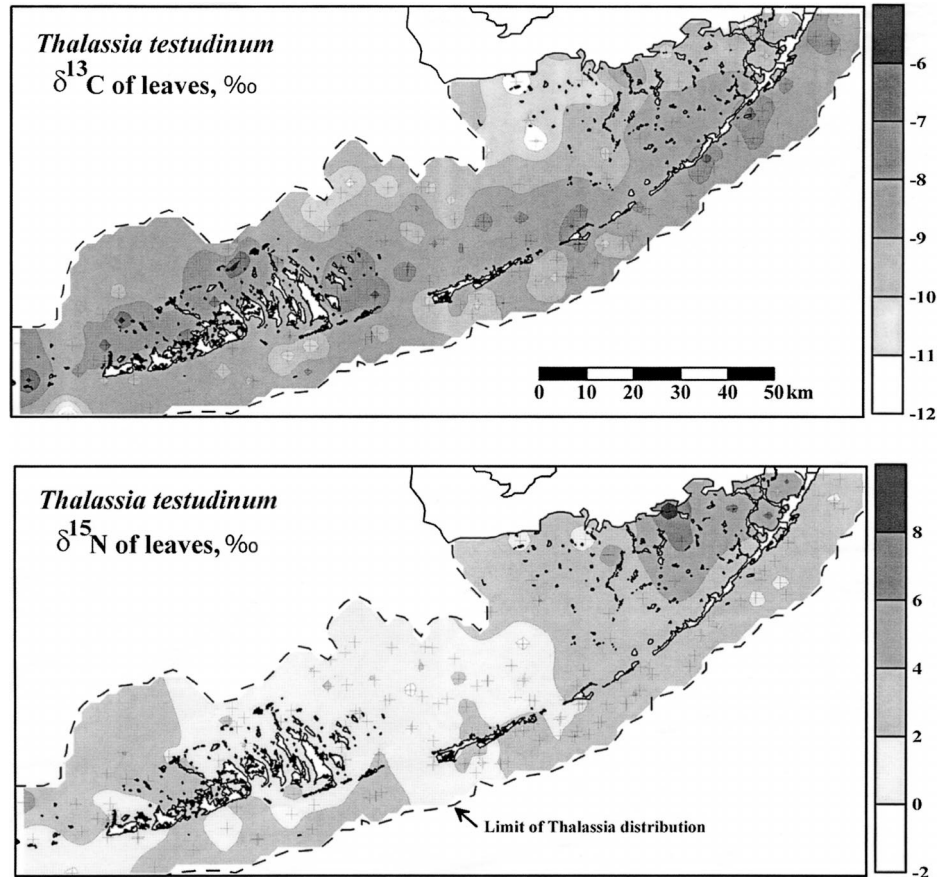
Fig. 10.   Spatial pattern of the $\delta^{13}C$ and $\delta^{15}N$ of green leaves of *Thalassia testudinum* in south Florida. All data was collected in the summer months to control for the seasonal pattern.

was also known. Both the C:P and N:P of *T. testudinum* leaves were positively correlated with $\delta^{13}C$ (Table 1). This relationship of decreasing $\delta^{13}C$ with increasing P content at the synoptic scale mirrored the relationship seen at the seasonal monitoring stations (Fig. 7). The relationship of increasing $\delta^{15}N$ with decreasing N content that was observed at the seasonal monitoring stations (Fig. 7) was also seen at the synoptic scale (Table 1). There was no significant relationship between the $\delta^{15}N$ and $\delta^{13}C$ of *T. testudinum* leaves from the 350 synoptic mapping stations (Table 1).

Discussion

Elemental and isotopic composition of leaves of the seagrass *T. testudinum* was highly variable across the 10,000 km² and 8 yr of this study, and the data presented herein generally expand the reported ranges of elemental and isotopic composition for this species (Table 2). The striking spatial and temporal patterns in the variability of elemental and isotopic content suggest that the measured variation is not merely random noise, but instead that the variation contains useful information about the effects of the environment on the nutrient content and physiological status of seagrasses. Our work calls into question the interpretation of studies about nutrient cycling and food webs in estuaries based on few samples collected at one time, since we document natural variability of elemental and isotopic content greater than the signals often used to imply changes in the structure or function of ecosystems.

Despite the tropical nature of the study site, there is substantial seasonal variability in the growth rate and abundance of the perennial *T. testudinum,* with enhanced summertime growth rates leading to 64% higher abundance in summer compared to winter (Fourqurean et al. 2001). These differences are correlated with seasonal patterns in both light availability and water temperature. It has been shown experimentally that decreases in light availability lead to lighter $\delta^{13}C$ values for seagrasses (Grice et al. 1996). Photosynthesis of *T. testudinum* in south Florida is rarely photosaturated (Fourqurean and Zieman 1991), so increases in light lead

TABLE 1. Correlations (nonparametric Spearman's ρ) between elemental ratios and stable isotopic composition of *Thalassia testudinum* leaves from the synoptic sampling. n = 836 for elemental ratios; n = 350 for stable isotope ratios. Values of Spearman's ρ are above the diagonal, p values are below the diagonal. Bold type indicates significant correlations.

	C:N	C:P	N:P	$\delta^{13}C$	$\delta^{15}N$
C:N	—	0.088	**−0.388**	−0.014	**0.126**
C:P	0.011	—	**0.860**	**0.294**	−0.000
N:P	**<0.001**	**<0.001**	—	**0.308**	−0.099
$\delta^{13}C$	0.787	**<0.001**	**<0.001**	—	−0.045
$\delta^{15}N$	**0.019**	0.998	0.066	0.371	—

to increases in photosynthetic C fixation, which should lead to decreased isotopic discrimination during photosynthesis and heavier values of $\delta^{13}C$ in plant tissues, if the increased rates cause a drawdown in the availability of CO_2 (Farquhar et al. 1989). We recorded a highly seasonal and repeatable change in the $\delta^{13}C$ of *T. testudinum,* with an average yearly increase of 1.4‰ from winter minima to summer maxima (Fig. 8). The seasonal increase in $\delta^{13}C$ values is a potential sign that seagrass photosynthesis may begin to be C limited in summer months in south Florida. A similar seasonality in stable C isotope ratios in phytoplankton has been used to infer a drawdown of CO_2 pools during productivity blooms (Laws et al. 1995; Ostrom et al. 1997). Previous studies of more limited duration have also documented some seasonal pat-

terns in $\delta^{13}C$ of seagrasses, and in more temperate locations with a greater seasonal difference in light and temperature, the seasonal difference in $\delta^{13}C$ can be more pronounced than we measured in this tropical ecosystem (Stephenson et al. 1984; Fourqurean et al. 1997).

It is also possible that factors other than seasonal changes in photosynthetic rates may be responsible for the seasonal pattern in $\delta^{13}C$ of seagrass leaves. A decrease in solubility of CO_2 with increasing temperatures can also lead to a smaller CO_2 pool and less isotopic discrimination and heavier $\delta^{13}C$ values of marine plants in summer months (Francois et al. 1993). It is also possible that the $\delta^{13}C$ of the dissolved inorganic carbon (DIC) in seawater may change as a result of seasonal changes in inputs of terrestrially-derived organic matter and the bal-

TABLE 2. Reported values of $\delta^{15}N$ and $\delta^{13}C$ of green leaves of *Thalassia testudinum.*

$\delta^{15}N$	$\delta^{13}C$	Location	Reference
This study			
−4.3 to 9.4	−13.5 to −5.2	South Florida	
South Florida			
	−12.5	Florida Keys	McMillan et al. 1980
	−16.3 to −7.3	South Florida	Lin et al. 1991
1.9 to 3.2	−14 to −7.0	South Florida	Zieman et al. 1984
	−15.3 to −8.7	South Florida	Fleming et al. 1990
3.4	−13.6	Florida Bay	Harrigan et al. 1989
−1.0 to 9.4		Florida Bay	Corbett et al. 1999
5.3 to 5.9	−9.1 to −8.5	Florida Bay	Orem et al. 1999
−1.2 to 3.7	−12.3 to −6.1	South Florida	Anderson and Fourqurean 2003
5.5	−10.5	Florida Keys	Schwamborn and Criales 2000
−1.2 to 9.4	−16.3 to −6.1		Range of literature values, south Florida
Other locations			
3.5	−13.2	Nicaragua	Macko 1981
3.9	−8.9	Texas	Macko 1981
4.3	−11.1	Jamaica	Macko 1981
2.9 to 5.7		Texas	Fry et al. 1987
	−11.0 to −8.3	Texas	McMillan et al. 1980
	−10.0 to −9.9	St. Croix, U.S. Virgin Islands	McMillan et al. 1980
	−10.9	Veracruz, Mexico	McMillan et al. 1980
	−9.04	Texas	Benedict and Scott 1976
	−11.4	Texas	Benedict et al. 1980
	−12.3 to −11.1	Belize	Ambler et al. 1994
	−8.9	Texas	Fry and Parker 1979
	−9.6 to −11.0	Tampa Bay	Durako and Hall 1992
−1.2 to 9.4	−16.3 to −6.1		Range of literature values

ance between ecosystem production and respiration. Swart et al. (2001) documents substantial variability in the $\delta^{13}C$ of the DIC in Florida Bay, but there were no clear seasonal patterns in this variability. Surge and Lohmann (2002) found that $\delta^{13}C$ of DIC was enriched by about 5‰ in summer compared to winter in mangrove-lined estuaries 200 km north of our study area.

Seasonal patterns in root-zone processes could influence the $\delta^{13}C$ of seagrass tissues. About 90% of the biomass of *T. testudinum* is in belowground structures (Fourqurean and Zieman 1991), and the lacunal system of seagrasses allows for diffusion of gases from the root zone to the leaves where photosynthesis occurs. Respiratory processes can dissolve carbonate sediments (Jensen et al. 1998; Burdige and Zimmerman 2002), and carbonate sediments are enriched in ^{13}C relative to seagrass tissues, providing for the possibility that our observed increases in $\delta^{13}C$ during the warm summer months could be a result of increased dissolution of carbonates in response to increased respiration in the sediments. Such an effect could also help explain the enrichment of the $\delta^{13}C$ of the DIC of surface waters observed in the region (Surge and Lohmann 2002).

Increased photosynthetic rates can result in higher growth rates for plants, but elements other than C are needed to synthesize new plant tissues. Given that the seagrass-specific Redfield ratio is approximately 550:25:1 for C:N:P (Atkinson and Smith 1983; Duarte 1990), on average 1 mol of N is required for every 22 mol of C and 1 mol of P for every 550 mol of C allocated to growth. The likely mechanism underlying the repeatable seasonal pattern in C:N and C:P ratios (Fig. 4) is that N and P can not be supplied from the environment in sufficient quantities to satisfy the growth demands of the seagrasses when photosynthetic rates, and the potential for growth, are high during the summer months. Additional evidence for seasonal nutrient limitation as a function of potential growth rate comes from the relationship between the mean C:N and C:P ratios and the amplitude of the seasonal signal of those ratios (Fig. 5). Sites with ample N to meet their growth demands, i.e., those with low average C:N ratios, have little seasonal variability compared to sites at which the high mean C:N ratios indicate N deficits. The same holds for the relationship between mean C:P ratios and the amplitude of the seasonal pattern in C:P ratios.

Sites with evidence of higher average photosynthetic rates are the ones that show deviation from seagrass Redfield N:P stoichiometry; sites with high N:P ratios, indicating P limitation, had the highest average $\delta^{13}C$ values (Fig. 7). This was likely a result

of relative light availability. Low light would be expected to produce low photosynthetic rates and isotopically depleted $\delta^{13}C$ in plant tissues. Such an effect can be seen along natural gradients in light availability (Cooper and DeNiro 1989), and experimental reductions in light availability lead to decreases in $\delta^{13}C$ of seagrasses (Abal et al. 1994; Grice et al. 1996). Low light levels also result in seagrass Redfield-like N:P ratios (Fourqurean and Rutten 2003); only at relatively high light levels (relative to nutrient supply) would one expect deviation from an N and P balance.

The cause of the seasonal cycle in $\delta^{15}N$ (Fig. 8) needs to be determined by careful experimentation. The two most likely causes are a seasonal variability in the $\delta^{15}N$ of the source dissolve inorganic nitrogen (DIN) or seasonal differences in the fractionation of the DIN pool during uptake. We feel that the most probable cause is a decreased fractionation in summer when plant growth demands outstrip the N supply and draw down the pool of available N, in a manner similar to the decrease in fractionation of the DIC pool in summer when photosynthetic rates are high. On average, there was a 0.8‰ repeatable seasonal pattern of isotopically light values in winter and heavy values in summer as determined by fitting the sine model to the station mean data. This degree of seasonal pattern was less than the mean annual range at all sites of 1.7‰ because of phasing differences among sites. At some sites, there was as much as a 3.4‰ average annual range, which is about the same range that had been previously documented for *T. testudinum* from all locations in the species' range outside of south Florida (Table 2). Evidence to support the hypothesized mechanism behind the increase in $\delta^{15}N$ values during summer months can also be seen in the relationship between C:N and $\delta^{15}N$ (Fig. 7); as the C:N increases indicating decreasing N availability, the $\delta^{15}N$ values also increased in a manner consistent with decreased fractionation of a DIN pool of constant $\delta^{15}N$ as the availability of N decreased. It is also possible that there are seasonal patterns in the $\delta^{15}N$ of the DIN source for seagrass growth, especially in the sediments underlying the seagrass beds. The $\delta^{15}N$ of soil DIN is a complex function of supply (e.g., deposition, nitrogen fixation, and remineralization), utilization (e.g., plant uptake and denitrification), and losses both advective and diffusive. Many of these processes strongly fractionate the DIN pool (see Handley and Scrimgeour 1997 for a review of this topic).

At the spatial resolution of the 30 monitoring sites, *T. testudinum* elemental content was a reflection of the relative availability of N and P in the water column (Fig. 3), suggesting that seagrass stoi-

chiometry does reflect the relative availability of nutrients in the environment. The long lifespan of seagrass shoots (often over 10 yr in south Florida, Peterson and Fourqurean 2001) results in seagrasses being long-term integrators of nutrient availability; we have found the spatial patterns in seagrass N:P to be much less variable in time than N:P in the water column. The slope of the relationship between water column and seagrass leaf N:P is 0.5, indicating that P is more available relative to N for benthic primary producers than in the water column. This is likely a result of the loss of N from the sediments through diffusion and denitrification, while P is retained relatively efficiently in the sediments as a result of the kinetics of phosphate sorption onto carbonate sediments (de Kanel and Morse 1978; Short et al. 1990).

The samples collected to describe fine-scale spatial variability in elemental and isotopic content of *T. testudinum* leaves were all collected in the summer months in order to control for the marked seasonality. Summer sampling also is the only appropriate time to collect elemental content data when assaying for regional pattern in elemental content, since the nutrient limitation signal is only expressed during periods of rapid growth. Our summer samples contained as much variation in elemental and isotopic content as the seasonal samples (Figs. 2 and 6), indicating spatial differences in environmental conditions were as important in determining elemental and isotopic content of the seagrass leaves as seasonal patterns in photosynthesis and growth. Across the landscape, there was no significant correlation between C:N and C:P, indicating that N and P availability were independently varying across the study area. If regional patterns in light availability were causing the patterns in C:N and C:P, then these quantities would be correlated. The general pattern in relative availability of N and P to seagrasses across the 10,000 km^2 of the study area was low N:P on the offshore areas on the Atlantic Ocean side of the Florida Keys, high N:P in the enclosed Florida Bay estuary, and relatively low N:P along the northern boundary of the study area in the Gulf of Mexico (Fig. 9). The spatial patterns in elemental ratios have been previously reported for Florida Bay (Fourqurean et al. 1992; Frankovich and Fourqurean 1997) and the back reef environment in the eastern third of the study area (Fourqurean and Zieman 2002), but this is the first documentation of the trend in the western Florida Keys. The prevailing view of ubiquitous P limitation of seagrass beds underlain by carbonate sediments (Short et al. 1990) is brought into question with these data, since broad areas offshore on the Atlantic Ocean side of the Florida Keys and west of the island of

Key West have N:P values less than 30, indicating potential N limitation. Experimental evidence supports the hypothesis of N limitation in the regions of low N:P in the eastern part of our study area (Ferdie and Fourqurean 2004), but this experimental evidence also indicates that, at intermediate N:P ratios, that seagrass Redfield ratios are imperfect predictors of the response of seagrass ecosystems to nutrient addition.

Areas closest to the islands of the Florida Keys had the heaviest $\delta^{13}C$ values for *T. testudinum* leaves (Fig. 10). These areas tend to be shallow (ca. 1–2 m) compared to areas farther from the islands on the Atlantic Ocean side of the Florida Keys, where $\delta^{13}C$ was lower by 2–3‰. This was a pattern to be expected if less light reached the seagrasses in the deeper offshore areas. The enclosed regions of Florida Bay also had isotopically light $\delta^{13}C$ values for *T. testudinum* compared to the shallow areas adjacent to the islands, despite the very shallow nature of Florida Bay (ca. 1 m average depth). It is likely that the isotopically light seagrass leaf carbon is a result of the influence of a DIC pool that has been influenced by CO_2 produced by the oxidation of organic matter derived from mangroves and other emergent and terrestrial plants (Lin et al. 1991; Swart et al. 2001). Seagrass stable C isotope values were positively correlated with both the N:P and C:P ratios (Table 1), indicating that areas with lower relative P availability were characterized by relatively heavy $\delta^{13}C$ values across the spatial extent of this study. This relationship mirrors that seen at the individual monitoring sites, where C:P and $\delta^{13}C$ values both increased during times of high productivity, as well as the relationship between the yearly mean N:P and yearly mean $\delta^{13}C$ at the 30 monitoring sites.

The broad spatial patterns in the $\delta^{15}N$ value of leaves of *T. testudinum* (Fig. 10) may have been caused by either regional patterns in the isotopic signature of the DIN available to the seagrasses, by different amount of fractionation of the available DIN pool during uptake, or a combination of these factors. The very high $\delta^{15}N$ values in north-central Florida Bay have been attributed to progressive denitrification of DIN advected into Florida Bay from the Gulf of Mexico and to seepage of ^{15}N-rich groundwater into the surface waters (Corbett et al. 1999). It is interesting to note that there was no signal of heavy $\delta^{15}N$ values close to the heavily populated Florida Keys, despite the fact that groundwater under the islands is human sewage-influenced (Lapointe et al. 1990) and likely enriched with ^{15}N. The very low $\delta^{15}N$ values ($< 2‰$) typical of the middle of our study area potentially indicate low average growth rates and/or high DIN availability, resulting in large fractionation of the DIN

pool. The low $\delta^{15}N$ values are largely coincident with an area known as the Sluiceway that allows terrestrially-influenced Gulf of Mexico water to pass through the Florida Keys to the Florida Straights (Schomer and Drew 1982). Another possible explanation of these low values is a DIN pool heavily influenced by fertilizer-laden runoff from the agricultural fields of Florida, because artificial fertilizers have a $\delta^{15}N$ of near 0‰, but as yet we have no data on the $\delta^{15}N$ of DIN from the surface water or sediment porewaters of this area. Large N inputs from the agricultural areas are not likely, since most of the DIN that enters the Everglades ecosystems that run off into this region is lost to uptake and denitrification before it reaches the Gulf of Mexico (Rudnick et al. 1999), but the potential for this low $\delta^{15}N$ signal to be an indicator of anthropogenic N merits further study.

Anthropogenically-increased N loading is one of the largest threats to the coastal ocean worldwide (Paerl 1997; Vitousek et al. 1997; Tilman et al. 2001). Given the increasing use of N isotopes in vegetation as an indicator of anthropogenic alterations of coastal N cycling, it is very important that we understand natural causes of spatial and temporal variability in ^{15}N:^{14}N. The data and patterns presented in this paper make it clear that there is no threshold $\delta^{15}N$ value for marine plants that can be used as an unambiguous indicator of human sewage pollution without a thorough understanding of local temporal and spatial variability. It would be possible to misinterpret a seasonal increase in $\delta^{15}N$ values associated with seasonal increases in growth rates as indication of an increase in the influence of human wastes, which generally have heavy $\delta^{15}N$ signatures. Likewise, sampling an area without synchronizing the season of sampling across the area could lead to spurious spatial patterns in N and C isotope ratios.

Enriched $\delta^{15}N$ values in benthic vegetation have been linked to anthropogenic N inputs that result in deleterious effects in N-limited marine ecosystems (McClelland et al. 1997; McClelland and Valiela 1998a). We found the opposite relationship to be true; there was an inverse relationship between plant-assessed N availability (C:N ratio) and the $\delta^{15}N$ values of seagrass tissues (Fig. 7 and Table 1). Apparently, there are many ways to induce a heavy $\delta^{15}N$ signature in a marine plant. Anthropogenic loading of ^{15}N-enriched sewage-derived DIN, progressive denitrification of an N source and the resulting isotopically-enriched remaining DIN (Fourqurean et al. 1997), and N limitation during periods of rapid growth can all lead to isotopically heavy $\delta^{15}N$ signatures.

Our unpublished data on other marine plants from south Florida and other seagrasses from other places in the world suggest that the variability we document herein is not peculiar to *T. testudinum*. The existence of a substantial amount of non-random variability in the elemental and isotopic composition of *T. testudinum* in south Florida augurs well for the utility of using elemental and stable isotopic content as indicators of ecosystem processes and for tracing elements in ecosystems, but it is important that the intra-annual and small-scale spatial variability of these plants is understood before interpreting the consequences of differences in samples.

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Sources of Unpublished Materials

Boyer, J. Unpublished data. Southeast Environmental Research Center, Florida International University, Miami, Florida 33199.