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Recommended Citation
Campbell, J.E., J.W. Fourqurean. 2009. Interspecific variation in the elemental and stable isotopic content of seagrasses in South Florida. Marine Ecology Progress Series 387: 109-123.

This material is based upon work supported by the National Science Foundation through the Florida Coastal Everglades Long-Term Ecological Research program under Cooperative Agreements #DBI-0620409 and #DEB-9910514. Any opinions, findings, conclusions, or recommendations expressed in the material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.
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Interspecific variation in the elemental and stable isotopic content of seagrass communities in South Florida

Running head: Interspecific variation in seagrass elemental and isotopic content

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Keywords: Seagrass, elemental content, stable isotopes, interspecific variation, carbon acquisition

ABSTRACT

The elemental (C, N and P) and isotopic ($\delta^{13}C$, $\delta^{15}N$) content of leaves of the seagrasses *Thalassia testudinum*, *Halodule wrightii*, and *Syringodium filiforme* were measured across a 10,000 km^2 survey of the seagrass communities of South Florida in 1999 and 2000. Trends at local and broad spatial scales were compared to examine interspecific variation in the seagrass characteristics often used as ecological indicators. The elemental and stable isotope contents of all species were variable and demonstrated marked interspecific variation. At broad spatial scales mean nitrogen: phosphorus (N:P) ratios were lowest for *T.testudinum* (36:5 ± 1.1) and *S. filiforme* (38:9 ± 1.3), and highest for *H. wrightii* (44:1 ± 1.8). Stable carbon isotope ratios ($\delta^{13}C$) were highest for *S. filiforme* (-6.2‰ ± 0.2‰), intermediate for *T. testudinum* (-8.6‰ ± 0.2‰),

and lowest for *H. wrightii* (-10.6‰ ± 0.3‰). Stable nitrogen isotopes ($\delta^{15}N$) were heaviest for *T. testudinum* (2.0‰ ± 0.1‰), and lightest for *H. wrightii* (1.0‰ ± 0.3‰) and *S. filiforme* (1.6‰ ± 0.2‰). Site depth was negatively correlated to $\delta^{13}C$ for all species, while $\delta^{15}N$ was positively correlated to depth for *H. wrightii* and *S. filiforme*. Similar trends were held for local comparisons, suggesting that taxon specific physiological/ecological properties strongly control interspecific variation in elemental and stable isotope content. Temporal trends in $\delta^{13}C$ were measured, and revealed that interspecific variation was displayed throughout the year. This work documents interspecific variation in the nutrient dynamics of three common seagrasses in South Florida, indicating that interpretation of elemental and stable isotope values needs to be species specific.

INTRODUCTION

The elemental and isotopic content of plant biomass can be used to characterize both the nutritional status and environmental conditions of macrophyte communities (McMillan et al. 1980, Atkinson & Smith 1983, Farquhar et al. 1989, Duarte 1992, Fourqurean et al. 2005, Fourqurean et al. 2007). These plant parameters, while reflecting the local availabilities of essential resources (nitrogen, phosphorus, carbon, and light), can additionally reflect interspecific variation, and the manner in which species interact with local resources. Taxonomic differences in growth rates, life-history strategies, physiology and morphology have the ability to influence resultant nitrogen:phosphorus (N:P) ratios and the stable isotopic content of plant material (Farquhar et al. 1989, Dawson et al. 2002, Agren 2004). While landscape patterns in elemental and isotopic content of submerged plants may be driven by large scale spatial variations in the stable isotopic composition of nutrient pools and the availabilities of nutrients and light; localized interspecific differences have been detected (Fourqurean et al. 2007), and may be attributed to physiological differences amongst sympatric plant species. Prior to using variations in the N:P ratios and stable isotopic compositions of benthic plants for inferring ecosystem processes, it is important to understand the factors which drive these variations at all spatial scales, and how taxonomic differences can be reflected within these parameters.

The N:P ratio of plant material is related to the availability of these elements in the environment relative to plant demand (Duarte 1990). Over landscape scales, spatial gradients in N or P availability are reflected by spatial patterns in plant nutrient content, as shown for seagrasses growing in coastal marine habitats (Fourqurean et al. 1992a, Fourqurean & Zieman 2002, Fourqurean et al. 2005). Nutrient addition experiments have supported the close link between environmental availabilities and tissue nutrient content, particularly in locations where

ambient elemental concentrations are low (Duarte 1990, Ferdie & Fourqurean 2004, Armitage et al. 2005). Other environmental factors can also influence the nutrient content of plant material. For example, increased light availability has been found to decrease the nutrient content of seagrass leaves because of enhanced growth rates leading to the depletion of nutrient sources (Abal et al. 1994). Thus, it has been widely documented that resource availability strongly controls intraspecific variation in the elemental ratios of benthic macrophytes. However, variation seen between plant species may be driven by factors other than environmental conditions, suggesting that ecological/physiological differences may drive interspecific variation at any particular location. While single species comparisons have been used across a broad range of seagrasses over large spatial scales, localized interspecific comparisons may provide important cues about the ecological differences occurring between sympatric species (Fourqurean et al. 2007).

The stable isotopic content of plant material has provided a powerful tool for the study and assessment of ecological processes. In addition to identifying nutrient sources and processing within ecosystems (Dawson et al. 2002), stable isotopes have aided in food web analysis and the study of energy flow amongst trophic levels (Peterson & Fry 1987). However, the factors regulating the stable isotopic content of primary producers are complex, and require detailed knowledge of spatial, temporal, and taxonomic variation. For example, the stable carbon isotope content of seagrass material is predominantly controlled by the environmental factors of carbon source, irradiance, and temperature (Durako & Hall 1992, Abal et al. 1994, Grice et al. 1996, Hemminga & Mateo 1996). Spatial and temporal variation in these factors have been found to influence the carbon isotopic content of seagrass species across landscape scales (Fourqurean et al. 1997, Fourqurean et al. 2005), highlighting the importance of documenting

background variation when applied to food web studies. Numerous studies have detailed the impact of environmental conditions on intraspecific variation in stable isotope parameters. However, interspecific divergence, particularly amongst sympatric seagrasses, has received less attention and may be attributable to physiological/ecological distinctions between species. Stable isotope comparisons between co-occurring species may reveal important cues pertaining to how specific species process local resources.

Seagrasses fractionate the available pool of inorganic carbon based upon the degree of carbon demand relative to the degree of carbon supply. Experimental evidence has shown that for a given carbon supply, plants grown under high light conditions display increased photosynthetic rates and increased carbon demand, resulting in reduced discrimination against ^{13}C and heavier isotopic signatures (Cooper & Deniro 1989, Durako & Hall 1992). Similarly, for a given light level, experiments have found enriched carbon isotope signatures ($\delta^{13}C$) with decreases in carbon supply (Durako & Sackett 1993). These relationships provide environmental information pertaining to the light and inorganic carbon status of plant material. However, interspecific variation in the mechanisms by which plants process carbon can additionally impact $\delta^{13}C$ values (Farquhar et al. 1989), thus individual plant physiology may contribute to overall variation in stable carbon isotope values. Utilization of bicarbonate (which is isotopically distinct from CO_2) may impact $\delta^{13}C$ ratios (Hemminga & Mateo 1996), and may further contribute to variation in isotope values. Bicarbonate use is dependent upon species specific seagrass physiology (Invers et al. 1999), thus interspecific differences in stable isotope values may be attributed to the varied physiologies associated with HCO_3^- uptake. Systematic differences in the $\delta^{13}C$ ratios of leaves of co-occurring seagrass species, as documented between the Mediterranean

species *Posidonia oceanica* and *Cymodocea nodosa* (Fourqurean et al 2007), would suggest fundamental differences in the way that species interact with the available DIC pool.

Seagrass stable nitrogen content additionally provides important information pertaining to the identity of the sources of dissolved inorganic nitrogen (DIN), and the various processes which serve to fractionate the available nutrient pool. For example, as bacterial processing (nitrification, denitrification, and nitrogen fixation) alters the ratio of ^{15}N:^{14}N in the DIN pool, the macrophytic composition of stable nitrogen isotopes is influenced, and thus can be utilized to infer degrees of nitrogen cycling (Peterson & Fry 1987, Dawson et al. 2002). Due to this microbial processing, sewage derived nutrient inputs are isotopically heavy, which can be used to detect possible anthropogenic contributions to the DIN pool.

Similar to carbon isotope discrimination, seagrasses can fractionate the source pool of dissolved inorganic nitrogen upon plant uptake (Fourqurean et al. 2005). The degree of nitrogen fractionation depends upon the size of the DIN pool relative to plant demand (Fourqurean et al. 2005). Seasonal fluctuations in the $\delta^{15}N$ value of seagrass tissues have been detected, and have been attributed to changes in both the isotopic signature of the source pool, and the degree of plant fractionation as a response to seasonal productivities (Fourqurean et al. 2005). However, altered DIN fractionation between multiple species has yet to be documented.

In this paper, we document interspecific divergence in the elemental and stable isotope content of three seagrass species in South Florida, and explore environmental correlates of these variations across both local and broad spatial scales. Our aim is to better characterize the sources of variation in elemental and isotopic ratios of plants often used as environmental indicators, and highlight the role that ecological/physiological characteristics play in determining species specific values. Seagrass properties (N:P, limitation index, $\delta^{13}C$, and $\delta^{15}N$) were compared across

136 sites in South Florida, representing a 10,000 km^2 survey area of the seagrass beds in the Florida Keys National Marine Sanctuary (FKNMS). We were particularly interested in documenting interspecific variation in the properties of seagrasses co-occurring under similar environmental conditions, within a given site. We hypothesize that the varied life history strategies of benthic macrophytes would be reflected in plant elemental content, with fast-growing, early successional species displaying N:P ratios further removed from ideal values as compared to slow-growing species from the same site. We additionally hypothesize that species-specific differences in carbon uptake strategies and plant physiologies have the ability to strongly regulate stable isotope parameters. Lastly, we hypothesize that fast growing, early successional species may exhibit larger seasonal variation in stable isotope values as a result of increased growth rates and elevated carbon and nitrogen demand. Interspecific comparisons at both local (within site) and broad (amongst all sites) scales allowed us to examine the spatial extent to which taxonomic variation is important, and its impact on the isotopic and elemental composition of benthic plants.

METHODS

The Florida Keys National Marine Sanctuary (FKNMS) is a shallow-water, marine ecosystem located at the southern tip of the Florida peninsula and comprised of seagrass beds, coral reefs, and mangrove communities (Fig. 1). Seagrass communities in the FKNMS are primarily composed of *Thalassia testudinum*, *Halodule wrightii*, and *Syringodium filiforme*. During the summer months of 1999, 80 sites were randomly selected across the FKNMS, and surveyed for seagrass abundance, nutrient content and isotopic composition. A repeat survey was conducted during the summer of 2000 at 56 different randomly generated sites, which only

quantified seagrass abundance and nutrient content. Seasonal variation in seagrass isotopic content was additionally assessed through a separate series of quarterly surveys conducted during 1999 and 2000. Within each year, a network of 30 permanent monitoring stations was sampled 4 times (see Fourqurean et al. 2001 for further description). The elemental and isotopic composition of *T. testudinum* has been previously described for this region (Fourqurean et al 2005). In order to examine interspecific variation, our current study incorporates a portion of those data on the elemental and isotopic content of *T. testudinum*, with new data for the other two seagrass species (*S. filiforme* and *H. wrightii*) common in South Florida.

From the selected random sites, three separate comparative analyses were conducted which spanned various spatial and seasonal scales. One analysis included all data from the 1999 (80 sites) and 2000 (56 sites) surveys, representing comparisons within the summer season over large spatial scales. Both monospecific (22%) and mixed species (78%) seagrass beds were included. A second analysis included data from the 1999 and 2000 summer surveys, however monospecific sites were excluded, thus interspecific comparisons were solely conducted within multi-species sites where seagrasses co-occurred under similar environmental conditions. Each multi-species site contained two or more seagrass species growing adjacently (< 50m apart), under similar light and depth regimes. Multi-species sites were grouped according to pairwise interspecific comparisons: *Thalassia testudinum*/*Halodule wrightii* (65 sites), *Halodule wrightii*/*Syringodium filiforme* (38 sites), and *Thalassia testudinum*/*Syringodium filiforme* (75 sites). The third analysis similarly conducted within-site interspecific comparisons, however only utilized data from the 30 permanent monitoring sites, allowing for comparisons of intra-annual variability between species pairs. Within this network, the number of sites for interspecific

comparison varied depending upon season; *T. testudinum*/*H. wrightii* (4-7 sites), *H. wrightii*/*S.filiforme* (5-8 sites), *T. testudinum*/*S. filiforme* (19-22 sites).

For all surveys, at each sampling site, short shoots of each seagrass species present were haphazardly collected along a 50m transect. When available, 6 shoots of *Thalassia testudinum*, 30 shoots of *Syringodium filiforme*, and 40 shoots of *Halodule wrightii* were harvested, placed on ice, and transported back to the lab. The less robust seagrass species required higher collection amounts to ensure enough biomass was available for all elemental and isotopic analyses. Seagrass leaves were separated according to species, cleaned of epiphytes through gentle scraping with a razor blade, and cut from their respective short shoots. Leaves were then dried to a constant weight at 80°C, ground to a fine powder with a mortar and pestle, and analyzed in duplicate for C and N content using a CHN analyzer (Fourqurean et al. 2005). Phosphorus content was determined through dry oxidation, acid hydrolysis extraction followed by a colorimetric analysis (Fourqurean et al. 1992a). Elemental ratios were calculated on a mole:mole basis.

All isotopic analyses were measured using standard elemental analyzer isotope ratio mass spectrometer procedures. The elemental analyzer was used to combust all organic material and subsequently reduce the formed gasses 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 nitrogen (AIR,N_2) and Vienna Pee Dee belemnite (V-PDB) for carbon. Analytical reproducibility of the reported δ values, based on sample replicates, was better than ± 0.08‰ for carbon and ± 0.2‰ for nitrogen. Care was taken to remove all visible carbonate material from the surface of the leaves. As a test of the efficacy

of our cleaning we acidified a subset of seagrass samples with the most enriched $\delta^{13}C$ values to drive off any remaining carbonate material, and then determined the $\delta^{13}C$ of this decalcified material. The differences in $\delta^{13}C$ between acidified and unacidified samples were small (< 0.3 ‰ on average).

The distributions of all seagrass elemental and isotopic parameters were checked for normality using the Kolmogorov-Smirov test (α=0.05). Standard linear regression was used to test the strength of the relationship between stable isotope variables ($\delta^{13}C$, $\delta^{15}N$) and site depth. Relationships among elemental and isotopic ratios were additionally assessed for all species, across all sites sampled in 1999, using non-parametric correlations (Spearman's ρ). Interspecific Sanctuary-wide species-specific differences in seagrass N:P ratios and isotopic values were assessed using a single factor analysis of variance (ANOVA). Upon detecting significance, post-hoc analysis was conducted using either a Tukey's HSD for equal variances, or a Dunnett's T3 test for unequal variances (significance $p < 0.05$). In addition to testing N:P ratios, a Limitation Index (L.I. = |30- N:P|) was calculated to quantify the degree of divergence from the ideal ca. 30:1 "Seagrass Redfield Ratio" identified by Atkinson and Smith (1983) and Duarte (1990). Larger L.I. values indicate greater degrees of nutrient limitation. Such a calculation is necessary because across the landscape of the FKNMS there are both N- and P-limited regions (Fourqurean et al 2005).

To conduct within-site interspecific comparisons, differences in N:P ratios, L.I., $\delta^{13}C$, and $\delta^{15}N$ were tested with a pair-wise Students t-test, significance $p < 0.05$. To describe temporal variation in seagrass isotopic content for species that co-occurred at the 30 permanent stations, we fit a sine model of the form $y = \text{mean} + \alpha[\sin(\text{time} + \Phi)]$, where α is the amplitude of a sine wave, and Φ is a phase angle (we used time values in radians for both time and Φ, where 2π

radians = 365d), to a seasonal time series using an iterative nonlinear curve fitting regression. Confidence intervals (95%) of model parameters were compared to test for significant differences in seasonal variation. We were interested in using the non-linear regression as a means of testing interspecific divergence in the mean and amplitude of seasonal seagrass $\delta^{13}C$ variation.

RESULTS

Interspecific variation in seagrass elemental and isotopic composition at broad scales across the FKNMS

In 1999, *Thalassia testudinum* occurred at 78 of the randomly selected sites (98%), while *Halodule wrightii* and *Syringodium filiforme* occurred at 31 sites (39%) and 37 sites (46%) respectively. In 2000, *T. testudinum* occurred at 56 sites (100%), while *H. wrightii* and *S. filiforme* occurred at 39 sites (70%) and 40 sites (71%) respectively. Nitrogen, phosphorus and carbon content varied within each species, across all sanctuary sites in both years (Table 1), however they did not vary significantly between years. In both years, coefficients of variation show that leaf phosphorus content (CV=0.28-0.37) displayed greater variation in all species compared to nitrogen (CV=0.15-0.25) and carbon content (CV=0.06-0.12). The N:P frequency distribution for *T. testudinum* was slightly skewed towards higher values (Fig. 2). Comparatively, *H. wrightii* displayed a normal distribution with the highest mean N:P ratios (44.1 ± 1.8), which were reflected in the highest mean L.I. of all species (15.3 ± 1.7, Fig. 3, Table 1). *Syringodium filiforme* displayed an intermediate N:P ratio of 38.9 ± 1.3, which was normally distributed, and an average limitation index value of 10.9 ± 1.1. *Thalassia testudinum* displayed the lowest mean N:P ratio (36.5 ± 1.1), and the lowest mean L.I. value (9.6 ± 0.9) of the species. Mean *T.*

testudinum N:P ratio and L.I. values were significantly lower than those of *Halodule wrightii*, yet similar to *Syringodium filiforme* (Table 1). Mean *H. wrightii* N:P ratio and L. I. values were similar to *S. filiforme* (ANOVA, $F = 7.89$, $p = 0.07$; ANOVA, $F = 6.25$, $p = 0.09$).

Halodule wrightii displayed the lowest $\delta^{13}C$ values of all species, with a mean of -10.6 ‰ ± 0.3‰, a range of -13.2‰ to -7.8‰, and a normal distribution (Fig. 4). *Halodule wrightii* $\delta^{13}C$ values were significantly lower than both *Thalassia testudinum* and *Syringodium filiforme* (Table 1; ANOVA, $F = 53.5$, $p < 0.001$). *Syringodium filiforme* displayed the highest $\delta^{13}C$ values with a mean of -6.2‰ ± 0.2‰, a range of -3.5‰ to -8.4‰, and a normal distribution. The $\delta^{13}C$ values of *S. filiforme* were significantly higher than those of *T. testudinum* (ANOVA, $F = 53.5$, $p < 0.001$). *Thalassia testudinum* displayed intermediate $\delta^{13}C$ values with a mean of -8.6‰ ± 0.2‰, a range of -13.0‰ to -5.3‰, whose values were normally distributed.

Stable nitrogen isotope values varied by 7.7‰, 7.5‰, and 6.3‰ for *Thalassia testudinum*, *Halodule wrightii*, and *Syringodium filiforme* respectively (Table 1). *Thalassia testudinum* displayed the highest $\delta^{15}N$ values of all species, with mean of 2.0‰ ± 0.2‰ and a normal distribution (Fig. 5). *Thalassia testudinum* displayed $\delta^{15}N$ values which were higher than those of *H. wrightii* (Table 1; ANOVA, $F = 5.08$, $p < 0.01$), yet similar to *S. filiforme* (ANOVA, $F = 5.08$, $p = 0.45$). *Halodule wrightii* displayed the lowest values, with a mean of 1.0‰ ± 0.3‰ and a normal distribution. *Halodule wrightii* $\delta^{15}N$ values were similar to those of *S. filiforme* (ANOVA, $F = 5.08$, $p = 0.18$). *Syringodium filiforme* displayed intermediate values, with a mean of 1.6‰ ± 0.3‰ and a normal distribution.

Across FKNMS, elemental and isotopic ratios of all three seagrass species were correlated with site depth (Table 2, Fig. 6). The $\delta^{13}C$ and N:P ratios were negatively correlated to site depth for all species, and displayed similar variation with depth amongst species. The $\delta^{15}N$

ratio was positively correlated with site depth for *Halodule wrightii* and *Syringodium filiforme*, yet there was no correlation for *Thalassia testudinum* (Fig. 6). The variation in $\delta^{15}N$ with depth was similar between *H. wrightii* and *S. filiforme*, and further analysis revealed that negative correlations between %N and $\delta^{15}N$ were restricted to the deeper offshore locations (>4.3m). *Syringodium filiforme* was the only species for which $\delta^{13}C$ was correlated to $\delta^{15}N$. Correlations between elemental and isotopic ratios were mixed depending upon species. In *T. testudinum*, N:P was positively correlated to $\delta^{13}C$, yet uncorrelated to $\delta^{15}N$. *Syringodium filiforme* showed a negative correlation between N:P and $\delta^{15}N$, yet no correlation between N:P and $\delta^{13}C$. In *H. wrightii*, N:P was both negatively correlated to $\delta^{15}N$, and positively correlated to $\delta^{13}C$. L.I. values were negatively correlated to both site depth and $\delta^{15}N$ for *H. wrightii* and *S. filiforme*, *T. testudinum* showed no correlation amongst these parameters. For all species L.I. was positively correlated to $\delta^{13}C$.

Interspecific variation in seagrass elemental and isotopic composition at local scales

Of the sites which contained both *Thalassia testudinum* and *Halodule wrightii* (65 sites), N:P ratios and L.I. values were significantly higher for *H. wrightii* ($p < 0.001$; Fig. 7). When averaged for both years, *T. testudinum* had an N:P ratio and an L.I. value of 35.9 ± 1.4 and 8.8 ± 1.1 respectively at sites where it co-occurred with *H. wrightii*, while *H. wrightii* had an N:P ratio and an L.I. value of 44.5 ± 1.9 and 15.8 ± 1.8 at the same sites. When growing in similar locations, significant differences were additionally detected in $\delta^{13}C$ signatures, with *T. testudinum* (-8.9 ± 0.4) displaying isotopically heavier values than *H. wrightii* (-10.4 ± 0.3) ($p < 0.001$)(Fig. 8). There were no consistent, statistically significant differences in $\delta^{15}N$ between these two species at sites where they co-occurred.

Halodule wrightii and *Syringodium filiforme* co-occurred at 38 sites, and did not differ in either N:P ratios or L.I. (Fig. 7). For both sampling years, N:P ratios and L.I. were 43.9 ± 2.6 and 15.8 ± 2.2 respectively for *H. wrightii*, and 42.3 ± 2.2 and 14.2 ± 1.9 respectively for *S. filiforme*. Significant differences were detected in $\delta^{13}C$ signatures, with *H. wrightii* displaying a value of -10.1‰ ± 0.3‰, as compared to the isotopically heavy *S. filiforme* (-5.7‰ ± 0.3‰) ($p < .001$)(Fig. 8). There were no consistent, statistical differences in $\delta^{15}N$ between *H. wrightii* and *S. filiforme* at sites where they co-occurred.

Thalassia testudinum and *Syringodium filiforme* co-occurred at 75 sites, and displayed significant differences in both N:P ratios and L.I. at those sites ($p < 0.001$, $p < 0.01$ respectively). For both years, *T. testudinum* displayed an N:P ratio and L.I. value of 34.2 ± 1.1 and 7.4 ± 0.9, respectively. While *S. filiforme* displayed an N:P ratio and L.I. of 38.8 ± 1.4 and 10.9 ± 1.2, respectively (Fig. 7). Additionally, $\delta^{13}C$ signatures were statistically distinct, with *T. testudinum* (-8.8‰ ± 0.2‰) displaying isotopically lighter values than *S. filiforme* (-6.3‰ ± 0.2‰) ($p < 0.001$) (Fig. 8). There were no statistical differences in $\delta^{15}N$ between *T. testudinum* and *S. filiforme* at sites where they co-occurred.

Temporal variation in seagrass isotopic content at local scales

There was marked seasonal variation in the $\delta^{13}C$ values of *Thalassia testudinum*, *Halodule wrightii*, and *Syringodium filiforme* (Fig. 9). $\delta^{13}C$ values for all three species were heaviest in the summer and fall months, and lightest in the winter months. The sine models described 51-73% of the variation in $\delta^{13}C$ values (Table 3). Parameter estimates for α and Φ were not significantly different between species comparisons, indicating no interspecific differences in the degree of seasonal variation, nor the seasonal timing of $\delta^{13}C$ values. Mean $\delta^{13}C$

values of the sine models were significantly different for all species comparisons, indicating that the interspecific differences we documented above with our FKNMS-wide summer surveys were maintained throughout the year. Seasonal trends in $\delta^{15}N$ values were not detected for any species.

DISCUSSION

The elemental and isotopic leaf content of the seagrasses *Thalassia testudinum*, *Halodule wrightii*, and *Syringodium filiforme* displayed marked interspecific variation at both local and broad spatial scales within the Florida Keys National Marine Sanctuary. Both within and between species variations were nonrandom, and demonstrated both the effects of environmental parameters and species-specific physiologies on the elemental and isotopic content of seagrass tissues. This study highlights the importance of seagrass species identity in the evaluation of plant nutrient and isotopic data for coastal monitoring efforts and food web analyses.

Relationships between elemental ratios, isotopic ratios, and water depth across the FKNMS

Significant differences in $\delta^{13}C$ were detected amongst all three seagrass species. The lightest $\delta^{13}C$ values were displayed by *Halodule wrightii*, while *Thalassia testudinum* and *Syringodium filiforme* displayed the intermediate and heaviest values respectively. Similarly, Lepoint et al. (2008) found that *Syringodium isoetifolium* was more enriched in ^{13}C compared to *Halodule* sp. and *Thalassia hemprichii* in Mozambique. The heaviest $\delta^{15}N$ was displayed by *T. testudinum*, which was significantly more positive than *H. wrightii*. The $\delta^{15}N$ value for *S. filiforme* was not statistically distinct from the other species. Large scale trends in the stable isotopic content of various seagrasses may provide information pertaining to physiological and

ecological properties of each species. However, data at this large scale should be interpreted with caution, as trends may be confounded by spatial variations in: (1) the distribution of various seagrass species and (2) environmental conditions.

All three seagrass species showed significant correlations between $\delta^{13}C$ values and site depth across the large spatial scale of the sanctuary (Fig. 6). Because light availability generally decreases with depth in the sea, this suggests that for each species, light plays an important role in regulating the $\delta^{13}C$ content of seagrass tissues, as demonstrated in both laboratory and field studies (Durako & Hall 1992, Abal et al. 1994, Grice et al. 1996). Likely this is because of reduced photosynthetic discrimination against the heavier isotope, leading to increased $\delta^{13}C$ values at high light levels. Within a species, isotopically heavy values may indicate the possibility of photosynthetic carbon limitation during periods of high irradiance (Fourqurean et al. 2005). Field studies have shown that the inverse relationship between light and depth are reflected in the $\delta^{13}C$ ratio of seagrass tissues elsewhere (Cooper & Deniro 1989, Lepoint et al. 2003, Fourqurean et al. 2007). In our study, $\delta^{13}C$ values decrease by 0.40, 0.29, and 0.25 ‰ m^{-1} in depth for *Thalassia testudinum*, *Halodule wrightii*, and *Syringodium filiforme* respectively. We don't think that the $\delta^{13}C$-depth relationships are a function of variations in the isotopic signature of the DIC pool, as has been observed in regions where mineralization of organic matter from C_3 mangroves causes isotopically depleted DIC that is incorporated into seagrass tissues (Fry & Sherr 1984, Fleming et al. 1990, Lin et al. 1991). The FKNMS sites that are in close proximity to terrestrial DIC sources display the heaviest $\delta^{13}C$ values, contrary to what would be expected if C_3 material was impacting the isotopic value of the DIC pool available to the seagrass community.

The $\delta^{15}N$ value of seagrass leaf material can be controlled by numerous factors. In addition to alterations in the isotopic composition of the source nitrogen pool, the relationship between plant nutrient demand and environmental availability represents a strong determinant of leaf isotope ratios. Reduced light as depth increases could, by reducing leaf growth rates and demand for N, influence leaf $\delta^{15}N$ as depressed demand increases discrimination against the heavier isotope. However, the net change in leaf $\delta^{15}N$ with increasing depth should be a consequence of the magnitude of the reduction in N demand relative to the changes in N availability. For instance, in cases where N availability remains constant with increasing depth, we would expect a decrease in leaf $\delta^{15}N$ with increasing depth due to a depressed demand for N and higher discrimination against ^{15}N. Alternatively, cases in which leaf $\delta^{15}N$ shows little change with depth may represent scenarios whereby both N demand and N availability concurrently decrease with depth, resulting in little alteration to ^{15}N discrimination. Working with other species of seagrasses, Grice et al (1996), Lepoint et al (2003), and Fourqurean et al (2007) all found no significant change in leaf $\delta^{15}N$ across large depth and light gradients, although they did not investigate the causal mechanisms for those observations. Lastly, if nitrogen availability decreases dramatically with increasing depth, we could, despite reductions in N demand, find higher leaf $\delta^{15}N$ values as depth increases. We believe this to be the case in our study, as the observed relationships between $\delta^{15}N$ and depth are a consequence of the distribution of deep-water sites within a landscape of variable nitrogen availability. The leaf $\delta^{15}N$ value of both *Halodule wrightii* and *Syringodium filiforme* was positively correlated to site depth, and negatively correlated to both N:P ratios and L.I. Such correlations suggest that as nitrogen becomes less available and nutrient limitation increases, these seagrasses reduce fractionation of the available DIN pool. Additionally, the negative correlation between %N and $\delta^{15}N$ was only

significant for the deeper sites under similar light regimes, further indicating that environmental availability was driving the relationship between depth and $\delta^{15}N$. This indicates that the reduced DIN discrimination in *H. wrightii* and *S. filiforme* increasingly occurs at deeper offshore locations, where primary production is N-limited (Fourqurean & Zieman 2002, Ferdie & Fourqurean 2004, Fourqurean et al. 2005). This trend, however, was not held for *Thalassia testudinum* which exhibited no correlation between $\delta^{15}N$ values and site depth or N:P ratios. Thus, these correlations were only evident for the fastest growing seagrasses (*H. wrightii* and *S. filiforme*), which may utilize nutrient resources more rapidly than *T. testudinum*, leading to decreases in DIN fractionation and higher L.I. compared to the slower-growing *T. testudinum*. Further experimentation is needed to detail these processes.

The fact that interspecific differences in stable carbon isotopes remain statistically distinct across large spatial scales may imply that seagrass physiology plays a dominant role in determining interspecific variation in $\delta^{13}C$. Average values for *Halodule wrightii* were 2.0 ‰ lighter than *Thalassia testudinum*, and 4.5 ‰ lighter than *Syringodium filiforme*. Such differences might be attributed to the varied mechanisms of bicarbonate acquisition and internal carbon recycling within marine macrophytes (Fry & Sherr 1984, Fry et al. 1985, Hemminga & Mateo 1996, Fourqurean et al. 2007), or caused by morphological variations in leaves (Lepoint et al. 2008). To various degrees, seagrass photosynthetic carbon demand may be met via a combination of diffusive CO_2 transport, and active HCO_3^- import (Invers et al. 1999, Invers et al. 2001). While dissolved CO_2 has an isotopic carbon signature of ca. -9 ‰, HCO_3^- in marine waters has an isotopic signature of 0 ‰. Preferential fixation of bicarbonate from the inorganic carbon pool should result in seagrass tissue which is enriched in ^{13}C (Hemminga & Mateo 1996). Interspecific variation in seagrass $\delta^{13}C$ values may reflect previously documented interspecific

variation in bicarbonate acquisition mechanisms, as some species display enhanced HCO_3^- utilization efficiencies (Bjork et al. 1997, Invers et al. 1999, Schwarz et al. 2000, Uku et al. 2005). Our field data suggest that *S. filiforme* may rely more on HCO_3^- as a carbon source than *H. wrightii* or *T. testudinum*, an hypothesis that should be explored.

Internal recycling of CO_2 has been proposed as an alternate mechanism which can influence the stable carbon isotope value of benthic macrophytes (Cooper 1989, Abal et al. 1994, Grice et al. 1996). Seagrass species which have enhanced lacunal volume may display increased recycling of internal carbon pools, and reduced isotopic discrimination. As internal recycling of CO_2 reduces the degree of carbon back-diffusion, RUBISCO operates in an increasingly closed environment, converting all inorganic carbon into organic products, resulting in isotopically heavier $\delta^{13}C$ signatures (Sharkey & Berry 1985). Internal recycling may therefore serve to complicate the relationship between the $\delta^{13}C$ of source DIC, and the $\delta^{13}C$ of seagrass tissues. Our data are consistent with more efficient internal recycling of CO_2 in *Syringodium filiforme* than *Halodule wrightii* or *Thalassia testudinum*. It is evident that the factors contributing to the characteristic $\delta^{13}C$ value of specific seagrass species are complex, and require studies focused on detailing the contributions that carbon acquisition mechanisms, seagrass lacunal volume, and rates of CO_2 recycling make to the overall isotopic signature.

Interspecific variation in $\delta^{15}N$ across FKNMS reveal significant differences between *Thalassia testudinum* and *Halodule wrightii*, however it remains unclear whether this trend is due to differences in the degree of isotope fractionation among species, or due to spatial trends in the isotopic composition of source DIN and seagrass abundance. Across a large spatial scale, *T. testudinum* was isotopically heavier than *H. wrightii*, indicating decreased fractionation amongst nitrogen isotopes. Successional studies have demonstrated *T. testudinum* as a climax species with

the ability to thrive in nutrient poor environments, and *H. wrightii* as an early successional species thriving under more eutrophic conditions (Fourqurean et al. 1995). Across large spatial scales, *T. testudinum* may monospecifically occupy the most oligotrophic locations with the lowest DIN pools, resulting in decreased fractionation of the nitrogen isotope, accounting for heavier $\delta^{15}N$ values; *H. wrightii*, occupying areas with higher DIN pools may have higher rates of fractionation, lowering $\delta^{15}N$ values. Such results are not contradictory to previous findings concerning the correlation between $\delta^{15}N$ and depth. Despite *H. wrightii* displaying decreased $\delta^{15}N$ fractionation with depth, overall this species remains isotopically lighter than *T. testudinum* because it is excluded from the highly oligotrophic deeper waters solely occupied by *T. testudinum*.

Elemental comparisons across FKNMS reveal that *Thalassia testudinum* had significantly lower N:P ratios and L.I. than *Halodule wrightii* ($p < 0.01$), while *Syringodium filiforme* was not statistically distinct from either *T. testudinum* or *H. wrightii*. Overall, intraspecific variation at this scale is attributable to the balance between the availability nutrient resources (N and P) and rates of seagrass productivity (Fourqurean et al. 2005). However interspecific differences in the N:P ratios of *T. testudinum* and *H. wrightii* may result from life history differences between these 2 species. The early successional status and higher growth rates of *H. wrightii* may account for increased deviation from seagrass Redfield N:P stoichiometry. Nutrient demand is higher for *H. wrightii* than for *T. testudinum* (Fourqurean et al. 1992b), thus despite possibly being limited to sites elevated in nutrient concentrations, the fast growth rates of *H. wrightii* still produce N:P ratios which are drastically altered from Seagrass Redfield values. Ecologically, *T. testudinum* is a late successional species, and the life history strategy of reduced growth rates may allow *T. testudinum* to produce biomass with N:P ratios closer to the ideal Redfield value of 30:1. The

extensive investment in underground biomass (root/rhizome complex) may additionally allow *T. testudinum* to exploit sediment nutrient pools unavailable to other species, thus bringing stoichiometric ratios closer to 30:1. However, across landscape scales, elemental variation due to life history differences are difficult to separate from elemental variation due to spatial trends in nutrients, light, and seagrass abundance. For example, if *T. testudinum* were relatively more abundant in deeper, lower light environments, then lower productivities and reduced N:P ratios may be attributable to this spatial environmental factor, and not species-specific physiological/ecological properties.

Interspecific variation at local scales within the FKNMS

Within site, local comparisons between species control for spatial gradients in abiotic factors (light and nutrients), hence differences in elemental and isotopic compositions do not reflect environmental variation, and may be attributed to differences in species specific physiological and ecological properties. Congruence or discordance of local trends with broad spatial trends reveals whether interspecific variation is due to physiological attributes or wide ranging abiotic variation. We find that at the local scale, interspecific trends in stable carbon isotopic content and elemental ratios are generally held, while trends in stable nitrogen isotopic content are not consistent with the trends observed at broad spatial scales.

Local scale interspecific trends in $\delta^{13}C$ agree with broad scale trends, revealing that species specific physiological attributes are dominant factors in controlling taxon specific $\delta^{13}C$ values. At the same location, *Halodule wrightii* was significantly lighter than both *Thalassia testudinum* and *Syringodium filiforme*, and *T. testudinum* was significantly lighter than *S. filiforme*, yet heavier than *H. wrightii* (Fig. 8), suggesting that the interspecific trends at the

broad scale of the FKNMS are functions of the physiological attributes of carbon acquisition. Utilizing this model, *H. wrightii* may represent a seagrass species which, while displaying HCO_3^- use, exhibits high rates of carbon back diffusion (low CO_2 recycling), and thus fails to fix a large portion of incorporated carbon, allowing for increased isotopic discrimination. Conversely, *S. filiforme* may represent a species which displays reduced carbon back diffusion (high CO_2 recycling), and thus fixes a large majority of imported carbon. The apparent dependence of seagrass $\delta^{13}C$ on specific carbon acquisition properties has terrestrial analogues, as seen in the $\delta^{13}C$ variation between C_3 and C_4 plants. In addition to utilizing PEP carboxylase to fix CO_2, which discriminates less against ^{13}C than RUBISCO, C_4 plants limit carbon diffusion out of the leaves with morphological adaptations (e.g. bundle sheaths), and variations in the rates of CO_2 back diffusion have been noted to impact the $\delta^{13}C$ values of C_4 vegetation (Farquhar et al. 1989). While seagrasses are all C_3 plants (Beer & Wetzel 1982), the degree of bicarbonate use, extent of internal recycling, and specific leaf morphology (Lepoint et al. 2008) are likely to play analogous roles in explaining interspecific variation in the $\delta^{13}C$ value of marine plants.

Local scale interspecific trends in stable nitrogen isotope values displayed non-significant differences amongst all seagrass species. The significant differences observed between *Thalassia testudinum* and *Halodule wrightii* at the broad scale were not observed within sites, indicating that $\delta^{15}N$ trends across FKNMS were predominantly a result of spatial variation in seagrass distribution and DIN pools, as opposed to interspecific physiological differences in nitrogen uptake and fractionation. However, we note that the reduced sampling effort of the within site comparisons may have limited our ability to detect a significant difference in $\delta^{15}N$ between *T. testudinum* and *H. wrightii*, warranting future studies.

Interspecific variation in elemental ratios revealed that, similar to broad scale patterns, *Thalassia testudinum* displayed significantly lower N:P ratios than *Halodule wrightii* when growing at the same location. At the local scale, it was additionally found that *T. testudinum* had significantly lower N:P ratios than *S. filiforme*, previously undocumented at broader scales. There remained no difference in N:P ratios between *H. wrightii* and *S. filiforme*. By removing spatial variation in environmental variables and seagrass distribution, interspecific differences in elemental composition reflect important differences in the ecology of these seagrass species. When co-occurring with *T. testudinum*, and exposed to similar nutrient and light conditions, rapidly growing early successional species (*H. wrightii* and S. *filiforme*) are further removed from Seagrass Redfield stoichiometry, indicating heavily nutrient limited growth (particularly with respect to phosphorus). The slower growth rates of *T. testudinum* may allow for reduced nutrient limited growth, and an enhanced ability to thrive under oligotrophic conditions. The altered elemental ratios of co-occurring seagrasses further suggests that nutrient limitation needs to be viewed in respect to a specific primary producer, and may not be applicable to other species within the same location.

Temporal variation in seagrass isotope content

As previously documented for *Thalassia testudinum* (Fourqurean et al. 2005) the $\delta^{13}C$ of *Halodule wrightii* and *Syringodium filiforme* fluctuate seasonally. Interspecific variation in seagrass $\delta^{13}C$ value was not limited to the summer conditions of high productivity, as we would expect if differential CO_2 acquisition were leading to different amounts of isotopic discrimination during summer periods of maximum photosynthesis. Interspecific differences in $\delta^{13}C$ were maintained throughout the year, with peaks during the highly productive summer

periods and lows during the less productive winter periods. The constant interspecific variation suggests that species specific $\delta^{13}C$ values are a result of physiologically based traits, and are unlikely the result of altered or adaptive carbon acquisition mechanisms during the summer months. Thus, despite seasonal variation, seagrass $\delta^{13}C$ values remain significantly distinct throughout the year, and maintain the similar trends (*S. filiforme* > *T. testudinum* > *H. wrighii*) found during the summer surveys.

Seasonal variation in seagrass $\delta^{13}C$ values have been correlated to seasonal patterns in productivity, governed both by light availability and temperature (Fourqurean et al. 2001, Fourqurean et al. 2005, Fourqurean et al. 2007). Drawdown of CO_2 pools during the summer months, combined with limited CO_2 diffusion rates, may decrease the degree of fractionation displayed by RUBISCO. Similar seasonal trends have been noted for a number of phytoplankton studies, and may indicate carbon limitation during periods of high productivity (Fogel et al. 1992). Alternately, it is possible that increased summer productivities may enhance bicarbonate use within all seagrass species, elevating $\delta^{13}C$ values and mitigating carbon limited photosynthesis. The amplitudes of the sine models were not statistically distinct among species, thus there was no difference in the degree of seasonal variation. These findings contrasted with our original hypotheses, which expected the fastest growing seagrass species (*H. wrightii* and *S. filiforme*) to exhibit the greatest amplitude in seasonal variation, suggesting that while taxonomic differences strongly influence the mean $\delta^{13}C$ value, seasonal environmental factors strongly control annual variation within each species. There were no interspecific differences in Φ, and therefore the timing of the seasonal response (summer peaks and winter lows) amongst species. It is evident that while seasonal responses are not different in all three seagrass species, their mean $\delta^{13}C$ value remains markedly distinct throughout the annual cycle.

Seasonal variation in seagrass $\delta^{15}N$ was undetected for all species. Seasonal cycles in stable nitrogen isotope content have been previously documented for *Thalassia testudinum* in South Florida (Fourqurean et al. 2005), thus the lack of a seasonal pattern in our study may be due to an insufficient sampling size for this particular isotope parameter.

The elemental and stable isotopic content of seagrass species in South Florida displays considerable interspecific variation at both broad and local spatial scales. Here we present the first documentation of species specific variation in South Florida; by which carbon isotopic values may be associated with physiological differences in carbon acquisition and elemental ratios may be associated with ecological differences in seagrass growth rates. As such, the use of these parameters as indicators of ecosystem properties needs to carefully consider the species of marine plant being studied. In addition to previously documented spatial and temporal variation, taxonomic differences in isotopic and elemental content can introduce additional variation of significant magnitude. Interspecific differences are non-random, thus understanding how seagrass species differ in isotopic and elemental content will aid in the interpretation of studies which examine the food web and nutrient dynamics of multi-species seagrass ecosystems.

ACKNOWLEDGEMENTS

This work was supported by the Florida Keys Water Quality Protection Program Seagrass Monitoring Project funded by grants from NOAA's South Florida Program (contract no. NA06NOS4780105) and the US EPA (Assistance Agreement X797468102). This manuscript benefited by discussions with post-docs, students and technicians in JWF's laboratory. Among the many people who collected and processed data as part of the larger project, we are especially indebted to L. Rutten, S. Escorcia and P. Parker. W.T. Anderson at the FIU Stable Isotope

Laboratory analyzed our stable isotope samples. This is contribution number xx from the Southeast Environmental Research Center at Florida International University.

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Table 1: Elemental and stable isotopic composition of seagrass leaves collected across FKNMS in 1999 and 2000 (L.I. = Limitation Index). Superscripts on means within a column identify significantly different groups (post-hoc tests, $p<0.05$)

	$\delta^{15}N$	$\delta^{13}C$	%C (% dry wt)	%N (% dry wt)	%P (% dry wt)	C:N	C:P	N:P	L.I.
Thalassia testudinum									
Mean	2.0 ^{A}	-8.6 ^{A}	39.2 ^{A}	1.9 ^{A}	0.13 ^{A}	24.1 ^{A}	870.8 ^{A}	36.5 ^{A}	9.6 ^{A}
n	78	78	134	134	134	134	134	134	134
SE	0.15	0.20	0.20	0.02	0.00	0.31	26.28	1.07	0.88
C.V.	0.70	0.20	0.06	0.15	0.28	0.15	0.35	0.34	1.07
Median	1.8	-8.3	39.6	1.9	0.13	24.0	783.7	34.4	6.2
Minimum	-2.2	-13.0	31.1	1.4	0.06	17.1	500.3	17.1	0.2
Maximum	5.4	-5.3	43.2	2.6	0.22	33.9	1902.3	76.5	46.5
Halodule wrightii									
Mean	1.0 ^{B}	-10.6 ^{B}	43.4 ^{B}	2.3 ^{B}	0.13 ^{A}	22.7 ^{A}	1014.1 ^{B}	44.1 ^{B}	15.3 ^{B}
n	31	31	70	70	70	70	70	70	70
SE	0.29	0.28	0.27	0.05	0.01	0.44	51.76	1.82	1.67
C.V.	1.70	0.15	0.05	0.17	0.37	0.16	0.43	0.35	0.91
Median	0.9	-10.8	43.9	2.26	0.12	22.5	890.9	39.7	9.7
Minimum	-3.5	-13.2	35.0	1.48	0.05	16.7	472.3	23.7	0.3
Maximum	4.0	-7.8	46.3	3.18	0.25	33.9	2572.2	94.9	64.9
Syringodium filiforme									
Mean	1.6 ^{AB}	-6.2 ^{C}	38.9 ^{A}	2.1 ^{C}	0.13 ^{A}	22.8 ^{A}	866.0 ^{A}	38.9 ^{AB}	10.9 ^{AB}
n	37	37	77	77	77	77	77	77	77
SE	0.25	0.21	0.55	0.06	0.00	0.56	27.89	1.35	1.13
C.V.	0.96	0.20	0.12	0.25	0.30	0.22	0.28	0.30	0.91
Median	1.6	-6.2	41.0	2.2	0.12	21.3	846.3	37.7	8.3
Minimum	-1.6	-8.4	27.0	0.9	0.06	15.9	417.8	19.2	0.2
Maximum	4.7	-3.5	44.4	3.2	0.24	36.7	1576.4	77.2	47.2
ANOVA statistics for differences between species									
Between Group MS (df)	11.1 (2)	165.8 (2)	502.5 (2)	2.9 (2)	0.0 (2)	60.9 (2)	553539.6 (2)	1337.4 (2)	783.2 (2)
Within Group MS (df)	2.1 (143)	2.6 (143)	10.5 (278)	.15 (278)	0.0 (278)	16.2 (278)	107213.3 (278)	169.4 (278)	125.3 (278)
F-ratio	5.2	65.0	48.1	19.0	0.2	3.7	5.2	7.9	6.3
P-value	**<0.01**	**<0.01**	**<0.01**	**<0.01**	0.855	**<0.05**	**<0.01**	**<0.01**	**<0.01**

Table 2: Correlations (non-parametric Spearman's ρ) among elemental content, stable isotopic ratios, and water depth for all three species. Correlation coefficients are designated above the diagonal, P values for the pairwise comparisons are below the diagonal. Significant ($P < 0.05$) correlations are indicated in bold.

Thalassia testudinum	Depth	$\delta^{15}N$	$\delta^{13}C$	C:N	C:P	N:P	L.I.
Depth		0.113	**-0.655**	**0.377**	-0.131	**-0.322**	-0.208
$\delta^{15}N$	0.326		0.162	**0.272**	-0.073	-0.182	-0.012
$\delta^{13}C$	**<0.001**	0.158		-0.050	**0.327**	**0.400**	**0.407**
C:N	**0.001**	**0.016**	0.665		**0.281**	-0.159	0.057
C:P	0.255	0.526	**0.003**	**0.013**		**0.871**	**0.624**
N:P	**0.004**	0.110	**<0.001**	0.163	**<0.001**		**0.700**
L.I.	0.067	0.919	**<0.001**	0.621	**<0.001**	**<0.001**	

Halodule wrightii	Depth	$\delta^{15}N$	$\delta^{13}C$	C:N	C:P	N:P	L.I.
Depth		**0.415**	**-0.609**	-0.090	**-0.391**	**-0.466**	**-0.547**
$\delta^{15}N$	**0.020**		-0.210	**0.494**	-0.052	**-0.407**	**-0.423**
$\delta^{13}C$	**<0.001**	0.258		**0.364**	**0.631**	**0.559**	**0.627**
C:N	0.632	**0.005**	**0.044**		**0.613**	0.173	0.163
C:P	**0.029**	0.781	**<0.001**	**<0.001**		**0.861**	**0.809**
N:P	**0.008**	**0.023**	**0.001**	0.353	**<0.001**		**0.958**
L.I.	**0.001**	**0.018**	**<0.001**	0.381	**<0.001**	**<0.001**	

Syringodium filiforme	Depth	$\delta^{15}N$	$\delta^{13}C$	C:N	C:P	N:P	L.I.
Depth		**0.650**	**-0.477**	0.321	-0.211	**-0.459**	**-0.500**
$\delta^{15}N$	**<0.001**		**-0.407**	**0.631**	0.041	**-0.390**	**-0.412**
$\delta^{13}C$	**0.003**	**0.012**		0.019	0.253	0.279	**0.360**
C:N	**0.052**	**<0.001**	0.909		**0.377**	-0.299	-0.203
C:P	0.210	0.812	0.131	**0.022**		**0.733**	**0.668**
N:P	**0.004**	**0.017**	0.095	0.072	**<0.001**		**0.885**
L.I.	**0.002**	**0.011**	**0.029**	0.228	**<0.001**	**<0.001**	

Table 3: Parameter estimates for non-linear regressions of pairwise seagrass $\delta^{13}C$ seasonal data from 1999-2000 at 30 permanent monitoring stations in Florida Keys National Marine Sanctuary.

non linear regression Species Comparison	equation	r^2	Parameter estimates (95% confidence interval) mean	amp	Φ
T.testudinum	Y = -8.94 + 0.54 sin(DOY radians + 3.71)	0.55	(-9.41 , -8.48)	(-.140 , 1.22)	(2.55 , 4.88)
H. wrightii	Y = -11.02 + 0.83 sin(DOY radians + 4.28)	0.56	(-11.79 , -10.24)	(-.208 , 1.87)	(2.92 , 5.64)
H. wrightii	Y= -10.90 +0.69 sin(DOY radians + 4.19)	0.55	(-11.55 , -10.25)	(-.193 , 1.57)	(2.83 , 5.55)
S. filiforme	Y= -6.67 + 0.83 sin(DOY radians + 4.28)	0.55	(-7.45 , -5.88)	(-.224 , 1.89)	(2.9 , 5.66)
T. testudinum	Y= -8.96 +0.43 sin(DOY radians + 3.59)	0.73	(-9.2 , -8.71)	(.068 , .797)	(2.84 , 4.35)
S. filiforme	Y= -6.99 +0.61 sin(DOY radians + 4.73)	0.51	(-7.62 , -6.36)	(-.216 , 1.43)	(3.16 , 6.31)

FIGURE CAPTIONS

Figure 1. Map of study area showing locations of both survey sites and permanent monitoring sites. Survey sites have been designated as either single- or multi-species seagrass beds.

Figure 2. *Thalassia testudinum, Halodule wrightii,* and *Syringodium filiforme*. Frequency distributions of elemental ratios analyzed during 1999 and 2000 across FKNMS.

Figure 3. *Thalassia testudinum, Halodule wrightii,* and *Syringodium filiforme*. Frequency distributions of Limitation Index values analyzed during 1999 and 2000 across FKNMS.

Figure 4. *Thalassia testudinum, Halodule wrightii,* and *Syringodium filiforme*. Frequency distributions of stable carbon isotopes analyzed during 1999 across FKNMS.

Figure 5. *Thalassia testudinum, Halodule wrightii,* and *Syringodium filiforme*. Frequency distributions of stable nitrogen isotopes analyzed during 1999 across FKNMS.

Figure 6. *Thalassia testudinum, Halodule wrightii,* and *Syringodium filiforme*. Relationship between the $\delta^{13}C$ and $\delta^{15}N$ isotope ratios and depth. Linear regression and 95% confidence interval of the regression are indicated.

Figure 7. *Thalassia testudinum, Halodule wrightii,* and *Syringodium filiforme*. Interspecific comparisons in N:P ratios and Limitation Index values where species co-occur. Error bars are ± 1 SE. Significant differences between species are indicated (paired T-tests, ** = $p < 0.01$). The numbers of sites at which the species pairs co-occurred are given over each pair of bars.

Figure 8. *Thalassia testudinum, Halodule wrightii,* and *Syringodium filiforme*. Interspecific comparisons in $\delta^{13}C$ and $\delta^{15}N$ isotope ratios where species co-occur. Error bars are ± 1 SE. Significant differences between species are indicated (paired T-tests, ** = $p < 0.01$).

The numbers of sites at which the species pairs co-occurred are given over each pair of bars.

Figure 9. *Thalassia testudinum, Halodule wrightii,* and *Syringodium filiforme*. Pairwise interspecific comparisons of seasonal patterns in $\delta^{13}C$ of green leaves at the 30 permanent monitoring stations for co-occurring species. Each point represents the mean of the sites where both species of the pairwise comparisons co-occurred. Error bars represent ± 1 SE. The best fit sine model of the form y = mean + amp x sin(time + Φ) is shown, where amp is the amplitude of a sine wave and Φ is a phase angle in radians (2π radians = 365 d).

Figure 1

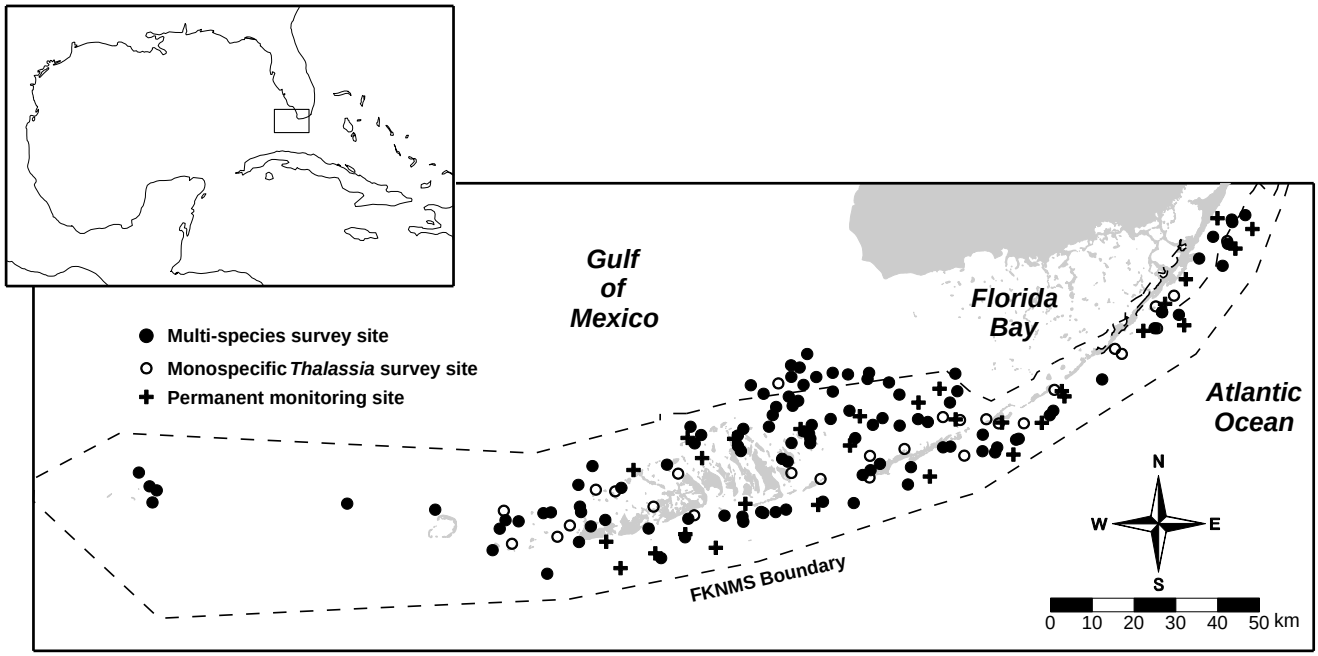

Gulf
of
Mexico
Florida
Bay
Atlantic
Ocean
Multi-species survey site
Monospecific *Thalassia* survey site
Permanent monitoring site
FKNMS Boundary
N
W
E
S
0 10 20 30 40 50 km

Figure 2

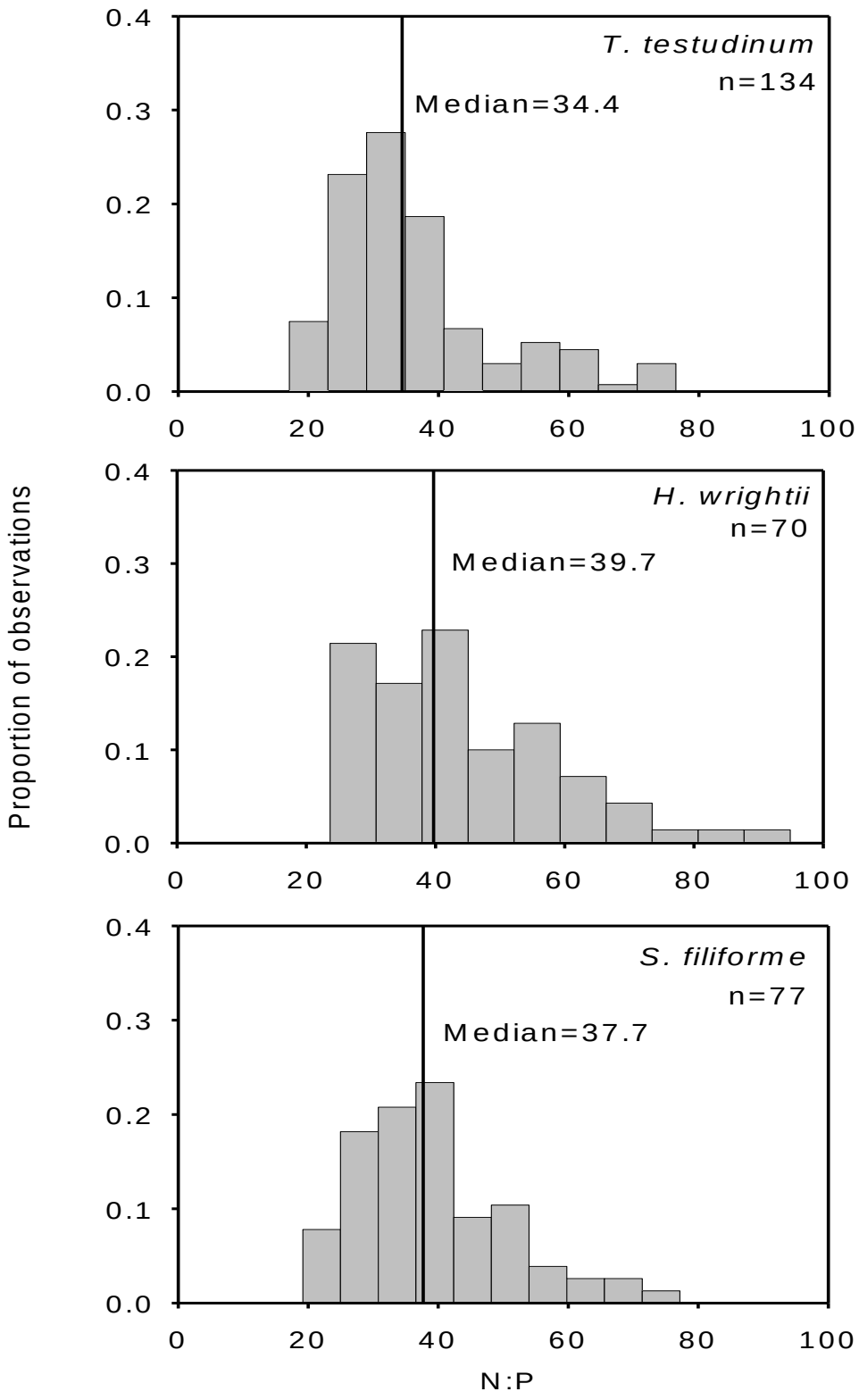

T. testudinum
n=134
Median=34.4
H. wrightii
n=70
Median=39.7
S. filiforme
n=77
Median=37.7
Proportion of observations
N:P
0.0
0.1
0.2
0.3
0.4
0
20
40
60
80
100

Figure 3

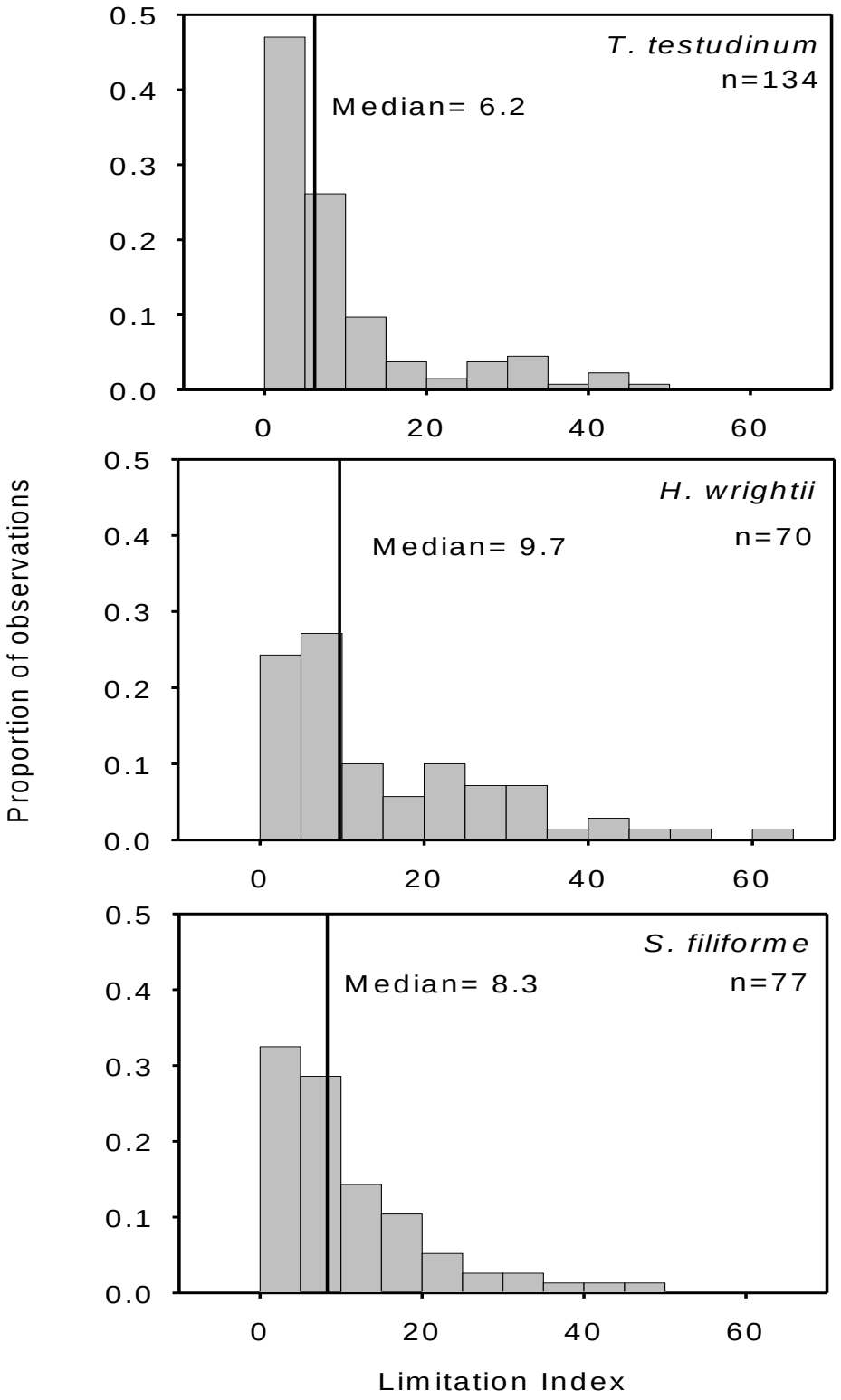

T. testudinum
n=134
Median= 6.2
H. wrightii
n=70
Median= 9.7
S. filiforme
n=77
Median= 8.3
Proportion of observations
Limitation Index
0.0
0.1
0.2
0.3
0.4
0.5
0
20
40
60

Figure 4

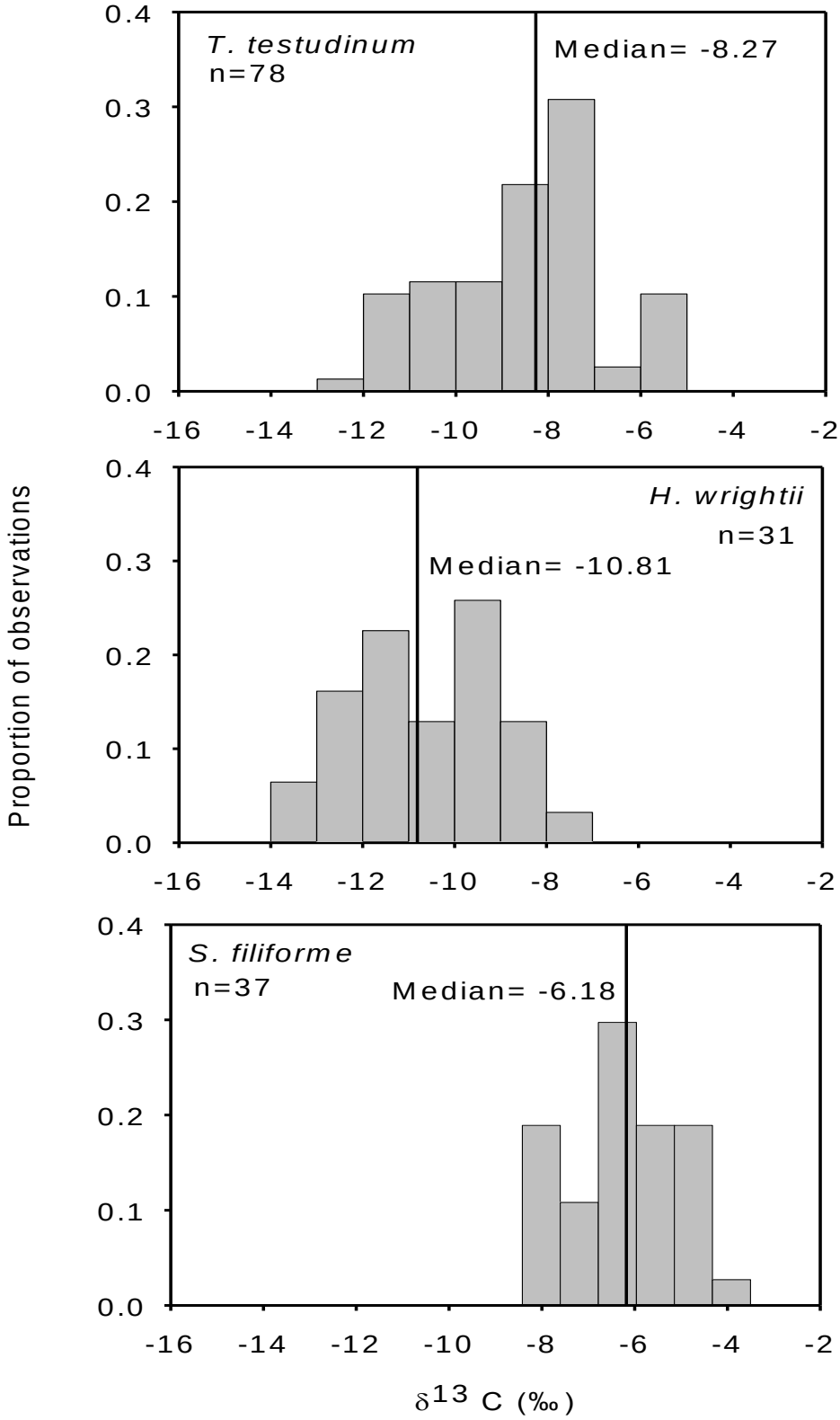

T. testudinum
n=78
Median= -8.27
H. wrightii
n=31
Median= -10.81
S. filiforme
n=37
Median= -6.18
Proportion of observations
0.0
0.1
0.2
0.3
0.4
-16
-14
-12
-10
-8
-6
-4
-2
δ13 C (‰)

Figure 5

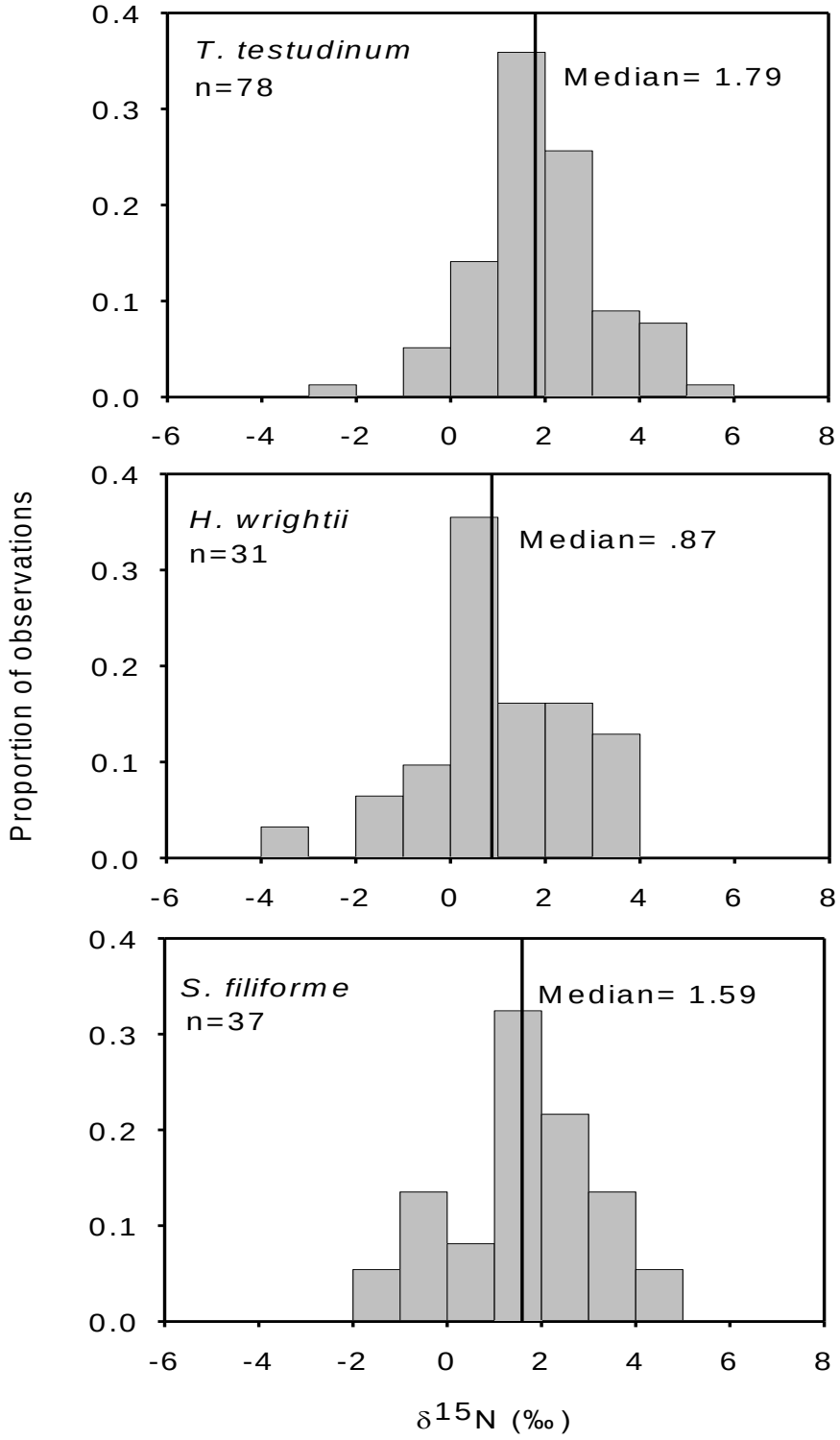

T. testudinum
n=78
Median= 1.79
H. wrightii
n=31
Median= .87
S. filiforme
n=37
Median= 1.59
Proportion of observations
0.0
0.1
0.2
0.3
0.4
-6
-4
-2
0
2
4
6
8
δ15N (‰)

Figure 6

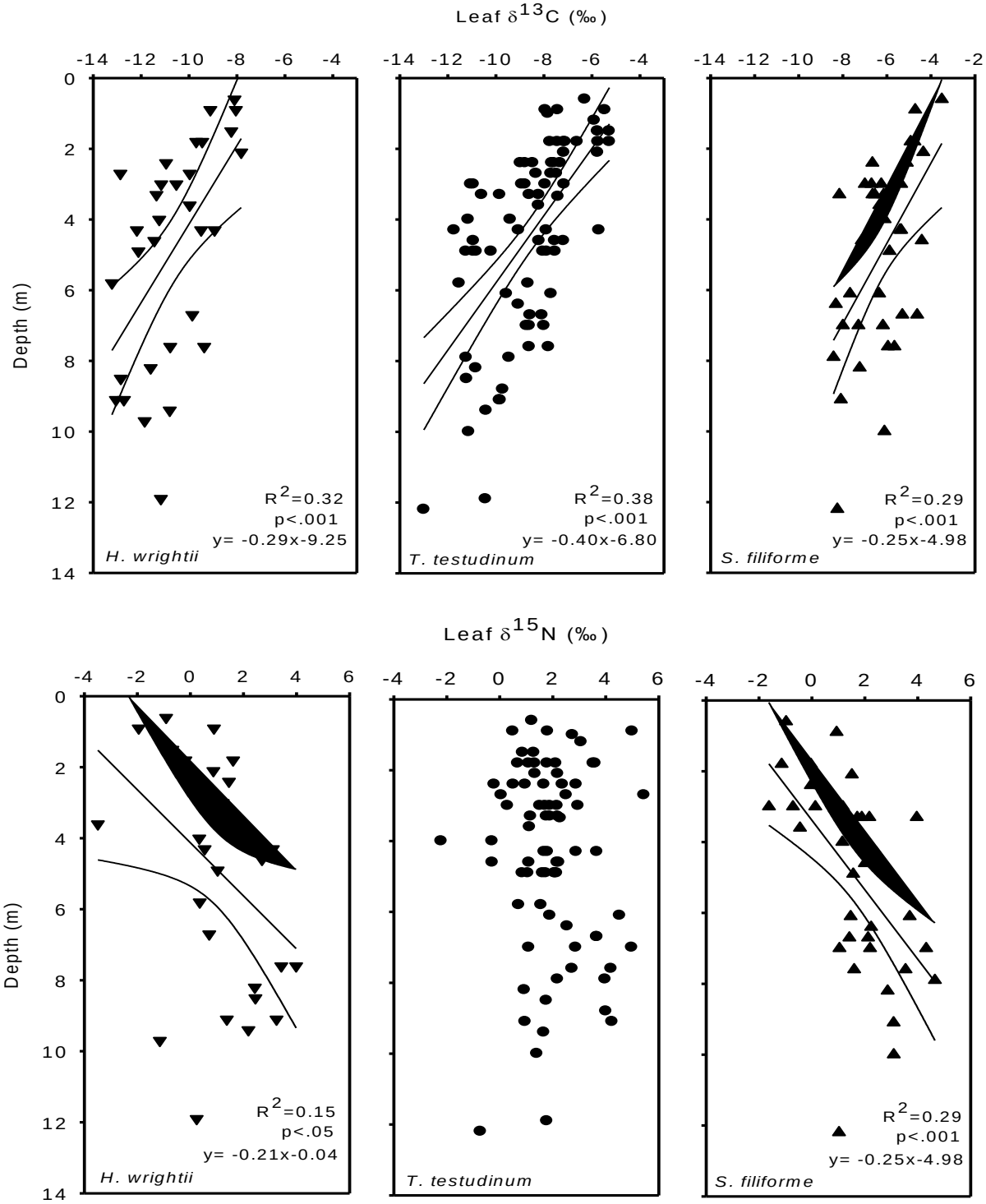

Leaf δ13C (‰)
Depth (m)
R2=0.32
p<.001
y= -0.29x-9.25
H. wrightii
R2=0.38
p<.001
y= -0.40x-6.80
T. testudinum
R2=0.29
p<.001
y= -0.25x-4.98
S. filiforme
Leaf δ15N (‰)
Depth (m)
R2=0.15
p<.05
y= -0.21x-0.04
H. wrightii
T. testudinum
R2=0.29
p<.001
y= -0.25x-4.98
S. filiforme

Figure 7

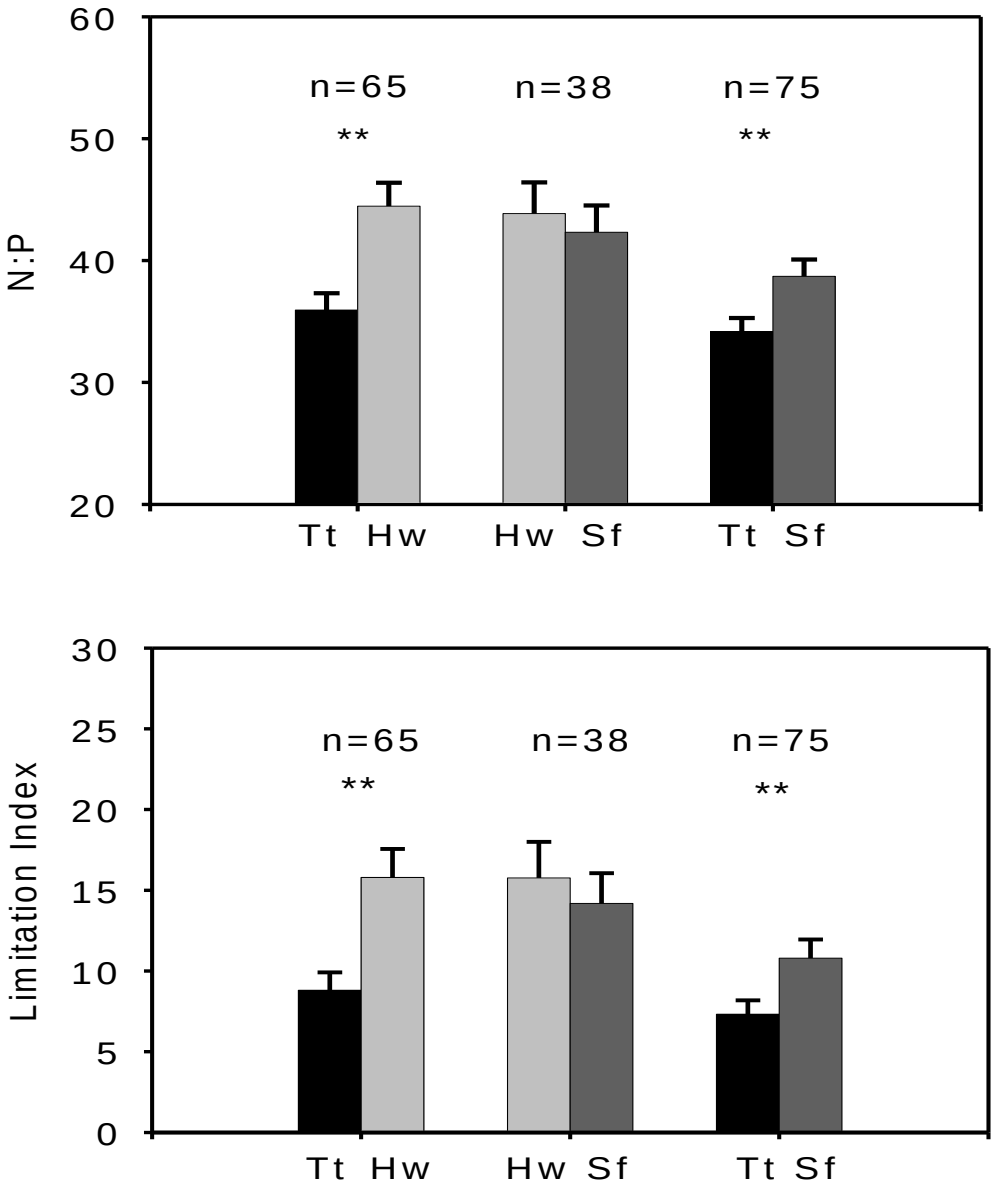

60
50
40
30
20
N:P
n=65
n=38
n=75
**
**
Tt Hw
Hw Sf
Tt Sf
30
25
20
15
10
5
0
Limitation Index
n=65
n=38
n=75
**
**
Tt Hw
Hw Sf
Tt Sf

Figure 8

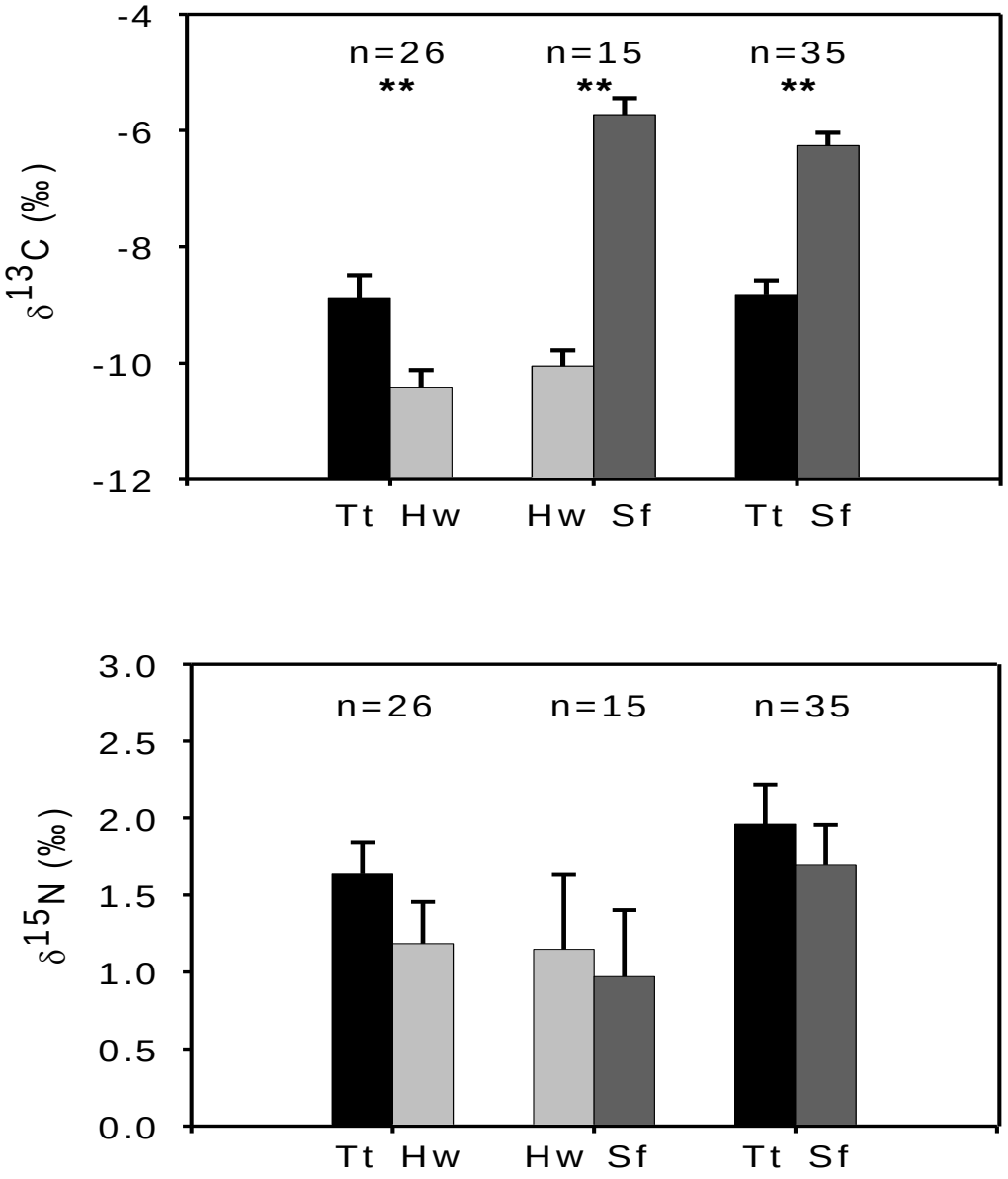
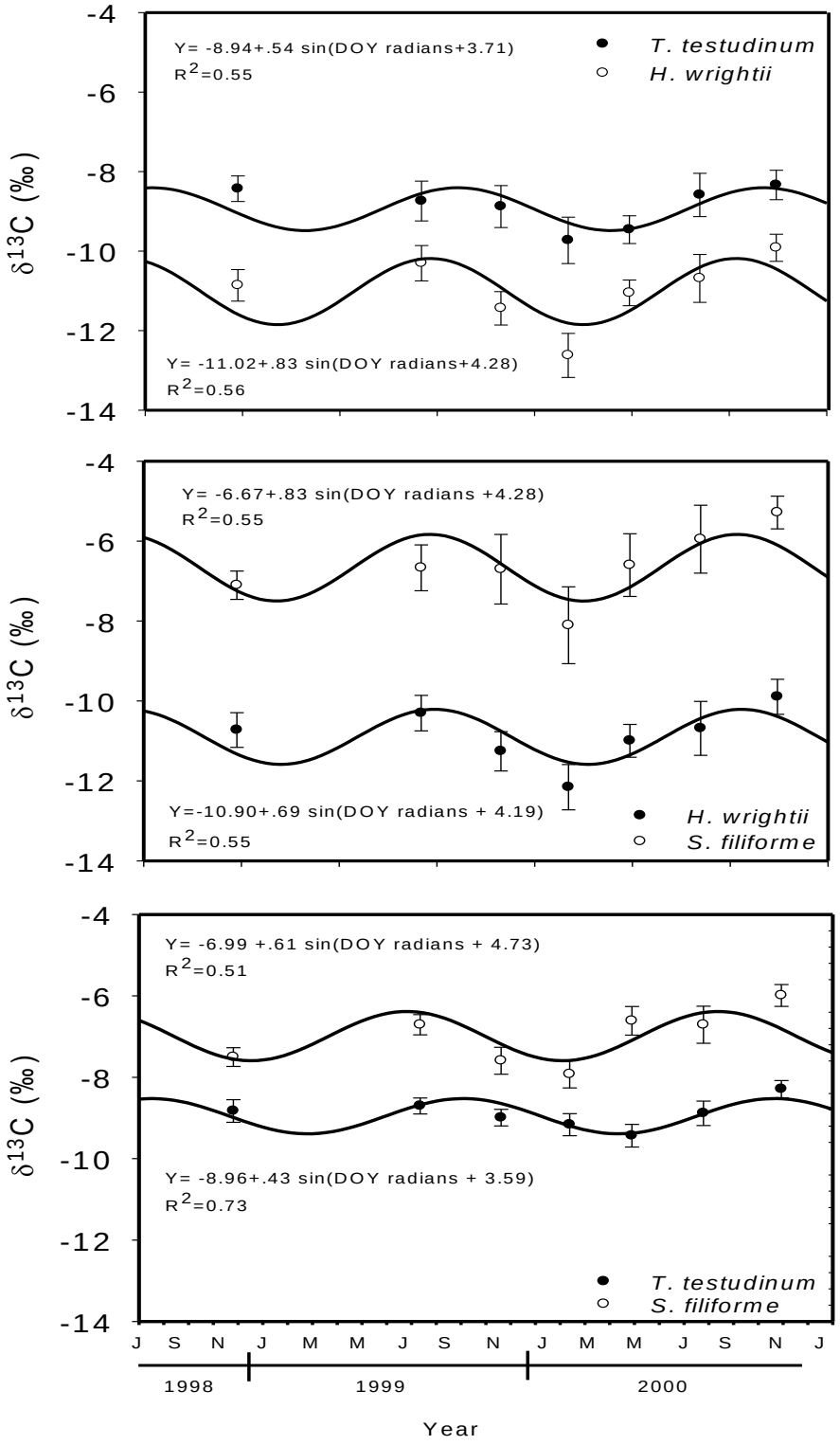

-4
-6
-8
-10
-12
δ13C (‰)
n=26
n=15
n=35
**
**
**
Tt Hw
Hw Sf
Tt Sf
3.0
2.5
2.0
1.5
1.0
0.5
0.0
δ15N (‰)
n=26
n=15
n=35
Tt Hw
Hw Sf
Tt Sf

Figure 9


Y= -8.94+.54 sin(DOY radians+3.71)
R2=0.55
T. testudinum
H. wrightii
Y= -11.02+.83 sin(DOY radians+4.28)
R2=0.56
Y= -6.67+.83 sin(DOY radians +4.28)
R2=0.55
Y=-10.90+.69 sin(DOY radians + 4.19)
R2=0.55
H. wrightii
S. filiforme
Y= -6.99 +.61 sin(DOY radians + 4.73)
R2=0.51
Y= -8.96+.43 sin(DOY radians + 3.59)
R2=0.73
T. testudinum
S. filiforme
δ13C (‰)
-4
-6
-8
-10
-12
-14
J S N J M M J S N J M M J S N J
1998
1999
2000
Year