2009

Interspecific variation in the elemental and stable isotope content of seagrasses in South Florida

Justin E. Campbell
Department of Biological Sciences, Florida International University, jcamp013@fiu.edu

James W. Fourqurean
Department of Biological Sciences, Marine Science Program and Southeast Environmental Research Center, Florida International University; Center for Tropical Plant Conservation, Fairchild Tropical Botanic Garden

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.

1 **Interspecific variation in the elemental and stable isotopic content of seagrass communities**

2 **in South Florida**

3

4 **Running head: Interspecific variation in seagrass elemental and isotopic content**

5

6 Justin E. Campbell[1*], James W. Fourqurean[1,2]

7 [1] *Department of Biological Sciences, Florida International University, Miami Florida 33199*

8 [2] *Fairchild Tropical Botanic Garden, Coral Gables, FL 33156*

9 *jcamp013@fiu.edu

10

13

14 **ABSTRACT**

15 The elemental (C, N and P) and isotopic ($\delta^{13}C$, $\delta^{15}N$) content of leaves of the seagrasses

16 *Thalassia testudinum*, *Halodule wrightii*, and *Syringodium filiforme* were measured across a

17 10,000 km^2 survey of the seagrass communities of South Florida in 1999 and 2000. Trends at

18 local and broad spatial scales were compared to examine interspecific variation in the seagrass

19 characteristics often used as ecological indicators. The elemental and stable isotope contents of

20 all species were variable and demonstrated marked interspecific variation. At broad spatial scales

21 mean nitrogen: phosphorus (N:P) ratios were lowest for *T.testudinum* (36:5 ± 1.1) and *S.*

22 *filiforme* (38:9 ± 1.3), and highest for *H. wrightii* (44:1 ± 1.8). Stable carbon isotope ratios ($\delta^{13}C$)

23 were highest for *S. filiforme* (-6.2‰ ± 0.2‰), intermediate for *T. testudinum* (-8.6‰ ± 0.2‰),

1   and lowest for *H. wrightii* (-10.6‰ ± 0.3‰). Stable nitrogen isotopes (δ^{15}N) were heaviest for *T.*

2   *testudinum* (2.0‰ ± 0.1‰), and lightest for *H. wrightii* (1.0‰ ± 0.3‰) and *S. filiforme* (1.6‰ ±

3   0.2‰). Site depth was negatively correlated to δ^{13}C for all species, while δ^{15}N was positively

4   correlated to depth for *H. wrightii* and *S. filiforme*. Similar trends were held for local

5   comparisons, suggesting that taxon specific physiological/ecological properties strongly control

6   interspecific variation in elemental and stable isotope content. Temporal trends in δ^{13}C were

7   measured, and revealed that interspecific variation was displayed throughout the year. This work

8   documents interspecific variation in the nutrient dynamics of three common seagrasses in South

9   Florida, indicating that interpretation of elemental and stable isotope values needs to be species

10   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

1   ambient elemental concentrations are low (Duarte 1990, Ferdie & Fourqurean 2004, Armitage et

2   al. 2005). Other environmental factors can also influence the nutrient content of plant material.

3   For example, increased light availability has been found to decrease the nutrient content of

4   seagrass leaves because of enhanced growth rates leading to the depletion of nutrient sources

5   (Abal et al. 1994). Thus, it has been widely documented that resource availability strongly

6   controls intraspecific variation in the elemental ratios of benthic macrophytes. However,

7   variation seen between plant species may be driven by factors other than environmental

8   conditions, suggesting that ecological/physiological differences may drive interspecific variation

9   at any particular location. While single species comparisons have been used across a broad

10   range of seagrasses over large spatial scales, localized interspecific comparisons may provide

11   important cues about the ecological differences occurring between sympatric species

12   (Fourqurean et al. 2007).

13   The stable isotopic content of plant material has provided a powerful tool for the study

14   and assessment of ecological processes. In addition to identifying nutrient sources and

15   processing within ecosystems (Dawson et al. 2002), stable isotopes have aided in food web

16   analysis and the study of energy flow amongst trophic levels (Peterson & Fry 1987). However,

17   the factors regulating the stable isotopic content of primary producers are complex, and require

18   detailed knowledge of spatial, temporal, and taxonomic variation. For example, the stable carbon

19   isotope content of seagrass material is predominantly controlled by the environmental factors of

20   carbon source, irradiance, and temperature (Durako & Hall 1992, Abal et al. 1994, Grice et al.

21   1996, Hemminga & Mateo 1996). Spatial and temporal variation in these factors have been

22   found to influence the carbon isotopic content of seagrass species across landscape scales

23   (Fourqurean et al. 1997, Fourqurean et al. 2005), highlighting the importance of documenting

1   background variation when applied to food web studies. Numerous studies have detailed the

2   impact of environmental conditions on intraspecific variation in stable isotope parameters.

3   However, interspecific divergence, particularly amongst sympatric seagrasses, has received less

4   attention and may be attributable to physiological/ecological distinctions between species. Stable

5   isotope comparisons between co-occurring species may reveal important cues pertaining to how

6   specific species process local resources.

7   Seagrasses fractionate the available pool of inorganic carbon based upon the degree of

8   carbon demand relative to the degree of carbon supply. Experimental evidence has shown that

9   for a given carbon supply, plants grown under high light conditions display increased

10   photosynthetic rates and increased carbon demand, resulting in reduced discrimination against

11   ^{13}C and heavier isotopic signatures (Cooper & Deniro 1989, Durako & Hall 1992). Similarly, for

12   a given light level, experiments have found enriched carbon isotope signatures ($\delta^{13}C$) with

13   decreases in carbon supply (Durako & Sackett 1993). These relationships provide environmental

14   information pertaining to the light and inorganic carbon status of plant material. However,

15   interspecific variation in the mechanisms by which plants process carbon can additionally impact

16   $\delta^{13}C$ values (Farquhar et al. 1989), thus individual plant physiology may contribute to overall

17   variation in stable carbon isotope values. Utilization of bicarbonate (which is isotopically distinct

18   from CO_2) may impact $\delta^{13}C$ ratios (Hemminga & Mateo 1996), and may further contribute to

19   variation in isotope values. Bicarbonate use is dependent upon species specific seagrass

20   physiology (Invers et al. 1999), thus interspecific differences in stable isotope values may be

21   attributed to the varied physiologies associated with HCO_3^- uptake. Systematic differences in the

22   $\delta^{13}C$ 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

1   136 sites in South Florida, representing a 10,000 km^2 survey area of the seagrass beds in the

2   Florida Keys National Marine Sanctuary (FKNMS). We were particularly interested in

3   documenting interspecific variation in the properties of seagrasses co-occurring under similar

4   environmental conditions, within a given site. We hypothesize that the varied life history

5   strategies of benthic macrophytes would be reflected in plant elemental content, with fast-

6   growing, early successional species displaying N:P ratios further removed from ideal values as

7   compared to slow-growing species from the same site. We additionally hypothesize that species-

8   specific differences in carbon uptake strategies and plant physiologies have the ability to strongly

9   regulate stable isotope parameters. Lastly, we hypothesize that fast growing, early successional

10   species may exhibit larger seasonal variation in stable isotope values as a result of increased

11   growth rates and elevated carbon and nitrogen demand. Interspecific comparisons at both local

12   (within site) and broad (amongst all sites) scales allowed us to examine the spatial extent to

13   which taxonomic variation is important, and its impact on the isotopic and elemental

14   composition of benthic plants.

15

16   **METHODS**

17   The Florida Keys National Marine Sanctuary (FKNMS) is a shallow-water, marine

18   ecosystem located at the southern tip of the Florida peninsula and comprised of seagrass beds,

19   coral reefs, and mangrove communities (Fig. 1). Seagrass communities in the FKNMS are

20   primarily composed of *Thalassia testudinum*, *Halodule wrightii*, and *Syringodium filiforme*.

21   During the summer months of 1999, 80 sites were randomly selected across the FKNMS, and

22   surveyed for seagrass abundance, nutrient content and isotopic composition. A repeat survey was

23   conducted during the summer of 2000 at 56 different randomly generated sites, which only

1   quantified seagrass abundance and nutrient content. Seasonal variation in seagrass isotopic

2   content was additionally assessed through a separate series of quarterly surveys conducted during

3   1999 and 2000. Within each year, a network of 30 permanent monitoring stations was sampled 4

4   times (see Fourqurean et al. 2001 for further description). The elemental and isotopic

5   composition of *T. testudinum* has been previously described for this region (Fourqurean et al

6   2005). In order to examine interspecific variation, our current study incorporates a portion of

7   those data on the elemental and isotopic content of *T. testudinum*, with new data for the other

8   two seagrass species (*S. filiforme* and *H. wrightii*) common in South Florida.

9   From the selected random sites, three separate comparative analyses were conducted

10   which spanned various spatial and seasonal scales. One analysis included all data from the 1999

11   (80 sites) and 2000 (56 sites) surveys, representing comparisons within the summer season over

12   large spatial scales. Both monospecific (22%) and mixed species (78%) seagrass beds were

13   included. A second analysis included data from the 1999 and 2000 summer surveys, however

14   monospecific sites were excluded, thus interspecific comparisons were solely conducted within

15   multi-species sites where seagrasses co-occurred under similar environmental conditions. Each

16   multi-species site contained two or more seagrass species growing adjacently (< 50m apart),

17   under similar light and depth regimes. Multi-species sites were grouped according to pairwise

18   interspecific comparisons: *Thalassia testudinum*/*Halodule wrightii* (65 sites), *Halodule*

19   *wrightii*/*Syringodium filiforme* (38 sites), and *Thalassia testudinum*/*Syringodium filiforme* (75

20   sites). The third analysis similarly conducted within-site interspecific comparisons, however only

21   utilized data from the 30 permanent monitoring sites, allowing for comparisons of intra-annual

22   variability between species pairs. Within this network, the number of sites for interspecific

8

1   comparison varied depending upon season; *T. testudinum*/*H. wrightii* (4-7 sites), *H.*

2   *wrightii*/*S.filiforme* (5-8 sites), *T. testudinum*/*S. filiforme* (19-22 sites).

3   For all surveys, at each sampling site, short shoots of each seagrass species present were

4   haphazardly collected along a 50m transect. When available, 6 shoots of *Thalassia testudinum*,

5   30 shoots of *Syringodium filiforme*, and 40 shoots of *Halodule wrightii* were harvested, placed

6   on ice, and transported back to the lab. The less robust seagrass species required higher

7   collection amounts to ensure enough biomass was available for all elemental and isotopic

8   analyses. Seagrass leaves were separated according to species, cleaned of epiphytes through

9   gentle scraping with a razor blade, and cut from their respective short shoots. Leaves were then

10   dried to a constant weight at 80°C, ground to a fine powder with a mortar and pestle, and

11   analyzed in duplicate for C and N content using a CHN analyzer (Fourqurean et al. 2005).

12   Phosphorus content was determined through dry oxidation, acid hydrolysis extraction followed

13   by a colorimetric analysis (Fourqurean et al. 1992a). Elemental ratios were calculated on a

14   mole:mole basis.

15   All isotopic analyses were measured using standard elemental analyzer isotope ratio mass

16   spectrometer procedures. The elemental analyzer was used to combust all organic material and

17   subsequently reduce the formed gasses into N_2 and CO_2, which were measured on a Finnigan

18   MAT Delta C IRMS in a continuous flow mode. The samples' isotopic ratios (R) are reported in

19   the standard delta notation (‰): δ (‰) = [(R_{sample}/$R_{standard}$)-1] x 1000. These results are presented

20   with respect to the international standards of atmospheric nitrogen (AIR,N_2) and Vienna Pee Dee

21   belemnite (V-PDB) for carbon. Analytical reproducibility of the reported δ values, based on

22   sample replicates, was better than $\pm$ 0.08‰ for carbon and $\pm$ 0.2‰ for nitrogen. Care was taken

23   to remove all visible carbonate material from the surface of the leaves. As a test of the efficacy

1   of our cleaning we acidified a subset of seagrass samples with the most enriched δ^{13}C values to

2   drive off any remaining carbonate material, and then determined the δ^{13}C of this decalcified

3   material. The differences in δ^{13}C between acidified and unacidified samples were small (< 0.3 ‰

4   on average).

5   The distributions of all seagrass elemental and isotopic parameters were checked for

6   normality using the Kolmogorov-Smirov test ($\alpha=0.05$). Standard linear regression was used to

7   test the strength of the relationship between stable isotope variables (δ^{13}C, δ^{15}N) and site depth.

8   Relationships among elemental and isotopic ratios were additionally assessed for all species,

9   across all sites sampled in 1999, using non-parametric correlations (Spearman's ρ). Interspecific

10   Sanctuary-wide species-specific differences in seagrass N:P ratios and isotopic values were

11   assessed using a single factor analysis of variance (ANOVA). Upon detecting significance, post-

12   hoc analysis was conducted using either a Tukey's HSD for equal variances, or a Dunnett's T3

13   test for unequal variances (significance $p < 0.05$). In addition to testing N:P ratios, a Limitation

14   Index (L.I. = |30- N:P|) was calculated to quantify the degree of divergence from the ideal ca.

15   30:1 "Seagrass Redfield Ratio" identified by Atkinson and Smith (1983) and Duarte (1990).

16   Larger L.I. values indicate greater degrees of nutrient limitation. Such a calculation is necessary

17   because across the landscape of the FKNMS there are both N- and P-limited regions (Fourqurean

18   et al 2005).

19   To conduct within-site interspecific comparisons, differences in N:P ratios, L.I., δ^{13}C, and

20   δ^{15}N were tested with a pair-wise Students t-test, significance $p < 0.05$. To describe temporal

21   variation in seagrass isotopic content for species that co-occurred at the 30 permanent stations,

22   we fit a sine model of the form y = mean + α[sin(time + Φ)], where α is the amplitude of a sine

23   wave, and Φ is a phase angle (we used time values in radians for both time and Φ, where 2π

1　radians = 365d), to a seasonal time series using an iterative nonlinear curve fitting regression.

2　Confidence intervals (95%) of model parameters were compared to test for significant

3　differences in seasonal variation. We were interested in using the non-linear regression as a

4　means of testing interspecific divergence in the mean and amplitude of seasonal seagrass $\delta^{13}C$

5　variation.

6

7　**RESULTS**

8　**Interspecific variation in seagrass elemental and isotopic composition at broad scales**

9　**across the FKNMS**

10　　　In 1999, *Thalassia testudinum* occurred at 78 of the randomly selected sites (98%), while

11　*Halodule wrightii* and *Syringodium filiforme* occurred at 31 sites (39%) and 37 sites (46%)

12　respectively. In 2000, *T. testudinum* occurred at 56 sites (100%), while *H. wrightii* and *S.*

13　*filiforme* occurred at 39 sites (70%) and 40 sites (71%) respectively. Nitrogen, phosphorus and

14　carbon content varied within each species, across all sanctuary sites in both years (Table 1),

15　however they did not vary significantly between years. In both years, coefficients of variation

16　show that leaf phosphorus content (CV=0.28-0.37) displayed greater variation in all species

17　compared to nitrogen (CV=0.15-0.25) and carbon content (CV=0.06-0.12). The N:P frequency

18　distribution for *T. testudinum* was slightly skewed towards higher values (Fig. 2). Comparatively,

19　*H. wrightii* displayed a normal distribution with the highest mean N:P ratios (44.1 ± 1.8), which

20　were reflected in the highest mean L.I. of all species (15.3 ± 1.7, Fig. 3, Table 1). *Syringodium*

21　*filiforme* displayed an intermediate N:P ratio of 38.9 ± 1.3, which was normally distributed, and

22　an average limitation index value of 10.9 ± 1.1. *Thalassia testudinum* displayed the lowest mean

23　N:P ratio (36.5 ± 1.1), and the lowest mean L.I. value (9.6 ± 0.9) of the species. Mean *T.*

1   *testudinum* N:P ratio and L.I. values were significantly lower than those of *Halodule wrightii*, yet

2   similar to *Syringodium filiforme* (Table 1). Mean *H. wrightii* N:P ratio and L. I. values were

3   similar to *S. filiforme* (ANOVA, F = 7.89, p = 0.07; ANOVA, F = 6.25, p = 0.09).

4   *Halodule wrightii* displayed the lowest δ^{13}C values of all species, with a mean of -10.6 ‰

5   ± 0.3‰, a range of -13.2‰ to -7.8‰, and a normal distribution (Fig. 4). *Halodule wrightii* δ^{13}C

6   values were significantly lower than both *Thalassia testudinum* and *Syringodium filiforme* (Table

7   1; ANOVA, F = 53.5, p < 0.001). *Syringodium filiforme* displayed the highest δ^{13}C values with a

8   mean of -6.2‰ ± 0.2‰, a range of -3.5‰ to -8.4‰, and a normal distribution. The δ^{13}C values

9   of *S. filiforme* were significantly higher than those of *T. testudinum* (ANOVA, F = 53.5, p <

10   0.001). *Thalassia testudinum* displayed intermediate δ^{13}C values with a mean of -8.6‰ ± 0.2‰,

11   a range of -13.0‰ to -5.3‰, whose values were normally distributed.

12   Stable nitrogen isotope values varied by 7.7‰, 7.5‰, and 6.3‰ for *Thalassia*

13   *testudinum*, *Halodule wrightii*, and *Syringodium filiforme* respectively (Table 1). *Thalassia*

14   *testudinum* displayed the highest δ^{15}N values of all species, with mean of 2.0‰ ± 0.2‰ and a

15   normal distribution (Fig. 5). *Thalassia testudinum* displayed δ^{15}N values which were higher than

16   those of *H. wrightii* (Table 1; ANOVA, F = 5.08, p < 0.01), yet similar to *S. filiforme* (ANOVA,

17   F = 5.08,p = 0.45). *Halodule wrightii* displayed the lowest values, with a mean of 1.0‰ ± 0.3‰

18   and a normal distribution. *Halodule wrightii* δ^{15}N values were similar to those of *S. filiforme*

19   (ANOVA, F = 5.08,p = 0.18). *Syringodium filiforme* displayed intermediate values, with a mean

20   of 1.6‰ ± 0.3‰ and a normal distribution.

21   Across FKNMS, elemental and isotopic ratios of all three seagrass species were

22   correlated with site depth (Table 2, Fig. 6). The δ^{13}C and N:P ratios were negatively correlated to

23   site depth for all species, and displayed similar variation with depth amongst species. The δ^{15}N

12

1 ratio was positively correlated with site depth for *Halodule wrightii* and *Syringodium filiforme*,

2 yet there was no correlation for *Thalassia testudinum* (Fig. 6). The variation in δ^{15}N with depth

3 was similar between *H. wrightii* and *S. filiforme*, and further analysis revealed that negative

4 correlations between %N and δ^{15}N were restricted to the deeper offshore locations (>4.3m).

5 *Syringodium filiforme* was the only species for which δ^{13}C was correlated to δ^{15}N. Correlations

6 between elemental and isotopic ratios were mixed depending upon species. In *T. testudinum*, N:P

7 was positively correlated to δ^{13}C, yet uncorrelated to δ^{15}N. *Syringodium filiforme* showed a

8 negative correlation between N:P and δ^{15}N, yet no correlation between N:P and δ^{13}C. In *H.*

9 *wrightii*, N:P was both negatively correlated to δ^{15}N, and positively correlated to δ^{13}C. L.I.

10 values were negatively correlated to both site depth and δ^{15}N for *H. wrightii* and *S. filiforme*, *T.*

11 *testudinum* showed no correlation amongst these parameters. For all species L.I. was positively

12 correlated to δ^{13}C.

13

14 **Interspecific variation in seagrass elemental and isotopic composition at local scales**

15   Of the sites which contained both *Thalassia testudinum* and *Halodule wrightii* (65 sites),

16 N:P ratios and L.I. values were significantly higher for *H. wrightii* ($p < 0.001$; Fig. 7). When

17 averaged for both years, *T. testudinum* had an N:P ratio and an L.I. value of 35.9 ± 1.4 and 8.8 ±

18 1.1 respectively at sites where it co-occurred with *H. wrightii*, while *H. wrightii* had an N:P ratio

19 and an L.I. value of 44.5 ± 1.9 and 15.8 ± 1.8 at the same sites. When growing in similar

20 locations, significant differences were additionally detected in δ^{13}C signatures, with *T.*

21 *testudinum* (-8.9 ± 0.4) displaying isotopically heavier values than *H. wrightii* (-10.4 ± 0.3) ($p <$

22 0.001)(Fig. 8). There were no consistent, statistically significant differences in δ^{15}N between

23 these two species at sites where they co-occurred.

1   *Halodule wrightii* and *Syringodium filiforme* co-occurred at 38 sites, and did not differ in

2   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

3   15.8 ± 2.2 respectively for *H. wrightii*, and 42.3 ± 2.2 and 14.2 ± 1.9 respectively for *S. filiforme*.

4   Significant differences were detected in δ^{13}C signatures, with *H. wrightii* displaying a value of

5   -10.1‰ ± 0.3‰, as compared to the isotopically heavy *S. filiforme* (-5.7‰ ± 0.3‰) (p <

6   .001)(Fig. 8). There were no consistent, statistical differences in δ^{15}N between *H. wrightii* and *S.*

7   *filiforme* at sites where they co-occurred.

8   *Thalassia testudinum* and *Syringodium filiforme* co-occurred at 75 sites, and displayed

9   significant differences in both N:P ratios and L.I. at those sites (p < 0.001, p < 0.01 respectively).

10   For both years, *T. testudinum* displayed an N:P ratio and L.I. value of 34.2 ± 1.1 and 7.4 ± 0.9,

11   respectively. While *S. filiforme* displayed an N:P ratio and L.I. of 38.8 ± 1.4 and 10.9 ± 1.2,

12   respectively (Fig. 7). Additionally, δ^{13}C signatures were statistically distinct, with *T. testudinum*

13   (-8.8‰ ± 0.2‰) displaying isotopically lighter values than *S. filiforme* (-6.3‰ ± 0.2‰) (p <

14   0.001) (Fig. 8). There were no statistical differences in δ^{15}N between *T. testudinum* and *S.*

15   *filiforme* at sites where they co-occurred.

16

17   **Temporal variation in seagrass isotopic content at local scales**

18   There was marked seasonal variation in the δ^{13}C values of *Thalassia testudinum*,

19   *Halodule wrightii*, and *Syringodium filiforme* (Fig. 9). δ^{13}C values for all three species were

20   heaviest in the summer and fall months, and lightest in the winter months. The sine models

21   described 51-73% of the variation in δ^{13}C values (Table 3). Parameter estimates for α and Φ

22   were not significantly different between species comparisons, indicating no interspecific

23   differences in the degree of seasonal variation, nor the seasonal timing of δ^{13}C values. Mean δ^{13}C

1   values of the sine models were significantly different for all species comparisons, indicating that

2   the interspecific differences we documented above with our FKNMS-wide summer surveys were

3   maintained throughout the year. Seasonal trends in δ^{15}N values were not detected for any

4   species.

5

6   **DISCUSSION**

7   The elemental and isotopic leaf content of the seagrasses *Thalassia testudinum*, *Halodule*

8   *wrightii*, and *Syringodium filiforme* displayed marked interspecific variation at both local and

9   broad spatial scales within the Florida Keys National Marine Sanctuary. Both within and

10   between species variations were nonrandom, and demonstrated both the effects of environmental

11   parameters and species-specific physiologies on the elemental and isotopic content of seagrass

12   tissues. This study highlights the importance of seagrass species identity in the evaluation of

13   plant nutrient and isotopic data for coastal monitoring efforts and food web analyses.

14

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

16   Significant differences in δ^{13}C were detected amongst all three seagrass species. The

17   lightest δ^{13}C values were displayed by *Halodule wrightii*, while *Thalassia testudinum* and

18   *Syringodium filiforme* displayed the intermediate and heaviest values respectively. Similarly,

19   Lepoint et al. (2008) found that *Syringodium isoetifolium* was more enriched in ^{13}C compared to

20   *Halodule* sp. and *Thalassia hemprichii* in Mozambique. The heaviest δ^{15}N was displayed by *T.*

21   *testudinum*, which was significantly more positive than *H. wrightii*. The δ^{15}N value for *S.*

22   *filiforme* was not statistically distinct from the other species. Large scale trends in the stable

23   isotopic content of various seagrasses may provide information pertaining to physiological and

1   ecological properties of each species. However, data at this large scale should be interpreted with

2   caution, as trends may be confounded by spatial variations in: (1) the distribution of various

3   seagrass species and (2) environmental conditions.

4   All three seagrass species showed significant correlations between δ^{13}C values and site

5   depth across the large spatial scale of the sanctuary (Fig. 6). Because light availability generally

6   decreases with depth in the sea, this suggests that for each species, light plays an important role

7   in regulating the δ^{13}C content of seagrass tissues, as demonstrated in both laboratory and field

8   studies (Durako & Hall 1992, Abal et al. 1994, Grice et al. 1996). Likely this is because of

9   reduced photosynthetic discrimination against the heavier isotope, leading to increased δ^{13}C

10   values at high light levels. Within a species, isotopically heavy values may indicate the

11   possibility of photosynthetic carbon limitation during periods of high irradiance (Fourqurean et

12   al. 2005). Field studies have shown that the inverse relationship between light and depth are

13   reflected in the δ^{13}C ratio of seagrass tissues elsewhere (Cooper & Deniro 1989, Lepoint et al.

14   2003, Fourqurean et al. 2007). In our study, δ^{13}C values decrease by 0.40, 0.29, and 0.25 ‰ m^{-1}

15   in depth for *Thalassia testudinum*, *Halodule wrightii*, and *Syringodium filiforme* respectively.

16   We don't think that the δ^{13}C-depth relationships are a function of variations in the isotopic

17   signature of the DIC pool, as has been observed in regions where mineralization of organic

18   matter from C_3 mangroves causes isotopically depleted DIC that is incorporated into seagrass

19   tissues (Fry & Sherr 1984, Fleming et al. 1990, Lin et al. 1991). The FKNMS sites that are in

20   close proximity to terrestrial DIC sources display the heaviest δ^{13}C values, contrary to what

21   would be expected if C_3 material was impacting the isotopic value of the DIC pool available to

22   the seagrass community.

1   The δ^{15}N value of seagrass leaf material can be controlled by numerous factors. In

2   addition to alterations in the isotopic composition of the source nitrogen pool, the relationship

3   between plant nutrient demand and environmental availability represents a strong determinant of

4   leaf isotope ratios. Reduced light as depth increases could, by reducing leaf growth rates and

5   demand for N, influence leaf δ^{15}N as depressed demand increases discrimination against the

6   heavier isotope. However, the net change in leaf δ^{15}N with increasing depth should be a

7   consequence of the magnitude of the reduction in N demand relative to the changes in N

8   availability. For instance, in cases where N availability remains constant with increasing depth,

9   we would expect a decrease in leaf δ^{15}N with increasing depth due to a depressed demand for N

10   and higher discrimination against ^{15}N. Alternatively, cases in which leaf δ^{15}N shows little change

11   with depth may represent scenarios whereby both N demand and N availability concurrently

12   decrease with depth, resulting in little alteration to ^{15}N discrimination. Working with other

13   species of seagrasses, Grice et al (1996), Lepoint et al (2003), and Fourqurean et al (2007) all

14   found no significant change in leaf δ^{15}N across large depth and light gradients, although they did

15   not investigate the causal mechanisms for those observations. Lastly, if nitrogen availability

16   decreases dramatically with increasing depth, we could, despite reductions in N demand, find

17   higher leaf δ^{15}N values as depth increases. We believe this to be the case in our study, as the

18   observed relationships between δ^{15}N and depth are a consequence of the distribution of deep-

19   water sites within a landscape of variable nitrogen availability. The leaf δ^{15}N value of both

20   *Halodule wrightii* and *Syringodium filiforme* was positively correlated to site depth, and

21   negatively correlated to both N:P ratios and L.I. Such correlations suggest that as nitrogen

22   becomes less available and nutrient limitation increases, these seagrasses reduce fractionation of

23   the available DIN pool. Additionally, the negative correlation between %N and δ^{15}N was only

17

1 significant for the deeper sites under similar light regimes, further indicating that environmental

2 availability was driving the relationship between depth and $\delta^{15}N$. This indicates that the reduced

3 DIN discrimination in *H. wrightii* and *S. filiforme* increasingly occurs at deeper offshore

4 locations, where primary production is N-limited (Fourqurean & Zieman 2002, Ferdie &

5 Fourqurean 2004, Fourqurean et al. 2005). This trend, however, was not held for *Thalassia*

6 *testudinum* which exhibited no correlation between $\delta^{15}N$ values and site depth or N:P ratios.

7 Thus, these correlations were only evident for the fastest growing seagrasses (*H. wrightii* and *S.*

8 *filiforme*), which may utilize nutrient resources more rapidly than *T. testudinum*, leading to

9 decreases in DIN fractionation and higher L.I. compared to the slower-growing *T. testudinum*.

10 Further experimentation is needed to detail these processes.

11 The fact that interspecific differences in stable carbon isotopes remain statistically

12 distinct across large spatial scales may imply that seagrass physiology plays a dominant role in

13 determining interspecific variation in $\delta^{13}C$. Average values for *Halodule wrightii* were 2.0 ‰

14 lighter than *Thalassia testudinum*, and 4.5 ‰ lighter than *Syringodium filiforme*. Such

15 differences might be attributed to the varied mechanisms of bicarbonate acquisition and internal

16 carbon recycling within marine macrophytes (Fry & Sherr 1984, Fry et al. 1985, Hemminga &

17 Mateo 1996, Fourqurean et al. 2007), or caused by morphological variations in leaves (Lepoint et

18 al. 2008). To various degrees, seagrass photosynthetic carbon demand may be met via a

19 combination of diffusive CO_2 transport, and active HCO_3^- import (Invers et al. 1999, Invers et al.

20 2001). While dissolved CO_2 has an isotopic carbon signature of ca. -9 ‰, HCO_3^- in marine

21 waters has an isotopic signature of 0 ‰. Preferential fixation of bicarbonate from the inorganic

22 carbon pool should result in seagrass tissue which is enriched in ^{13}C (Hemminga & Mateo 1996).

23 Interspecific variation in seagrass $\delta^{13}C$ values may reflect previously documented interspecific

1   variation in bicarbonate acquisition mechanisms, as some species display enhanced HCO_3^-

2   utilization efficiencies (Bjork et al. 1997, Invers et al. 1999, Schwarz et al. 2000, Uku et al.

3   2005). Our field data suggest that *S. filiforme* may rely more on HCO_3^- as a carbon source than

4   *H. wrightii* or *T. testudinum*, an hypothesis that should be explored.

5   Internal recycling of CO_2 has been proposed as an alternate mechanism which can

6   influence the stable carbon isotope value of benthic macrophytes (Cooper 1989, Abal et al. 1994,

7   Grice et al. 1996). Seagrass species which have enhanced lacunal volume may display increased

8   recycling of internal carbon pools, and reduced isotopic discrimination. As internal recycling of

9   CO_2 reduces the degree of carbon back-diffusion, RUBISCO operates in an increasingly closed

10 environment, converting all inorganic carbon into organic products, resulting in isotopically

11 heavier $\delta^{13}C$ signatures (Sharkey & Berry 1985). Internal recycling may therefore serve to

12 complicate the relationship between the $\delta^{13}C$ of source DIC, and the $\delta^{13}C$ of seagrass tissues. Our

13 data are consistent with more efficient internal recycling of CO_2 in *Syringodium filiforme* than

14 *Halodule wrightii* or *Thalassia testudinum*. It is evident that the factors contributing to the

15 characteristic $\delta^{13}C$ value of specific seagrass species are complex, and require studies focused on

16 detailing the contributions that carbon acquisition mechanisms, seagrass lacunal volume, and

17 rates of CO_2 recycling make to the overall isotopic signature.

18   Interspecific variation in $\delta^{15}N$ across FKNMS reveal significant differences between

19 *Thalassia testudinum* and *Halodule wrightii*, however it remains unclear whether this trend is

20 due to differences in the degree of isotope fractionation among species, or due to spatial trends in

21 the isotopic composition of source DIN and seagrass abundance. Across a large spatial scale, *T.*

22 *testudinum* was isotopically heavier than *H. wrightii*, indicating decreased fractionation amongst

23 nitrogen isotopes. Successional studies have demonstrated *T. testudinum* as a climax species with

1 the ability to thrive in nutrient poor environments, and *H. wrightii* as an early successional

2 species thriving under more eutrophic conditions (Fourqurean et al. 1995). Across large spatial

3 scales, *T. testudinum* may monospecifically occupy the most oligotrophic locations with the

4 lowest DIN pools, resulting in decreased fractionation of the nitrogen isotope, accounting for

5 heavier δ^{15}N values; *H. wrightii*, occupying areas with higher DIN pools may have higher rates

6 of fractionation, lowering δ^{15}N values. Such results are not contradictory to previous findings

7 concerning the correlation between δ^{15}N and depth. Despite *H. wrightii* displaying decreased

8 δ^{15}N fractionation with depth, overall this species remains isotopically lighter than *T. testudinum*

9 because it is excluded from the highly oligotrophic deeper waters solely occupied by *T.*

10 *testudinum*.

11   Elemental comparisons across FKNMS reveal that *Thalassia testudinum* had significantly

12 lower N:P ratios and L.I. than *Halodule wrightii* (p <0.01), while *Syringodium filiforme* was not

13 statistically distinct from either *T. testudinum* or *H. wrightii*. Overall, intraspecific variation at

14 this scale is attributable to the balance between the availability nutrient resources (N and P) and

15 rates of seagrass productivity (Fourqurean et al. 2005). However interspecific differences in the

16 N:P ratios of *T. testudinum* and *H. wrightii* may result from life history differences between these

17 2 species. The early successional status and higher growth rates of *H. wrightii* may account for

18 increased deviation from seagrass Redfield N:P stoichiometry. Nutrient demand is higher for *H.*

19 *wrightii* than for *T. testudinum* (Fourqurean et al. 1992b), thus despite possibly being limited to

20 sites elevated in nutrient concentrations, the fast growth rates of *H. wrightii* still produce N:P

21 ratios which are drastically altered from Seagrass Redfield values. Ecologically, *T. testudinum* is

22 a late successional species, and the life history strategy of reduced growth rates may allow *T.*

23 *testudinum* to produce biomass with N:P ratios closer to the ideal Redfield value of 30:1. The

1   extensive investment in underground biomass (root/rhizome complex) may additionally allow *T.*

2   *testudinum* to exploit sediment nutrient pools unavailable to other species, thus bringing

3   stoichiometric ratios closer to 30:1. However, across landscape scales, elemental variation due to

4   life history differences are difficult to separate from elemental variation due to spatial trends in

5   nutrients, light, and seagrass abundance. For example, if *T. testudinum* were relatively more

6   abundant in deeper, lower light environments, then lower productivities and reduced N:P ratios

7   may be attributable to this spatial environmental factor, and not species-specific

8   physiological/ecological properties.

9

10   **Interspecific variation at local scales within the FKNMS**

11   Within site, local comparisons between species control for spatial gradients in abiotic

12   factors (light and nutrients), hence differences in elemental and isotopic compositions do not

13   reflect environmental variation, and may be attributed to differences in species specific

14   physiological and ecological properties. Congruence or discordance of local trends with broad

15   spatial trends reveals whether interspecific variation is due to physiological attributes or wide

16   ranging abiotic variation. We find that at the local scale, interspecific trends in stable carbon

17   isotopic content and elemental ratios are generally held, while trends in stable nitrogen isotopic

18   content are not consistent with the trends observed at broad spatial scales.

19   Local scale interspecific trends in $\delta^{13}C$ agree with broad scale trends, revealing that

20   species specific physiological attributes are dominant factors in controlling taxon specific $\delta^{13}C$

21   values. At the same location, *Halodule wrightii* was significantly lighter than both *Thalassia*

22   *testudinum* and *Syringodium filiforme*, and *T. testudinum* was significantly lighter than *S.*

23   *filiforme*, yet heavier than *H. wrightii* (Fig. 8), suggesting that the interspecific trends at the

21

1   broad scale of the FKNMS are functions of the physiological attributes of carbon acquisition.

2   Utilizing this model, *H. wrightii* may represent a seagrass species which, while displaying HCO_3^-

3   use, exhibits high rates of carbon back diffusion (low CO_2 recycling), and thus fails to fix a large

4   portion of incorporated carbon, allowing for increased isotopic discrimination. Conversely, *S.*

5   *filiforme* may represent a species which displays reduced carbon back diffusion (high CO_2

6   recycling), and thus fixes a large majority of imported carbon. The apparent dependence of

7   seagrass $\delta^{13}C$ on specific carbon acquisition properties has terrestrial analogues, as seen in the

8    $\delta^{13}C$ variation between C_3 and C_4 plants. In addition to utilizing PEP carboxylase to fix CO_2,

9   which discriminates less against ^{13}C than RUBISCO, C_4 plants limit carbon diffusion out of the

10   leaves with morphological adaptations (e.g. bundle sheaths), and variations in the rates of CO_2

11   back diffusion have been noted to impact the $\delta^{13}C$ values of C_4 vegetation (Farquhar et al. 1989).

12   While seagrasses are all C_3 plants (Beer & Wetzel 1982), the degree of bicarbonate use, extent of

13   internal recycling, and specific leaf morphology (Lepoint et al. 2008) are likely to play analogous

14   roles in explaining interspecific variation in the $\delta^{13}C$ value of marine plants.

15   Local scale interspecific trends in stable nitrogen isotope values displayed non-significant

16   differences amongst all seagrass species. The significant differences observed between *Thalassia*

17   *testudinum* and *Halodule wrightii* at the broad scale were not observed within sites, indicating

18   that $\delta^{15}N$ trends across FKNMS were predominantly a result of spatial variation in seagrass

19   distribution and DIN pools, as opposed to interspecific physiological differences in nitrogen

20   uptake and fractionation. However, we note that the reduced sampling effort of the within site

21   comparisons may have limited our ability to detect a significant difference in $\delta^{15}N$ between *T.*

22   *testudinum* and *H. wrightii*, warranting future studies.

1   Interspecific variation in elemental ratios revealed that, similar to broad scale patterns,

2   *Thalassia testudinum* displayed significantly lower N:P ratios than *Halodule wrightii* when

3   growing at the same location. At the local scale, it was additionally found that *T. testudinum* had

4   significantly lower N:P ratios than *S. filiforme*, previously undocumented at broader scales.

5   There remained no difference in N:P ratios between *H. wrightii* and *S. filiforme*. By removing

6   spatial variation in environmental variables and seagrass distribution, interspecific differences in

7   elemental composition reflect important differences in the ecology of these seagrass species.

8   When co-occurring with *T. testudinum*, and exposed to similar nutrient and light conditions,

9   rapidly growing early successional species (*H. wrightii* and S. *filiforme*) are further removed

10   from Seagrass Redfield stoichiometry, indicating heavily nutrient limited growth (particularly

11   with respect to phosphorus). The slower growth rates of *T. testudinum* may allow for reduced

12   nutrient limited growth, and an enhanced ability to thrive under oligotrophic conditions. The

13   altered elemental ratios of co-occurring seagrasses further suggests that nutrient limitation needs

14   to be viewed in respect to a specific primary producer, and may not be applicable to other species

15   within the same location.

16

17   **Temporal variation in seagrass isotope content**

18   As previously documented for *Thalassia testudinum* (Fourqurean et al. 2005) the δ^{13}C of

19   *Halodule wrightii* and *Syringodium filiforme* fluctuate seasonally. Interspecific variation in

20   seagrass δ^{13}C value was not limited to the summer conditions of high productivity, as we would

21   expect if differential CO_2 acquisition were leading to different amounts of isotopic

22   discrimination during summer periods of maximum photosynthesis. Interspecific differences in

23   δ^{13}C were maintained throughout the year, with peaks during the highly productive summer

23

1   periods and lows during the less productive winter periods. The constant interspecific variation

2   suggests that species specific $\delta^{13}C$ values are a result of physiologically based traits, and are

3   unlikely the result of altered or adaptive carbon acquisition mechanisms during the summer

4   months. Thus, despite seasonal variation, seagrass $\delta^{13}C$ values remain significantly distinct

5   throughout the year, and maintain the similar trends (*S. filiforme* > *T. testudinum* > *H. wrighii*)

6   found during the summer surveys.

7   Seasonal variation in seagrass $\delta^{13}C$ values have been correlated to seasonal patterns in

8   productivity, governed both by light availability and temperature (Fourqurean et al. 2001,

9   Fourqurean et al. 2005, Fourqurean et al. 2007). Drawdown of CO_2 pools during the summer

10   months, combined with limited CO_2 diffusion rates, may decrease the degree of fractionation

11   displayed by RUBISCO. Similar seasonal trends have been noted for a number of phytoplankton

12   studies, and may indicate carbon limitation during periods of high productivity (Fogel et al.

13   1992). Alternately, it is possible that increased summer productivities may enhance bicarbonate

14   use within all seagrass species, elevating $\delta^{13}C$ values and mitigating carbon limited

15   photosynthesis. The amplitudes of the sine models were not statistically distinct among species,

16   thus there was no difference in the degree of seasonal variation. These findings contrasted with

17   our original hypotheses, which expected the fastest growing seagrass species (*H. wrightii* and *S.*

18   *filiforme*) to exhibit the greatest amplitude in seasonal variation, suggesting that while taxonomic

19   differences strongly influence the mean $\delta^{13}C$ value, seasonal environmental factors strongly

20   control annual variation within each species. There were no interspecific differences in Φ, and

21   therefore the timing of the seasonal response (summer peaks and winter lows) amongst species.

22   It is evident that while seasonal responses are not different in all three seagrass species, their

23   mean $\delta^{13}C$ value remains markedly distinct throughout the annual cycle.

1 Seasonal variation in seagrass $\delta^{15}N$ was undetected for all species. Seasonal cycles in

2 stable nitrogen isotope content have been previously documented for *Thalassia testudinum* in

3 South Florida (Fourqurean et al. 2005), thus the lack of a seasonal pattern in our study may be

4 due to an insufficient sampling size for this particular isotope parameter.

5 The elemental and stable isotopic content of seagrass species in South Florida displays

6 considerable interspecific variation at both broad and local spatial scales. Here we present the

7 first documentation of species specific variation in South Florida; by which carbon isotopic

8 values may be associated with physiological differences in carbon acquisition and elemental

9 ratios may be associated with ecological differences in seagrass growth rates. As such, the use of

10 these parameters as indicators of ecosystem properties needs to carefully consider the species of

11 marine plant being studied. In addition to previously documented spatial and temporal variation,

12 taxonomic differences in isotopic and elemental content can introduce additional variation of

13 significant magnitude. Interspecific differences are non-random, thus understanding how

14 seagrass species differ in isotopic and elemental content will aid in the interpretation of studies

15 which examine the food web and nutrient dynamics of multi-species seagrass ecosystems.

16

1   Laboratory analyzed our stable isotope samples. This is contribution number xx from the

2   Southeast Environmental Research Center at Florida International University.

3

4   **REFERENCES**

Abal EG, Loneragan N, Bowen P, Perry CJ, Udy JW, Dennison WC (1994) Physiological and Morphological Responses of the Seagrass *Zostera-Capricorni Aschers* to Light-Intensity. Journal of Experimental Marine Biology and Ecology 178:113-129

Agren GI (2004) The C : N : P stoichiometry of autotrophs - theory and observations. Ecology Letters 7:185-191

Armitage AR, Frankovich TA, Heck KL, Fourqurean JW (2005) Experimental nutrient enrichment causes complex changes in seagrass, microalgae, and macroalgae community structure in Florida Bay. Estuaries 28:422-434

Atkinson MJ, Smith SV (1983) C-N-P Ratios of Benthic Marine Plants. Limnology and Oceanography 28:568-574

Beer S, Wetzel RG (1982) Photosynthetic Carbon Fixation Pathways in *Zostera Marina* and 3 Florida Seagrasses. Aquatic Botany 13:141-146

Bjork M, Weil A, Semesi S, Beer S (1997) Photosynthetic utilisation of inorganic carbon by seagrasses from Zanzibar, East Africa. Marine Biology 129:363-366

Cooper LW (1989) Patterns of Carbon Isotopic Variability in Eelgrass, *Zostera Marina* L, from Izembek Lagoon, Alaska. Aquatic Botany 34:329-339

Cooper LW, Deniro MJ (1989) Stable Carbon Isotope Variability in the Seagrass *Posidonia Oceanica* - Evidence for Light Intensity Effects. Marine Ecology-Progress Series 50:225-229

Dawson TE, Mambelli S, Plamboeck AH, Templer PH, Tu KP (2002) Stable isotopes in plant ecology. Annual Review of Ecology and Systematics 33:507-559

Duarte CM (1990) Seagrass Nutrient Content. Marine Ecology-Progress Series 67:201-207

Duarte CM (1992) Nutrient Concentration of Aquatic Plants - Patterns across Species. Limnology and Oceanography 37:882-889

Durako MJ, Hall MO (1992) Effects of Light on the Stable Carbon Isotope Composition of the Seagrass *Thalassia Testudinum*. Marine Ecology-Progress Series 86:99-101

Durako MJ, Sackett WM (1993) Effects of $CO_{2(Aq)}$ on the Carbon Isotopic Composition of the Seagrass *Thalassia Testudinum Banks-Ex-Konig* (Hydrocharitaceae). Journal of Experimental Marine Biology and Ecology 169:167-180

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon Isotope Discrimination and Photosynthesis. Annual Review of Plant Physiology and Plant Molecular Biology 40:503-537

Ferdie M, Fourqurean JW (2004) Responses of seagrass communities to fertilization along a gradient of relative availability of nitrogen and phosphorus in a carbonate environment. Limnology and Oceanography 49:2082-2094

Fleming M, Lin GH, Sternberg LDL (1990) Influence of Mangrove Detritus in an Estuarine Ecosystem. Bulletin of Marine Science 47:663-669

Fogel ML, Cifuentes LA, Velinsky DJ, Sharp JH (1992) Relationship of Carbon Availability in Estuarine Phytoplankton to Isotopic Composition. Marine Ecology-Progress Series 82:291-300

Fourqurean JW, Escorcia SP, Anderson WT, Zieman JC (2005) 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. Estuaries 28:447-461

Fourqurean JW, Marba N, Duarte CM, Diaz-Almela E, Ruiz-Halpern S (2007) Spatial and temporal variation in the elemental and stable isotopic content of the seagrasses *Posidonia oceanica* and *Cymodocea nodosa* from the Illes Balears, Spain. Marine Biology 151:219-232

Fourqurean JW, Moore TO, Fry B, Hollibaugh JT (1997) Spatial and temporal variation in C:N:P ratios, δ^{15}N and δ^{13}C of eelgrass *Zostera marina* as indicators of ecosystem processes, Tomales Bay, California, USA. Marine Ecology-Progress Series 157:147-157

Fourqurean JW, Powell GVN, Kenworthy WJ, Zieman JC (1995) The Effects of Long-Term Manipulation of Nutrient Supply on Competition between the Seagrasses *Thalassia Testudinum* and *Halodule Wrightii* in Florida Bay. Oikos 72:349-358

Fourqurean JW, Willsie A, Rose CD, Rutten LM (2001) Spatial and temporal pattern in seagrass community composition and productivity in south Florida. Marine Biology 138:341-354

Fourqurean JW, Zieman JC (2002) Nutrient content of the seagrass *Thalassia testudinum* reveals regional patterns of relative availability of nitrogen and phosphorus in the Florida Keys USA. Biogeochemistry 61:229-245

Fourqurean JW, Zieman JC, Powell GVN (1992a) Phosphorus Limitation of Primary Production in Florida Bay - Evidence from C-N-P Ratios of the Dominant Seagrass *Thalassia Testudinum*. Limnology and Oceanography 37:162-171

Fourqurean JW, Zieman JC, Powell GVN (1992b) Relationships between Porewater Nutrients and Seagrasses in a Subtropical Carbonate Environment. Marine Biology 114:57-65

Fry B, Macko SA, Zieman JC (1985) Review of Stable Isotopic Investigations of Food Webs in Seagrass Meadows. Florida Marine Research Publications 42

Fry B, Sherr EB (1984) δ^{13}C Measurements as Indicators of Carbon Flow in Marine and Fresh-Water Ecosystems. Contributions in Marine Science 27:13-47

Grice AM, Loneragan NR, Dennison WC (1996) Light intensity and the interactions between physiology, morphology and stable isotope ratios in five species of seagrass. Journal of Experimental Marine Biology and Ecology 195:91-110

Hemminga MA, Mateo MA (1996) Stable carbon isotopes in seagrasses: Variability in ratios and use in ecological studies. Marine Ecology-Progress Series 140:285-298

Invers O, Perez M, Romero J (1999) Bicarbonate utilization in seagrass photosynthesis: role of carbonic anhydrase in *Posidonia oceanica* (L) Delile and *Cymodocea nodosa* (Ucria) Ascherson. Journal of Experimental Marine Biology and Ecology 235:125-133

Invers O, Zimmerman RC, Alberte RS, Perez M, Romero J (2001) Inorganic carbon sources for seagrass photosynthesis: an experimental evaluation of bicarbonate use in species inhabiting temperate waters. Journal of Experimental Marine Biology and Ecology 265:203-217

Lepoint G, Dauby P, Fontaine M, Bouquegneau JM, Gobert S (2003) Carbon and nitrogen isotopic ratios of the seagrass *Posidonia oceanica*: Depth-related variations. Botanica Marina 46:555-561

Lepoint G, Frederich B, Gobert S, Parmentier E (2008) Isotopic ratios and elemental contents as indicators of seagrass C processing and sewage influence in a tropical macrotidal ecosystem (Madagascar, Mozambique Channel). Scientia Marina 72:109-117

Lin GH, Banks T, Sternberg L (1991) Variation in $\delta^{13}C$ Values for the Seagrass *Thalassia Testudinum* and Its Relations to Mangrove Carbon. Aquatic Botany 40:333-341

McMillan C, Parker PL, Fry B (1980) ^{13}C /^{12}C Ratios in Seagrasses. Aquatic Botany 9:237-249

Peterson BJ, Fry B (1987) Stable Isotopes in Ecosystem Studies. Annual Review of Ecology and Systematics 18:293-320

Schwarz AM, Bjork M, Buluda T, Mtolera H, Beer S (2000) Photosynthetic utilisation of carbon and light by two tropical seagrass species as measured in situ. Marine Biology 137:755-761

Sharkey T, Berry JA (1985) Carbon isotope fractionation of algae as influenced by an inducible CO_2 concentrating mechanism. In Lucas, W.J.,Berry, J.A. (eds.) Inorganic carbon uptake by aquatic photosynthetic organisms. American Society of Plant Physiology, Rockville, MD, p. 389-402.

Uku J, Beer S, Bjork M (2005) Buffer sensitivity of photosynthetic carbon utilisation in eight tropical seagrasses. Marine Biology 147:1085-1090

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	δ^{15}N	δ^{13}C	C:N	C:P	N:P	L.I.
Depth		0.113	**-0.655**	**0.377**	-0.131	**-0.322**	-0.208
δ^{15}N	0.326		0.162	**0.272**	-0.073	-0.182	-0.012
δ^{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	δ^{15}N	δ^{13}C	C:N	C:P	N:P	L.I.
Depth		**0.415**	**-0.609**	-0.090	**-0.391**	**-0.466**	**-0.547**
δ^{15}N	**0.020**		-0.210	**0.494**	-0.052	**-0.407**	**-0.423**
δ^{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	δ^{15}N	δ^{13}C	C:N	C:P	N:P	L.I.
Depth		**0.650**	**-0.477**	0.321	-0.211	**-0.459**	**-0.500**
δ^{15}N	**<0.001**		**-0.407**	**0.631**	0.041	**-0.390**	**-0.412**
δ^{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 δ^{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 δ^{13}C and δ^{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 δ^{13}C and δ^{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 δ^{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 $\pm$ 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).
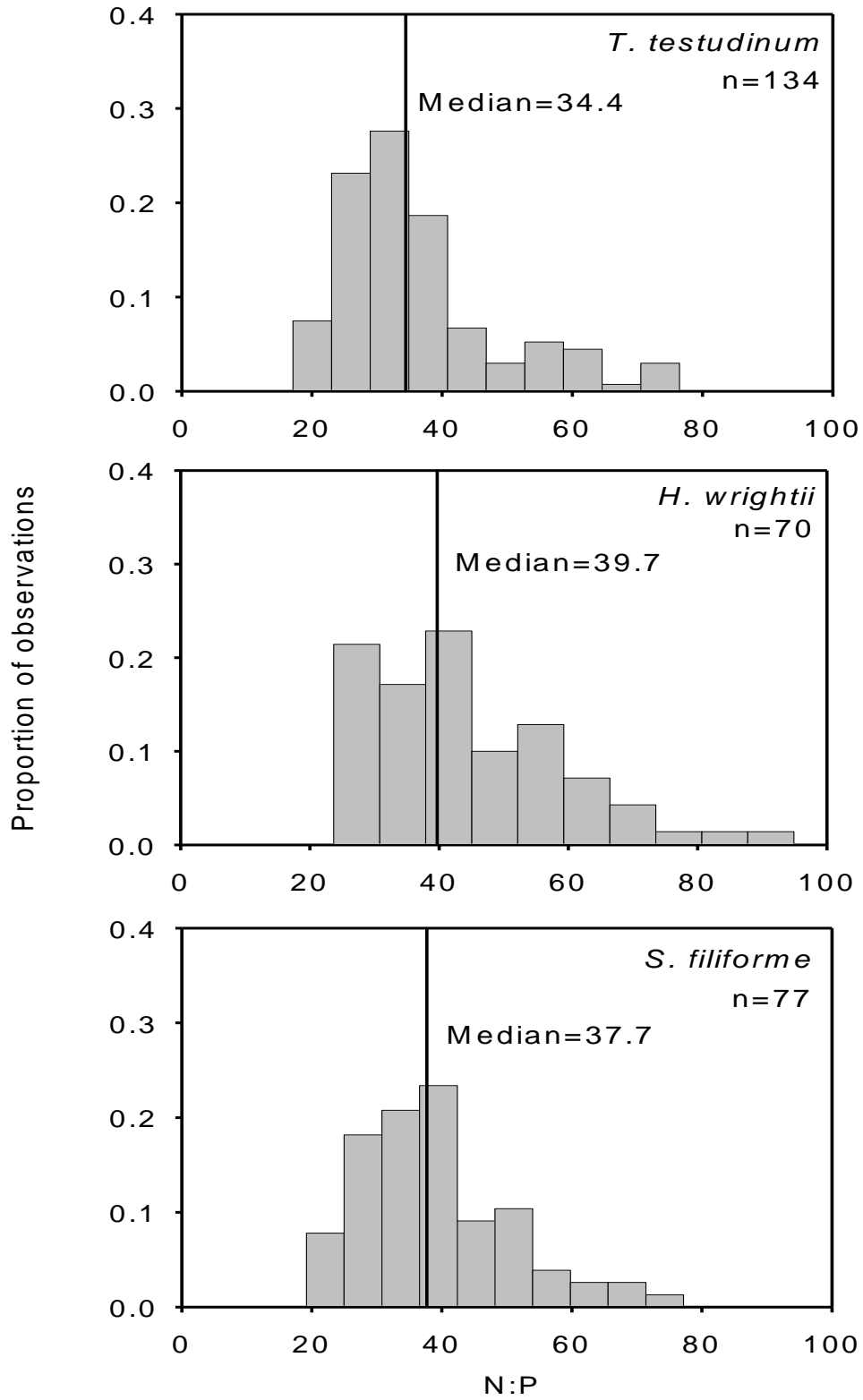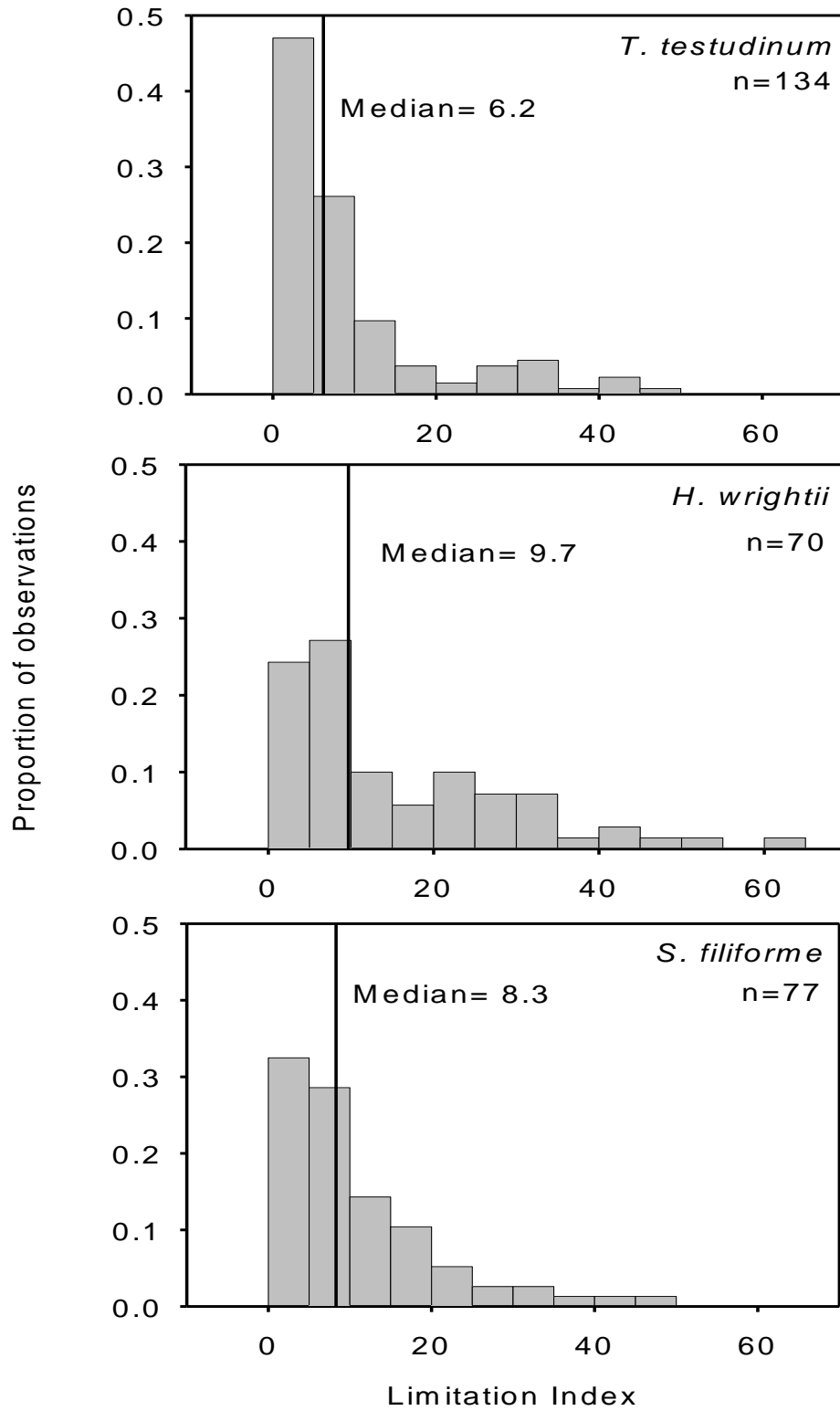
Figure 1

Legend:
- ● Multi-species survey site
- ○ Monospecific *Thalassia* survey site
- + Permanent monitoring site

Gulf of Mexico
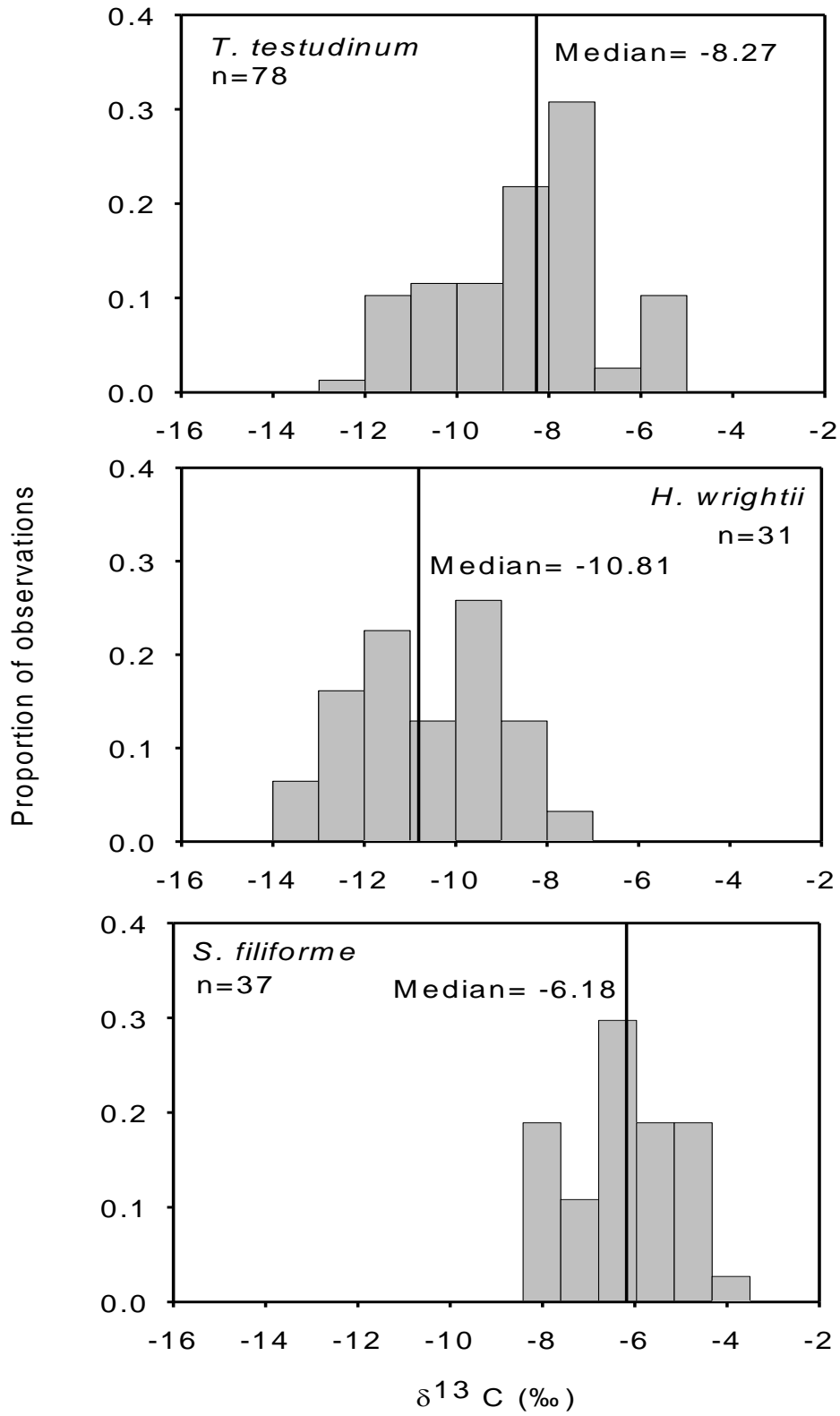
Florida Bay

Atlantic Ocean
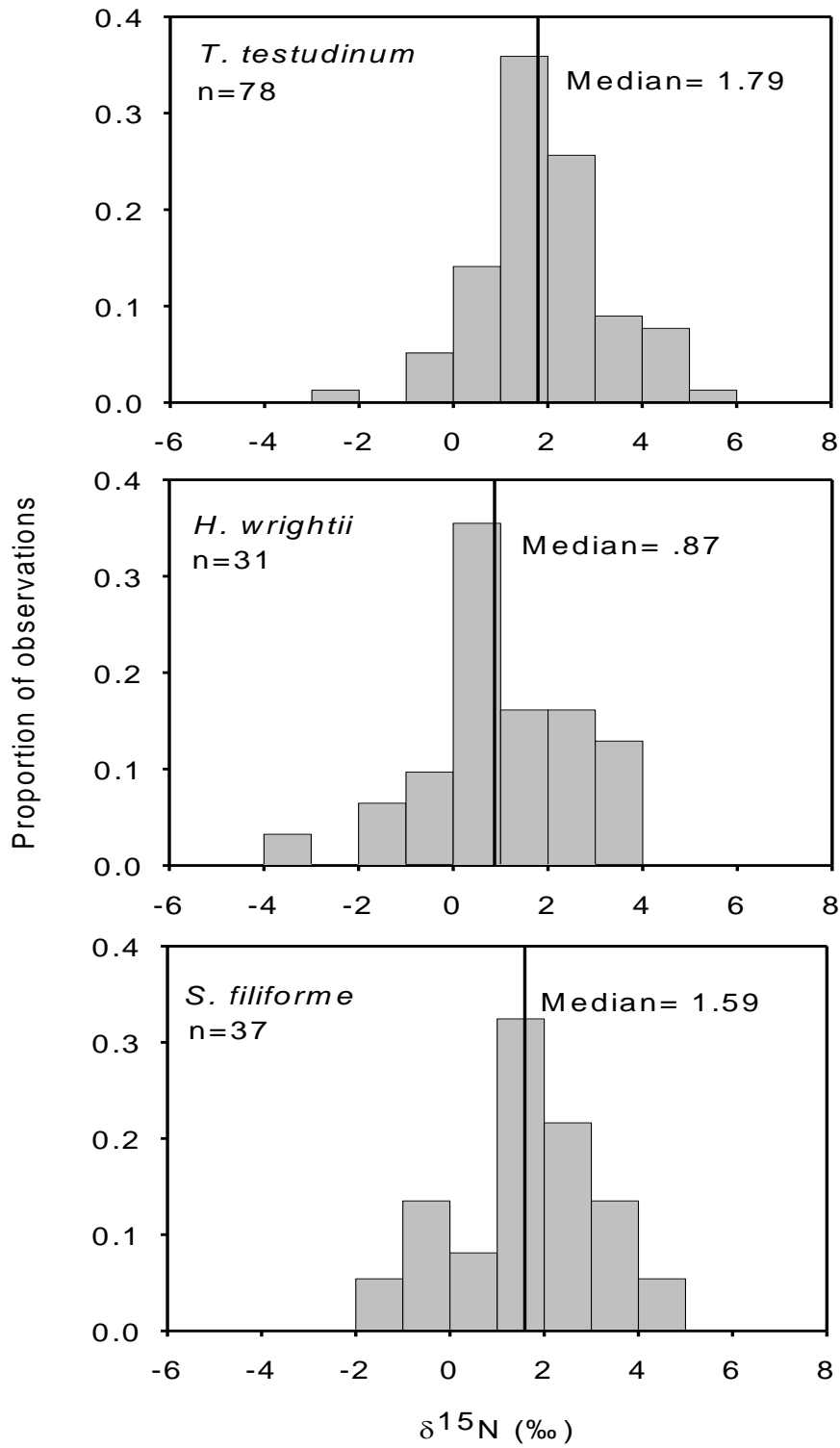
FKNMS Boundary

N
W — E
S

0 10 20 30 40 50 km
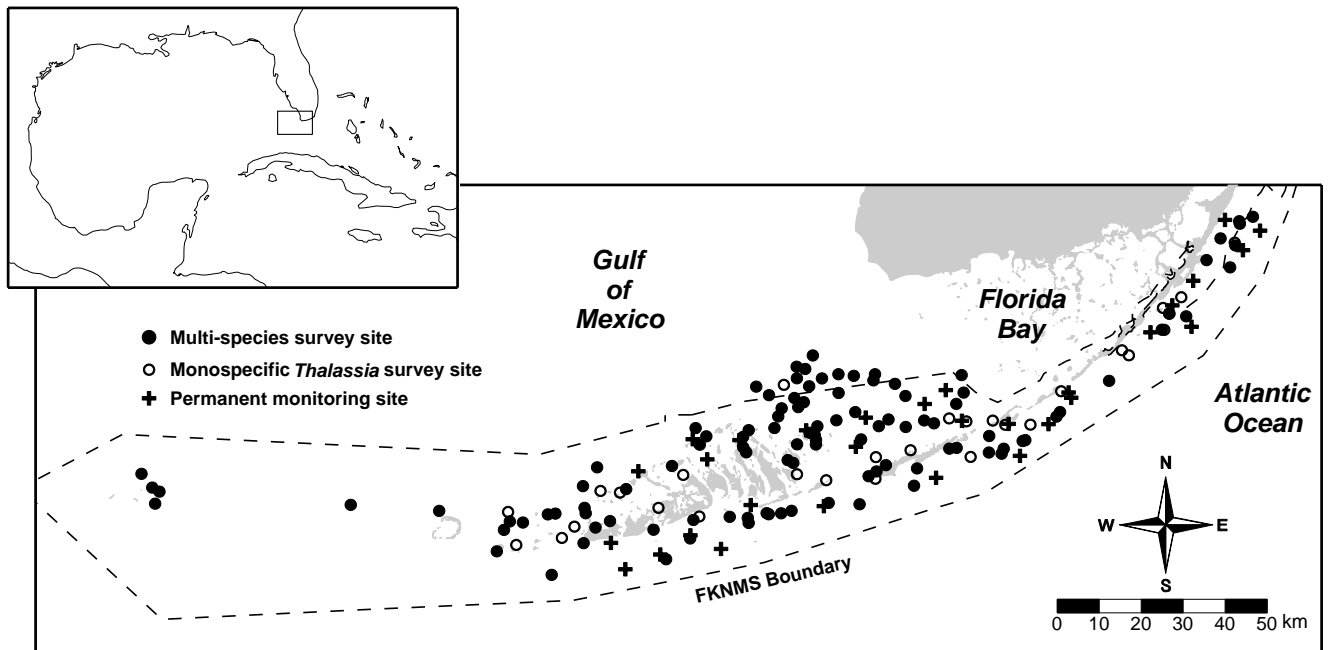
Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Leaf $\delta^{13}C$ (‰)

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 $\delta^{15}N$ (‰)

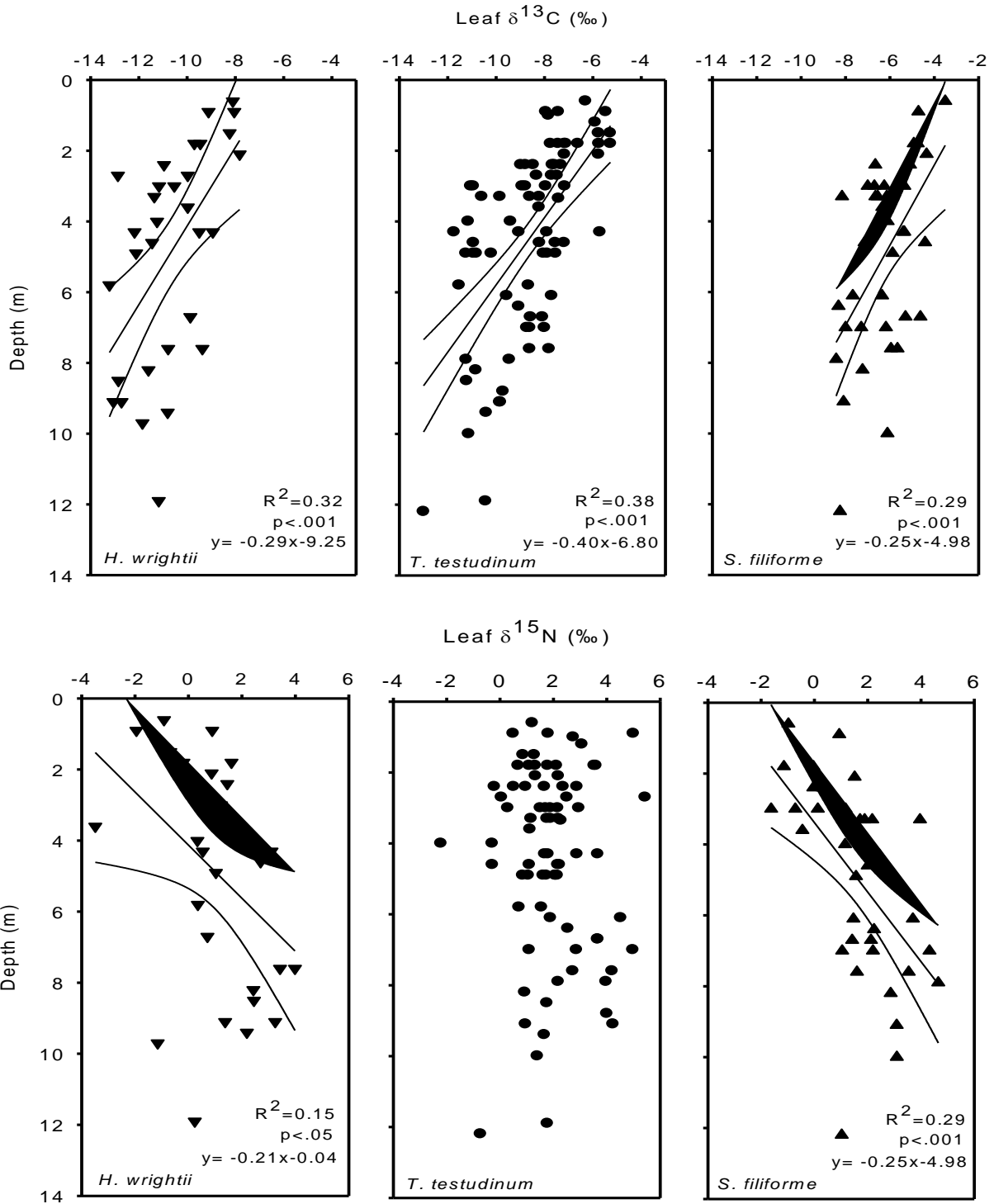

R²=0.15
p<.05
y= -0.21x-0.04

H. wrightii

T. testudinum

R²=0.29
p<.001
y= -0.25x-4.98

S. filiforme

Figure 7

Figure 8

Figure 9