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

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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

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12 acquisition

13

14 **ABSTRACT**

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

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

17 10,000 km<sup>2</sup> 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* ( $36:5 \pm 1.1$ ) and *S.*

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

23 were highest for *S. filiforme* ( $-6.2\text{‰} \pm 0.2\text{‰}$ ), intermediate for *T. testudinum* ( $-8.6\text{‰} \pm 0.2\text{‰}$ ),

1 and lowest for *H. wrightii* ( $-10.6‰ \pm 0.3‰$ ). Stable nitrogen isotopes ( $\delta^{15}\text{N}$ ) were heaviest for *T.*  
2 *testudinum* ( $2.0‰ \pm 0.1‰$ ), and lightest for *H. wrightii* ( $1.0‰ \pm 0.3‰$ ) and *S. filiforme* ( $1.6‰ \pm$   
3  $0.2‰$ ). Site depth was negatively correlated to  $\delta^{13}\text{C}$  for all species, while  $\delta^{15}\text{N}$  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  $\delta^{13}\text{C}$  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.

# 1 INTRODUCTION

2           The elemental and isotopic content of plant biomass can be used to characterize both the  
3 nutritional status and environmental conditions of macrophyte communities (McMillan et al.  
4 1980, Atkinson & Smith 1983, Farquhar et al. 1989, Duarte 1992, Fourqurean et al. 2005,  
5 Fourqurean et al. 2007). These plant parameters, while reflecting the local availabilities of  
6 essential resources (nitrogen, phosphorus, carbon, and light), can additionally reflect interspecific  
7 variation, and the manner in which species interact with local resources. Taxonomic differences  
8 in growth rates, life-history strategies, physiology and morphology have the ability to influence  
9 resultant nitrogen:phosphorus (N:P) ratios and the stable isotopic content of plant material  
10 (Farquhar et al. 1989, Dawson et al. 2002, Agren 2004). While landscape patterns in elemental  
11 and isotopic content of submerged plants may be driven by large scale spatial variations in the  
12 stable isotopic composition of nutrient pools and the availabilities of nutrients and light;  
13 localized interspecific differences have been detected (Fourqurean et al. 2007), and may be  
14 attributed to physiological differences amongst sympatric plant species. Prior to using variations  
15 in the N:P ratios and stable isotopic compositions of benthic plants for inferring ecosystem  
16 processes, it is important to understand the factors which drive these variations at all spatial  
17 scales, and how taxonomic differences can be reflected within these parameters.

18           The N:P ratio of plant material is related to the availability of these elements in the  
19 environment relative to plant demand (Duarte 1990). Over landscape scales, spatial gradients in  
20 N or P availability are reflected by spatial patterns in plant nutrient content, as shown for  
21 seagrasses growing in coastal marine habitats (Fourqurean et al. 1992a, Fourqurean & Zieman  
22 2002, Fourqurean et al. 2005). Nutrient addition experiments have supported the close link  
23 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  $^{13}\text{C}$  and heavier isotopic signatures (Cooper & Deniro 1989, Durako & Hall 1992). Similarly, for  
12 a given light level, experiments have found enriched carbon isotope signatures ( $\delta^{13}\text{C}$ ) 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  $\delta^{13}\text{C}$  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  $\text{CO}_2$ ) may impact  $\delta^{13}\text{C}$  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  $\text{HCO}_3^-$  uptake. Systematic differences in the  
22  $\delta^{13}\text{C}$  ratios of leaves of co-occurring seagrass species, as documented between the Mediterranean

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

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

11         Similar to carbon isotope discrimination, seagrasses can fractionate the source pool of  
12 dissolved inorganic nitrogen upon plant uptake (Fourqurean et al. 2005). The degree of nitrogen  
13 fractionation depends upon the size of the DIN pool relative to plant demand (Fourqurean et al.  
14 2005). Seasonal fluctuations in the  $\delta^{15}\text{N}$  value of seagrass tissues have been detected, and have  
15 been attributed to changes in both the isotopic signature of the source pool, and the degree of  
16 plant fractionation as a response to seasonal productivities (Fourqurean et al. 2005). However,  
17 altered DIN fractionation between multiple species has yet to be documented.

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

1 136 sites in South Florida, representing a 10,000 km<sup>2</sup> 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

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<sub>2</sub> and CO<sub>2</sub>, 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 (‰):  $\delta (\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}})-1] \times 1000$ . These results are presented  
20 with respect to the international standards of atmospheric nitrogen (AIR,N<sub>2</sub>) and Vienna Pee Dee  
21 belemnite (V-PDB) for carbon. Analytical reproducibility of the reported  $\delta$  values, based on  
22 sample replicates, was better than  $\pm 0.08\text{‰}$  for carbon and  $\pm 0.2\text{‰}$  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  $\delta^{13}\text{C}$  values to  
2 drive off any remaining carbonate material, and then determined the  $\delta^{13}\text{C}$  of this decalcified  
3 material. The differences in  $\delta^{13}\text{C}$  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-Smirnov test ( $\alpha=0.05$ ). Standard linear regression was used to  
7 test the strength of the relationship between stable isotope variables ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) 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  $\rho$ ). 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. =  $|30 - \text{N:P}|$ ) 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.,  $\delta^{13}\text{C}$ , and  
20  $\delta^{15}\text{N}$  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  $y = \text{mean} + \alpha[\sin(\text{time} + \Phi)]$ , where  $\alpha$  is the amplitude of a sine  
23 wave, and  $\Phi$  is a phase angle (we used time values in radians for both time and  $\Phi$ , where  $2\pi$

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  $\delta^{13}\text{C}$   
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 ( $44.1 \pm 1.8$ ), which  
20 were reflected in the highest mean L.I. of all species ( $15.3 \pm 1.7$ , Fig. 3, Table 1). *Syringodium*  
21 *filiforme* displayed an intermediate N:P ratio of  $38.9 \pm 1.3$ , which was normally distributed, and  
22 an average limitation index value of  $10.9 \pm 1.1$ . *Thalassia testudinum* displayed the lowest mean  
23 N:P ratio ( $36.5 \pm 1.1$ ), and the lowest mean L.I. value ( $9.6 \pm 0.9$ ) 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  $\delta^{13}\text{C}$  values of all species, with a mean of  $-10.6\text{‰}$   
5  $\pm 0.3\text{‰}$ , a range of  $-13.2\text{‰}$  to  $-7.8\text{‰}$ , and a normal distribution (Fig. 4). *Halodule wrightii*  $\delta^{13}\text{C}$   
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  $\delta^{13}\text{C}$  values with a  
8 mean of  $-6.2\text{‰} \pm 0.2\text{‰}$ , a range of  $-3.5\text{‰}$  to  $-8.4\text{‰}$ , and a normal distribution. The  $\delta^{13}\text{C}$  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  $\delta^{13}\text{C}$  values with a mean of  $-8.6\text{‰} \pm 0.2\text{‰}$ ,  
11 a range of  $-13.0\text{‰}$  to  $-5.3\text{‰}$ , whose values were normally distributed.

12 Stable nitrogen isotope values varied by  $7.7\text{‰}$ ,  $7.5\text{‰}$ , and  $6.3\text{‰}$  for *Thalassia*  
13 *testudinum*, *Halodule wrightii*, and *Syringodium filiforme* respectively (Table 1). *Thalassia*  
14 *testudinum* displayed the highest  $\delta^{15}\text{N}$  values of all species, with mean of  $2.0\text{‰} \pm 0.2\text{‰}$  and a  
15 normal distribution (Fig. 5). *Thalassia testudinum* displayed  $\delta^{15}\text{N}$  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  $1.0\text{‰} \pm 0.3\text{‰}$   
18 and a normal distribution. *Halodule wrightii*  $\delta^{15}\text{N}$  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  $1.6\text{‰} \pm 0.3\text{‰}$  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  $\delta^{13}\text{C}$  and N:P ratios were negatively correlated to  
23 site depth for all species, and displayed similar variation with depth amongst species. The  $\delta^{15}\text{N}$

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  $\delta^{15}\text{N}$  with depth  
3 was similar between *H. wrightii* and *S. filiforme*, and further analysis revealed that negative  
4 correlations between %N and  $\delta^{15}\text{N}$  were restricted to the deeper offshore locations (>4.3m).  
5 *Syringodium filiforme* was the only species for which  $\delta^{13}\text{C}$  was correlated to  $\delta^{15}\text{N}$ . Correlations  
6 between elemental and isotopic ratios were mixed depending upon species. In *T. testudinum*, N:P  
7 was positively correlated to  $\delta^{13}\text{C}$ , yet uncorrelated to  $\delta^{15}\text{N}$ . *Syringodium filiforme* showed a  
8 negative correlation between N:P and  $\delta^{15}\text{N}$ , yet no correlation between N:P and  $\delta^{13}\text{C}$ . In *H.*  
9 *wrightii*, N:P was both negatively correlated to  $\delta^{15}\text{N}$ , and positively correlated to  $\delta^{13}\text{C}$ . L.I.  
10 values were negatively correlated to both site depth and  $\delta^{15}\text{N}$  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  $\delta^{13}\text{C}$ .

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  $35.9 \pm 1.4$  and  $8.8 \pm$   
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  $44.5 \pm 1.9$  and  $15.8 \pm 1.8$  at the same sites. When growing in similar  
20 locations, significant differences were additionally detected in  $\delta^{13}\text{C}$  signatures, with *T.*  
21 *testudinum* ( $-8.9 \pm 0.4$ ) displaying isotopically heavier values than *H. wrightii* ( $-10.4 \pm 0.3$ ) ( $p <$   
22  $0.001$ )(Fig. 8). There were no consistent, statistically significant differences in  $\delta^{15}\text{N}$  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  $43.9 \pm 2.6$  and  
3  $15.8 \pm 2.2$  respectively for *H. wrightii*, and  $42.3 \pm 2.2$  and  $14.2 \pm 1.9$  respectively for *S. filiforme*.  
4 Significant differences were detected in  $\delta^{13}\text{C}$  signatures, with *H. wrightii* displaying a value of  
5  $-10.1\text{‰} \pm 0.3\text{‰}$ , as compared to the isotopically heavy *S. filiforme* ( $-5.7\text{‰} \pm 0.3\text{‰}$ ) ( $p <$   
6  $.001$ )(Fig. 8). There were no consistent, statistical differences in  $\delta^{15}\text{N}$  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  $34.2 \pm 1.1$  and  $7.4 \pm 0.9$ ,  
11 respectively. While *S. filiforme* displayed an N:P ratio and L.I. of  $38.8 \pm 1.4$  and  $10.9 \pm 1.2$ ,  
12 respectively (Fig. 7). Additionally,  $\delta^{13}\text{C}$  signatures were statistically distinct, with *T. testudinum*  
13 ( $-8.8\text{‰} \pm 0.2\text{‰}$ ) displaying isotopically lighter values than *S. filiforme* ( $-6.3\text{‰} \pm 0.2\text{‰}$ ) ( $p <$   
14  $0.001$ ) (Fig. 8). There were no statistical differences in  $\delta^{15}\text{N}$  between *T. testudinum* and *S.*  
15 *filiforme* at sites where they co-occurred.

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

18           There was marked seasonal variation in the  $\delta^{13}\text{C}$  values of *Thalassia testudinum*,  
19 *Halodule wrightii*, and *Syringodium filiforme* (Fig. 9).  $\delta^{13}\text{C}$  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  $\delta^{13}\text{C}$  values (Table 3). Parameter estimates for  $\alpha$  and  $\Phi$   
22 were not significantly different between species comparisons, indicating no interspecific  
23 differences in the degree of seasonal variation, nor the seasonal timing of  $\delta^{13}\text{C}$  values. Mean  $\delta^{13}\text{C}$

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  $\delta^{15}\text{N}$  values were not detected for any  
4 species.

## 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.

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

16 Significant differences in  $\delta^{13}\text{C}$  were detected amongst all three seagrass species. The  
17 lightest  $\delta^{13}\text{C}$  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  $^{13}\text{C}$  compared to  
20 *Halodule* sp. and *Thalassia hemprichii* in Mozambique. The heaviest  $\delta^{15}\text{N}$  was displayed by *T.*  
21 *testudinum*, which was significantly more positive than *H. wrightii*. The  $\delta^{15}\text{N}$  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  $\delta^{13}\text{C}$  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  $\delta^{13}\text{C}$  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  $\delta^{13}\text{C}$   
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  $\delta^{13}\text{C}$  ratio of seagrass tissues elsewhere (Cooper & Deniro 1989, Lepoint et al.  
14 2003, Fourqurean et al. 2007). In our study,  $\delta^{13}\text{C}$  values decrease by 0.40, 0.29, and 0.25 ‰  $\text{m}^{-1}$   
15 in depth for *Thalassia testudinum*, *Halodule wrightii*, and *Syringodium filiforme* respectively.  
16 We don't think that the  $\delta^{13}\text{C}$ -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  $\text{C}_3$  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  $\delta^{13}\text{C}$  values, contrary to what  
21 would be expected if  $\text{C}_3$  material was impacting the isotopic value of the DIC pool available to  
22 the seagrass community.

1           The  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  as depressed demand increases discrimination against the  
6 heavier isotope. However, the net change in leaf  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  with increasing depth due to a depressed demand for N  
10 and higher discrimination against  $^{15}\text{N}$ . Alternatively, cases in which leaf  $\delta^{15}\text{N}$  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  $^{15}\text{N}$  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  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  values as depth increases. We believe this to be the case in our study, as the  
18 observed relationships between  $\delta^{15}\text{N}$  and depth are a consequence of the distribution of deep-  
19 water sites within a landscape of variable nitrogen availability. The leaf  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  was only

1 significant for the deeper sites under similar light regimes, further indicating that environmental  
2 availability was driving the relationship between depth and  $\delta^{15}\text{N}$ . 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  $\delta^{15}\text{N}$  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  $\delta^{13}\text{C}$ . 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  $\text{CO}_2$  transport, and active  $\text{HCO}_3^-$  import (Invers et al. 1999, Invers et al.  
20 2001). While dissolved  $\text{CO}_2$  has an isotopic carbon signature of ca. -9 ‰,  $\text{HCO}_3^-$  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  $^{13}\text{C}$  (Hemminga & Mateo 1996).  
23 Interspecific variation in seagrass  $\delta^{13}\text{C}$  values may reflect previously documented interspecific

1 variation in bicarbonate acquisition mechanisms, as some species display enhanced  $\text{HCO}_3^-$   
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  $\text{HCO}_3^-$  as a carbon source than  
4 *H. wrightii* or *T. testudinum*, an hypothesis that should be explored.

5 Internal recycling of  $\text{CO}_2$  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  $\text{CO}_2$  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  $\delta^{13}\text{C}$  signatures (Sharkey & Berry 1985). Internal recycling may therefore serve to  
12 complicate the relationship between the  $\delta^{13}\text{C}$  of source DIC, and the  $\delta^{13}\text{C}$  of seagrass tissues. Our  
13 data are consistent with more efficient internal recycling of  $\text{CO}_2$  in *Syringodium filiforme* than  
14 *Halodule wrightii* or *Thalassia testudinum*. It is evident that the factors contributing to the  
15 characteristic  $\delta^{13}\text{C}$  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  $\text{CO}_2$  recycling make to the overall isotopic signature.

18 Interspecific variation in  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  values; *H. wrightii*, occupying areas with higher DIN pools may have higher rates  
6 of fractionation, lowering  $\delta^{15}\text{N}$  values. Such results are not contradictory to previous findings  
7 concerning the correlation between  $\delta^{15}\text{N}$  and depth. Despite *H. wrightii* displaying decreased  
8  $\delta^{15}\text{N}$  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  $\delta^{13}\text{C}$  agree with broad scale trends, revealing that  
20 species specific physiological attributes are dominant factors in controlling taxon specific  $\delta^{13}\text{C}$   
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

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  $\text{HCO}_3^-$   
3 use, exhibits high rates of carbon back diffusion (low  $\text{CO}_2$  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  $\text{CO}_2$   
6 recycling), and thus fixes a large majority of imported carbon. The apparent dependence of  
7 seagrass  $\delta^{13}\text{C}$  on specific carbon acquisition properties has terrestrial analogues, as seen in the  
8  $\delta^{13}\text{C}$  variation between  $\text{C}_3$  and  $\text{C}_4$  plants. In addition to utilizing PEP carboxylase to fix  $\text{CO}_2$ ,  
9 which discriminates less against  $^{13}\text{C}$  than RUBISCO,  $\text{C}_4$  plants limit carbon diffusion out of the  
10 leaves with morphological adaptations (e.g. bundle sheaths), and variations in the rates of  $\text{CO}_2$   
11 back diffusion have been noted to impact the  $\delta^{13}\text{C}$  values of  $\text{C}_4$  vegetation (Farquhar et al. 1989).  
12 While seagrasses are all  $\text{C}_3$  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  $\delta^{13}\text{C}$  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  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  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  $\delta^{13}\text{C}$  of  
19 *Halodule wrightii* and *Syringodium filiforme* fluctuate seasonally. Interspecific variation in  
20 seagrass  $\delta^{13}\text{C}$  value was not limited to the summer conditions of high productivity, as we would  
21 expect if differential  $\text{CO}_2$  acquisition were leading to different amounts of isotopic  
22 discrimination during summer periods of maximum photosynthesis. Interspecific differences in  
23  $\delta^{13}\text{C}$  were maintained throughout the year, with peaks during the highly productive summer



1 periods and lows during the less productive winter periods. The constant interspecific variation  
2 suggests that species specific  $\delta^{13}\text{C}$  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  $\delta^{13}\text{C}$  values remain significantly distinct  
5 throughout the year, and maintain the similar trends (*S. filiforme* > *T. testudinum* > *H. wrightii*)  
6 found during the summer surveys.

7         Seasonal variation in seagrass  $\delta^{13}\text{C}$  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  $\text{CO}_2$  pools during the summer  
10 months, combined with limited  $\text{CO}_2$  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  $\delta^{13}\text{C}$  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  $\delta^{13}\text{C}$  value, seasonal environmental factors strongly  
20 control annual variation within each species. There were no interspecific differences in  $\Phi$ , 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  $\delta^{13}\text{C}$  value remains markedly distinct throughout the annual cycle.

1           Seasonal variation in seagrass  $\delta^{15}\text{N}$  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

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3

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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,  $p < 0.05$ )

	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	%C (% dry wt)	%N (% dry wt)	%P (% dry wt)	C:N	C:P	N:P	L.I.
<b><i>Thalassia testudinum</i></b>									
Mean	2.0 <sup>A</sup>	-8.6 <sup>A</sup>	39.2 <sup>A</sup>	1.9 <sup>A</sup>	0.13 <sup>A</sup>	24.1 <sup>A</sup>	870.8 <sup>A</sup>	36.5 <sup>A</sup>	9.6 <sup>A</sup>
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
<b><i>Halodule wrightii</i></b>									
Mean	1.0 <sup>B</sup>	-10.6 <sup>B</sup>	43.4 <sup>B</sup>	2.3 <sup>B</sup>	0.13 <sup>A</sup>	22.7 <sup>A</sup>	1014.1 <sup>B</sup>	44.1 <sup>B</sup>	15.3 <sup>B</sup>
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
<b><i>Syringodium filiforme</i></b>									
Mean	1.6 <sup>AB</sup>	-6.2 <sup>C</sup>	38.9 <sup>A</sup>	2.1 <sup>C</sup>	0.13 <sup>A</sup>	22.8 <sup>A</sup>	866.0 <sup>A</sup>	38.9 <sup>AB</sup>	10.9 <sup>AB</sup>
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
<b>ANOVA statistics for differences between species</b>									
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  $\rho$ ) 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.

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

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

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

Table 3: Parameter estimates for non-linear regressions of pairwise seagrass  $\delta^{13}\text{C}$  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	$\Phi$
<i>T. testudinum</i>	$Y = -8.94 + 0.54 \sin(\text{DOY radians} + 3.71)$	0.55	(-9.41 , -8.48)	(-.140 , 1.22)	(2.55 , 4.88)
<i>H. wrightii</i>	$Y = -11.02 + 0.83 \sin(\text{DOY radians} + 4.28)$	0.56	(-11.79 , -10.24)	(-.208 , 1.87)	(2.92 , 5.64)
<i>H. wrightii</i>	$Y = -10.90 + 0.69 \sin(\text{DOY radians} + 4.19)$	0.55	(-11.55 , -10.25)	(-.193 , 1.57)	(2.83 , 5.55)
<i>S. filiforme</i>	$Y = -6.67 + 0.83 \sin(\text{DOY radians} + 4.28)$	0.55	(-7.45 , -5.88)	(-.224 , 1.89)	(2.9 , 5.66)
<i>T. testudinum</i>	$Y = -8.96 + 0.43 \sin(\text{DOY radians} + 3.59)$	0.73	(-9.2 , -8.71)	(.068 , .797)	(2.84 , 4.35)
<i>S. filiforme</i>	$Y = -6.99 + 0.61 \sin(\text{DOY radians} + 4.73)$	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  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  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  $\pm 1$  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  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotope ratios where species co-occur. Error bars are  $\pm 1$  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  $\delta^{13}\text{C}$  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  $y = \text{mean} + \text{amp} \times \sin(\text{time} + \Phi)$  is shown, where amp is the amplitude of a sine wave and  $\Phi$  is a phase angle in radians ( $2\pi$  radians = 365 d).

Figure 1

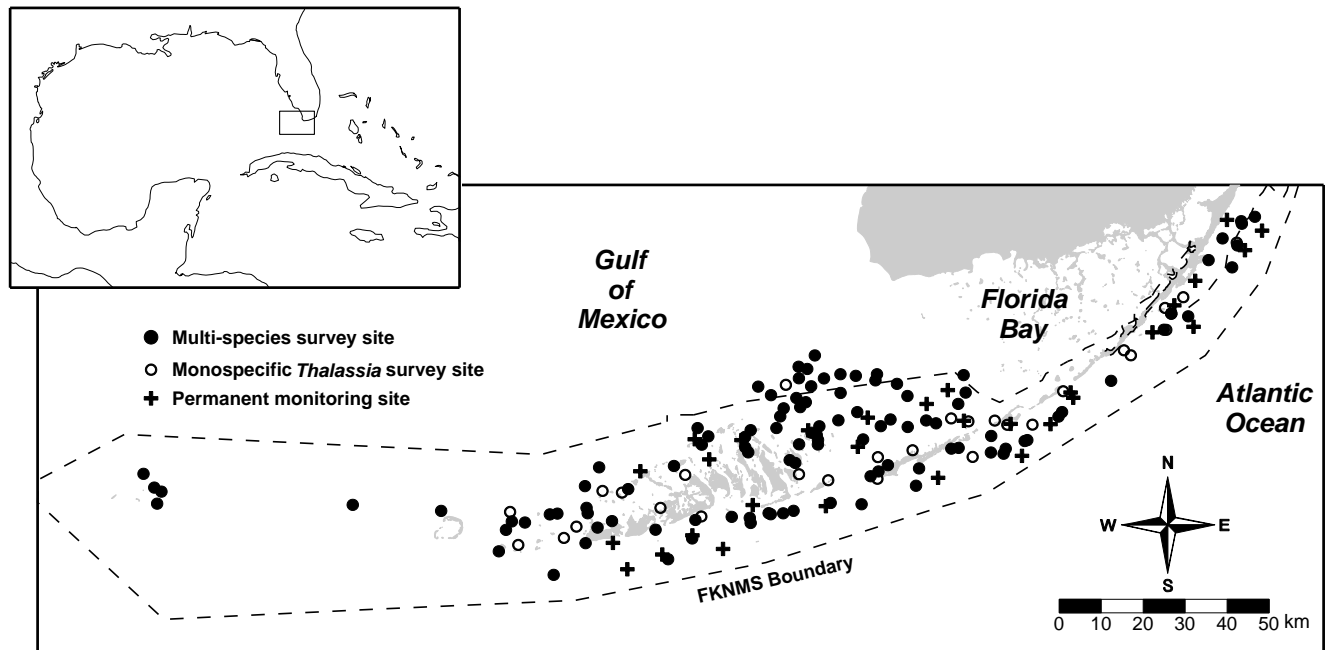


Figure 2

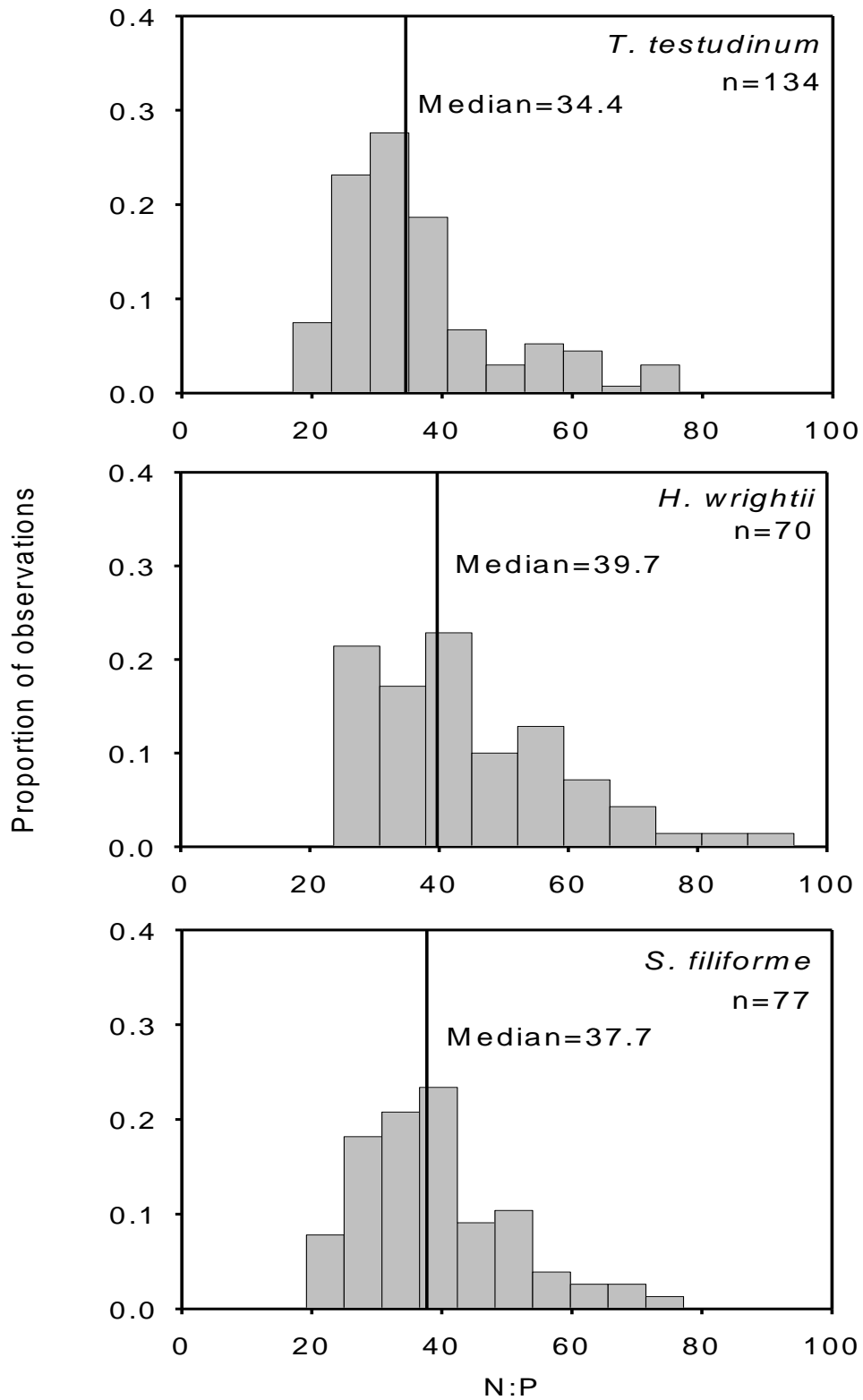


Figure 3

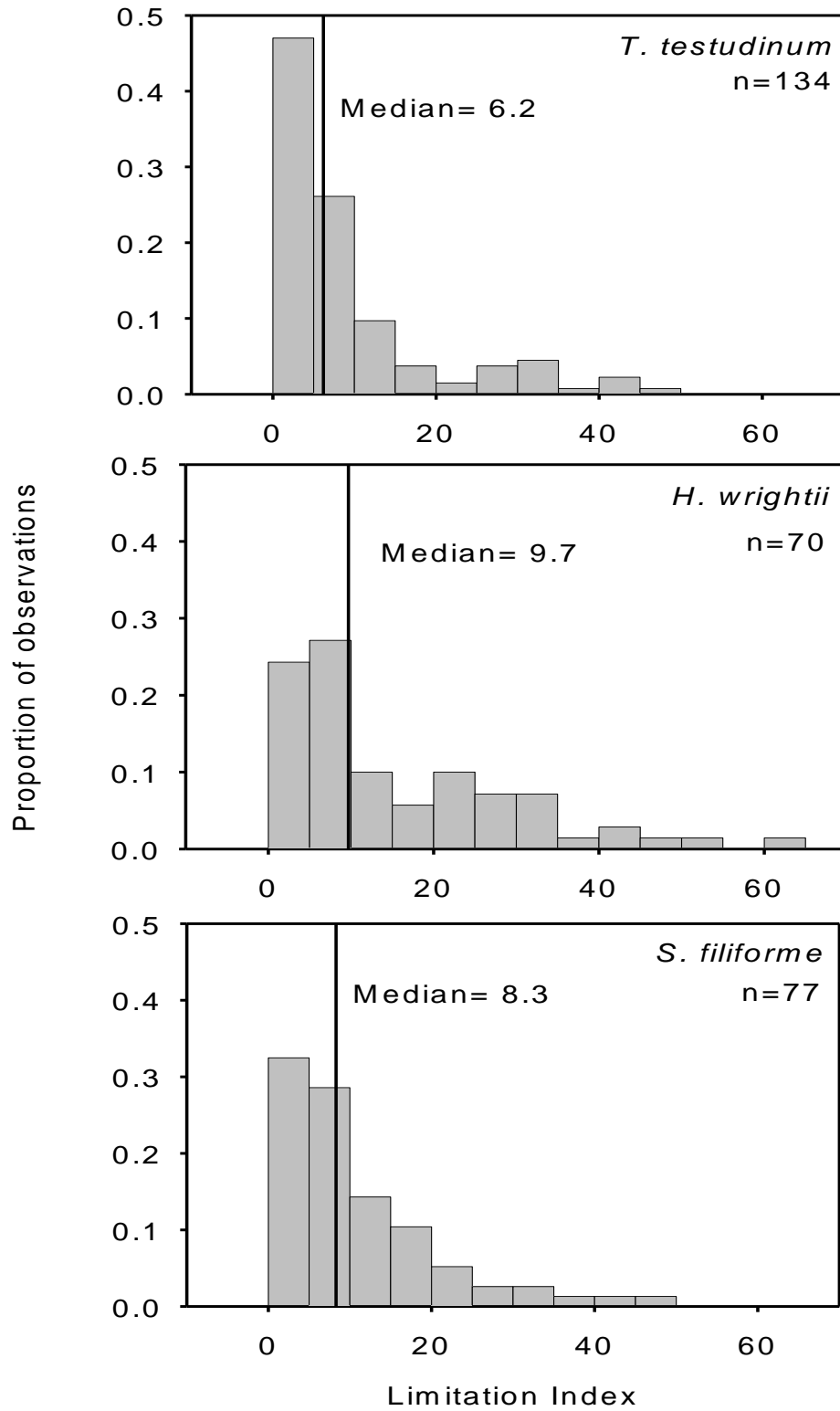


Figure 4

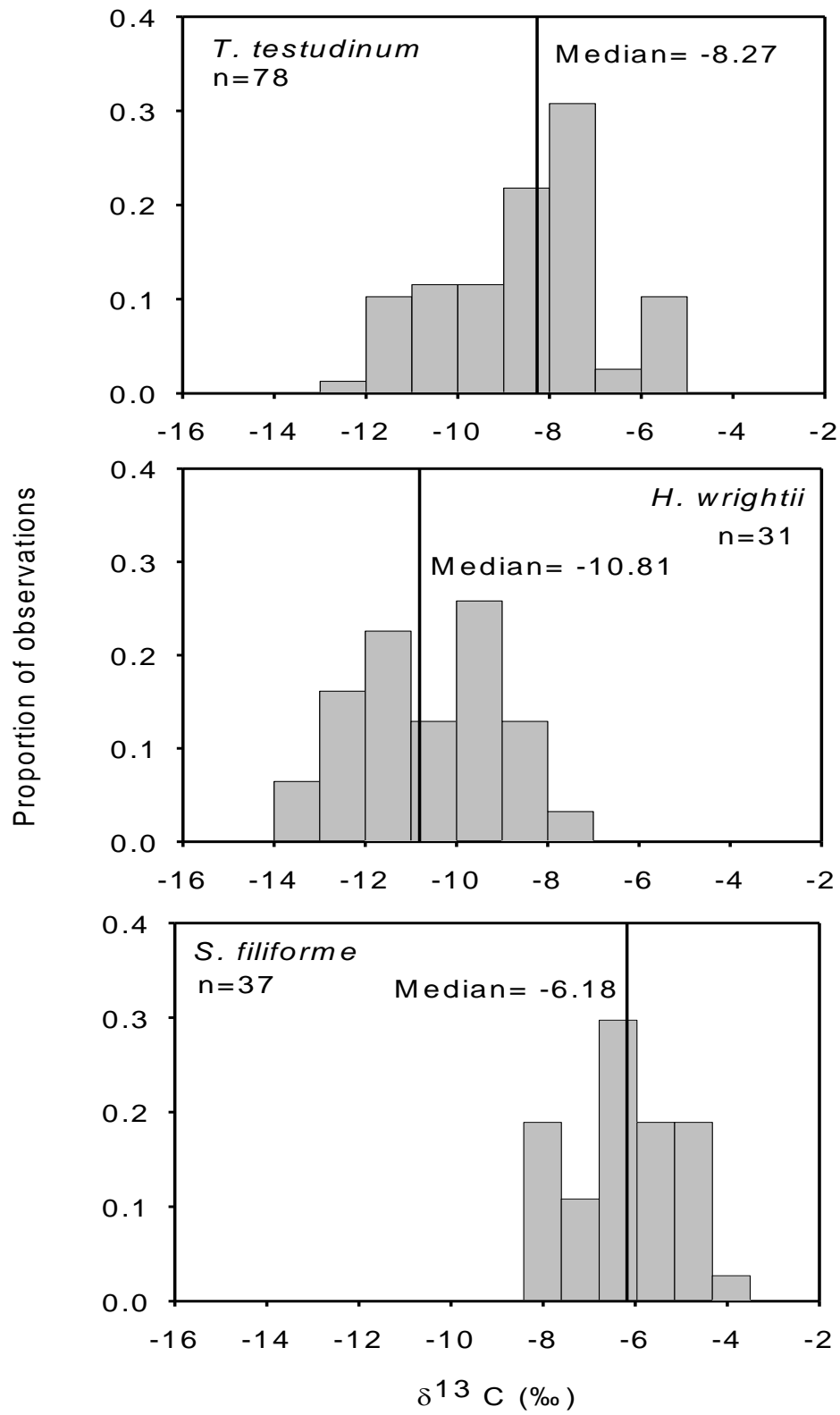


Figure 5

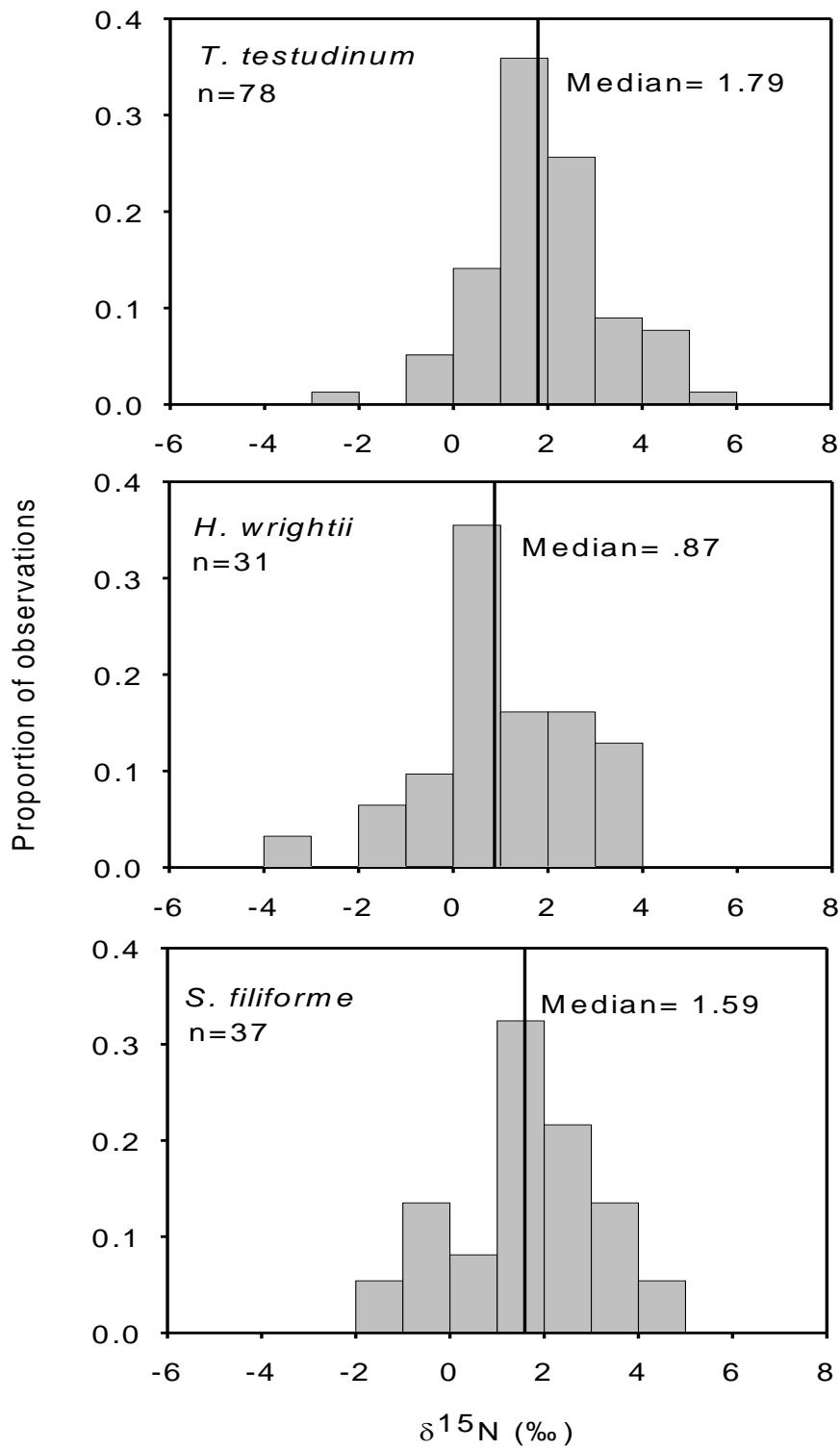


Figure 6

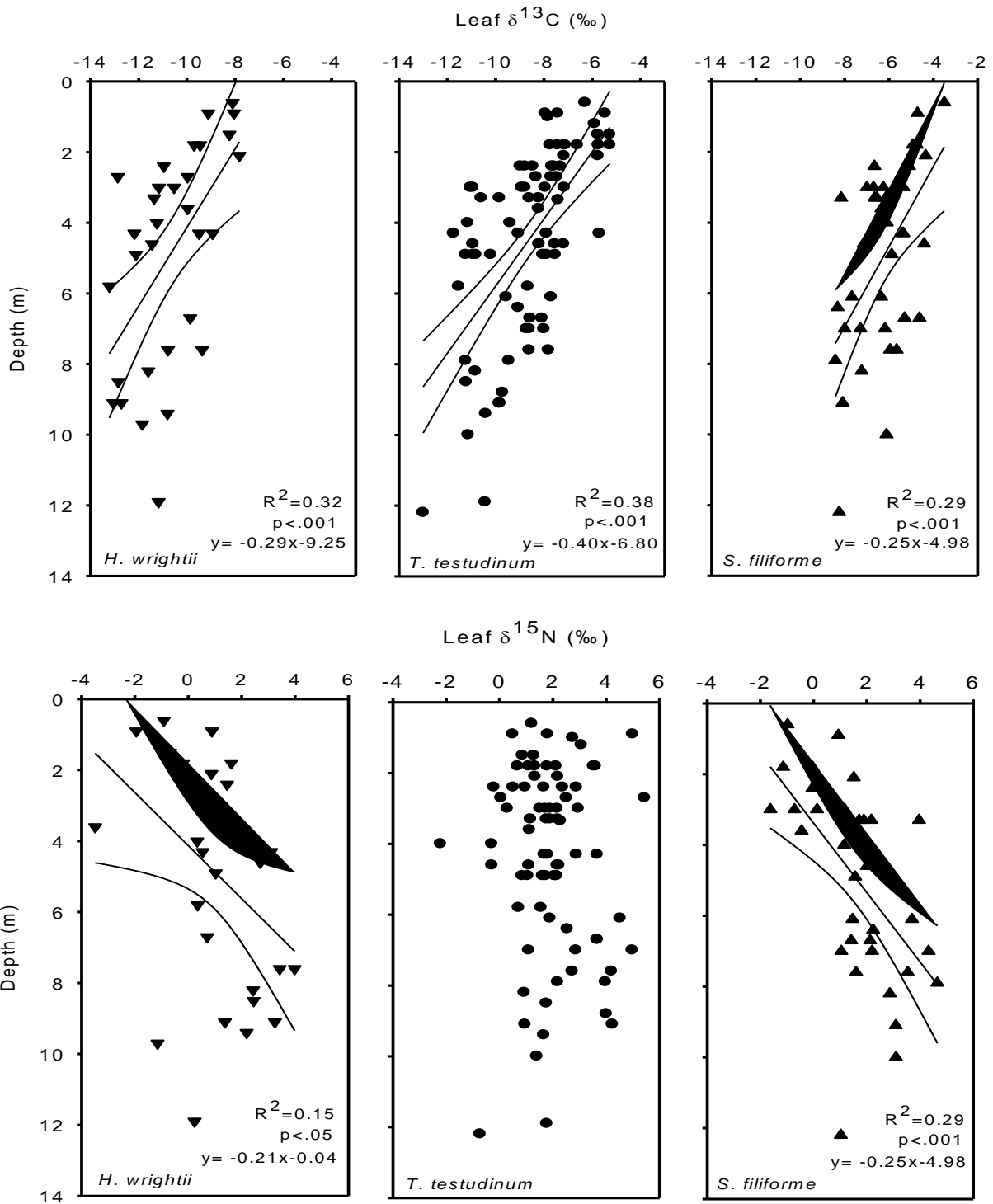




Figure 7

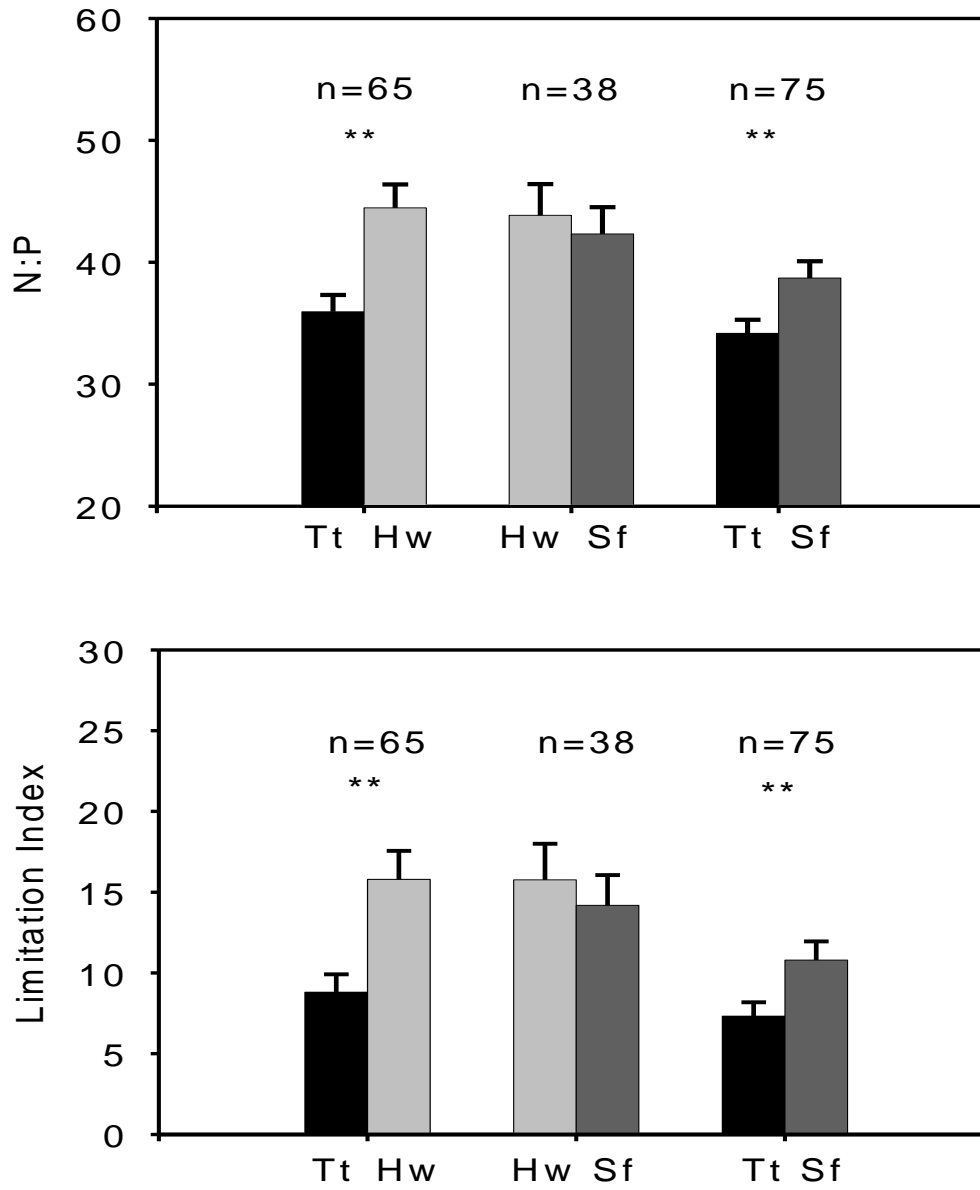


Figure 8

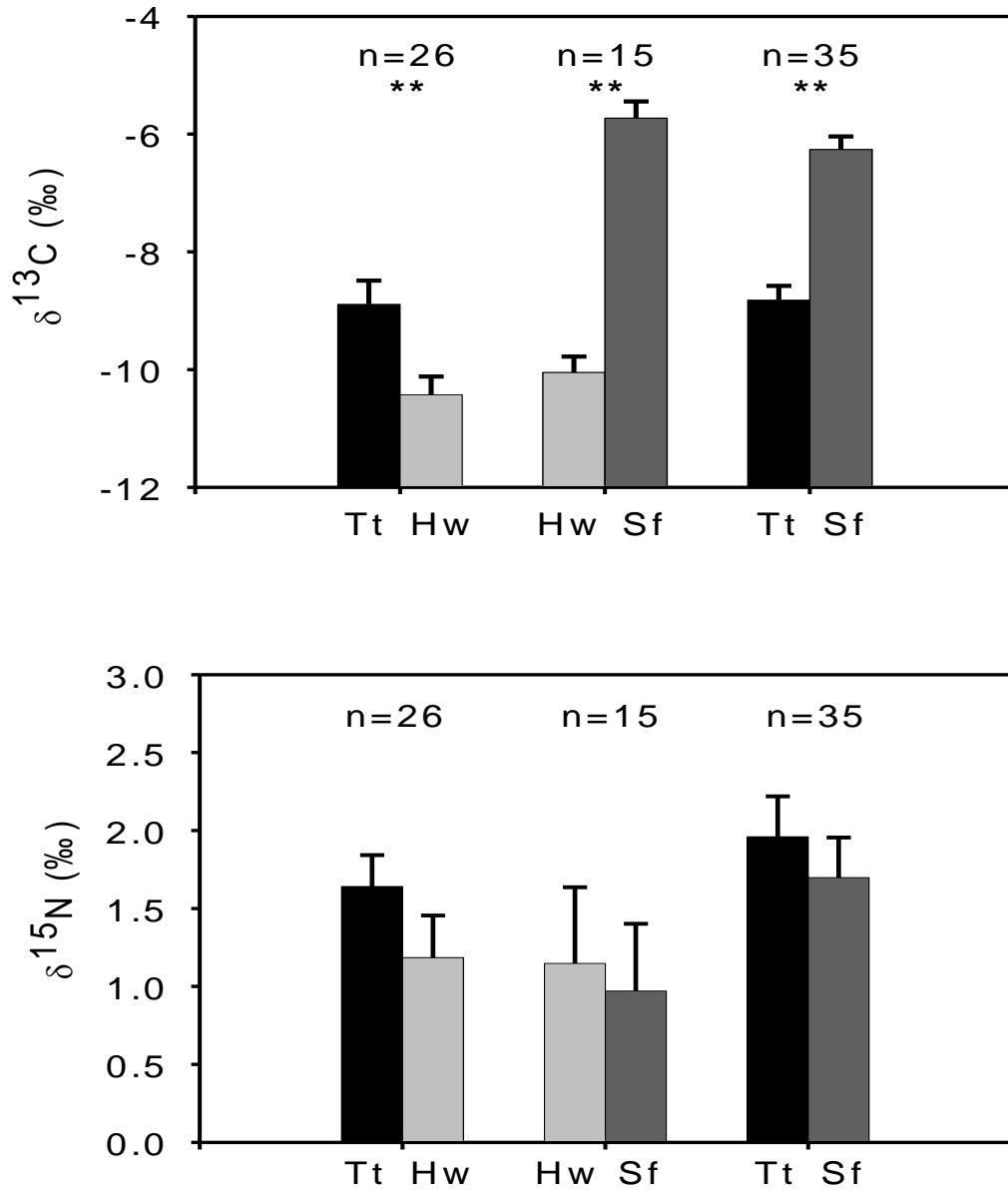


Figure 9

