Title: Negative relationships between the nutrient and carbohydrate content of the seagrass

Thalassia testudinum

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Abstract

This study documents relationships between plant nutrient content and rhizome carbohydrate content of a widely distributed seagrass species, *Thalassia testudinum*, in Florida. Five distinct seagrass beds were sampled for leaf nitrogen, leaf phosphorus, and rhizome carbohydrate content from 1997-1999. All variables displayed marked intra- and inter- regional variation. Elemental ratios (mean N:P ± S.E.) were lowest for Charlotte Harbor (9.9 ± 0.2) and highest for Florida Bay (53.5 ± 0.9), indicating regional shifts in the nutrient content of plant material. Rhizome carbohydrate content (mean ± S.E.) was lowest for Anclote Keys (21.8 ± 1.6 mg g^{-1}FM), and highest for Homosassa Bay (40.7 ± 1.7 mg g^{-1}FM). Within each region, significant negative correlations between plant nutrient and rhizome carbohydrate content were detected; thus, nutrient-replete plants displayed low carbohydrate content, while nutrient-deplete plants displayed high carbohydrate content. Spearman's rank correlations between nutrient and carbohydrate content varied from a minimum in Tampa Bay (ρ =-0.2) to a maximum in Charlotte Harbor (ρ =-0.73). Linear regressions on log-transformed data revealed similar trends. This consistent trend across five distinct regions suggests that nutrient supply may play an important role in the regulation of carbon storage within seagrasses. Here we present a new hypothesis for studies which aim to explain the carbohydrate dynamics of benthic plants.

1. Introduction

The ability of plants to endure disturbance events strongly depends upon their capacity to support essential growth and maintenance functions during unfavorable environmental conditions. Non-structural carbohydrate reserves play an important role in the resilience of perennial plants by serving as a "rescue mechanism", allowing plants to sustain respiration or rebuild damaged tissue in response to disturbance (McPherson and Williams, 1998; Landhausser and Lieffers, 2002; Poorter et al., 2010). Thus, understanding the dynamics of carbon storage, and the factors which influence carbohydrate reserves in plants may help elucidate their potential for resilience in disturbance- prone environments.

Non-structural carbohydrates build within plant storage organs by two distinct processes: true reserve formation and reserve accumulation (Chapin et al., 1990). The former process involves a metabolically regulated formation of storage carbohydrates at the expense of current plant growth, while the latter process results in a passive buildup of carbohydrates due to environmental factors (i.e. water and/or nutrient limitation) which constrain growth and reduce carbon demand (Chapin et al., 1990). Thus, the availability of external resources can strongly regulate storage dynamics, particularly in the latter case of reserve accumulation. In terrestrial plants, declines in nutrient availability can inhibit the production of new biomass, and increase stores of non-structural carbohydrates (Mooney et al., 1995; Wyka, 2000; Knox and Clarke, 2005). While the dynamics of carbohydrate storage have been studied for some marine plants, few studies have addressed the role that nutrients might play in regulating storage reserves.

Seagrasses allocate a substantial portion of their biomass to belowground storage organs (rhizomes), and like terrestrial plants, these structures serve as a carbohydrate reserve to support plant growth and maintenance during periods of low photosynthetic capacity (either due to

shading events or losses to herbivory). The extensive allocation of biomass to belowground structures suggests that these organs play an important role in the carbon dynamics of these plants, and may similarly be subjected to the processes of reserve accumulation.

This study examines the relationship between plant nutrient content and rhizome carbohydrate content in the seagrass, *Thalassia testudinum*, across multiple spatial scales in Florida. Here we present preliminary observational data to suggest that, in addition to other abiotic factors, nutrients may play an important role in regulating the size of carbohydrate reserves. Due to reserve accumulation, we hypothesized that nutrient-poor seagrasses would display increased rhizome carbohydrate content as compared to nutrient replete seagrasses. Nutrient-carbohydrate relationships were examined by sampling *T. testudinum* within five spatially distinct regions in Florida, and quantifying both the seagrass nutrient content and rhizome carbohydrate content across multiple years. Our observations suggest that future studies may need to consider the process of reserve accumulation, and the role of nutrient availability in the regulation of carbohydrate reserves.

2. Methods

2.1. Study site and sampling

Five distinct regions in Florida were selected to examine the relationship between nutrient and carbohydrate content of *T. testudinum*: Homosassa Bay (N28° 45', W82° 44'), Anclote Keys (N28° 12', W82° 47'), Tampa Bay (N27° 40', W82° 42'), Charlotte Harbor (N26° 48', W82° 08') and Florida Bay (N24° 58', W80° 50'). Within each region, 30 spatially-distributed, randomly-selected points (distributed over a 0.25 km^2 grid) were sampled during the summer seasons of 1997, 1998, and 1999 (see Carlson et al., 2003 for general description). At each sampling point, intact seagrass shoots were collected using a single, 6-inch diameter sediment

core, transported back to the lab on ice, and frozen until further chemical analyses. Secchi depth, temperature, and salinity were additionally recorded at each site. To quantify water clarity, the ratio between Secchi depth and site depth (Secchi ratio) was calculated; thus values near unity indicate conditions whereby the Secchi disc was visibly resting on the bottom. A portion of these data were previously used to assess regional indicators of seagrass health (Carlson et al., 2003), and examine large-scale patterns in relative nutrient availability across the eastern Gulf of Mexico (Fourqurean and Cai, 2001). The analyses presented herein are novel applications of these data.

2.2. Plant chemical analysis

Seagrass shoots were washed free of sediment, and separated into aboveground and belowground material. Leaf material was gently cleaned of epiphytes using a razor blade, dried to a constant weight at $80\,^{\circ}C$, and ground to a fine powder. Carbon (C) and nitrogen (N) content of leaf material was analyzed in duplicate using a CHN analyzer (Fisons NA1500). Leaf phosphorus (P) content was determined through a dry oxidation, acid hydrolysis extraction followed by a colorimetric analysis (Fourqurean et al., 1992). All elemental ratios were calculated on a mole : mole basis. Belowground, rhizome non-structural carbohydrate content (sucrose and hexose) was determined using sequential extraction methods (Zimmerman et al., 1995).

2.3. Statistical methods

Spearman's rank correlation and standard linear regression were used to test the strength of the relationship between seagrass leaf nutrient content and rhizome carbohydrate content across all sampling years. Linear regressions on log transformed data were produced for the nutrient (either N or P) which provided the highest correlation with rhizome carbohydrate

5

content for each respective region. Residuals from all linear regressions were tested for normality with a non-parametric Kolmogorov-Smirnov test (a=0.05).

3. Results

3.1 Site characteristics

Site depths displayed minor variation amongst the five sampling regions. Depths were generally lowest for Tampa Bay, and highest for Anclote Keys (Table 1). Due to the shallow depths and relatively clear water, most regions displayed Secchi ratios near 1, and exhibited minor intra-regional variation. Regional comparisons reveal that water clarity was highest for Tampa Bay, Homosassa Bay, and Florida Bay. Anclote Keys and Charlotte Harbor displayed slightly reduced water clarity, whereby average Secchi depths were 65% and 79% of the recorded site depth, respectively. Site temperatures were lowest for Homosassa Bay, and highest for Tampa Bay, while salinity was lowest in Charlotte Harbor and highest in Florida Bay.

3.2. Plant chemical characteristics

The nutrient and carbohydrate content of *Thalassia testudinum* displayed significant intra- and inter- regional variation for all sampling years (1997-1999). Across all regions, leaf nitrogen content (%N of dry mass) ranged from 1.60% to 3.96%, while leaf phosphorus content (%P of dry mass) ranged from 0.06% to 1.08%. Within each region, both leaf %N and %P were highest in Charlotte Harbor and lowest in Florida Bay (Table 1). Across all sites, carbon content displayed relatively little variation. Throughout the study, %P had higher coefficients of variation (0.16 - 0.26), as compared to %N (0.09 - 0.17) and %C (0.04 - 0.06). Regional variation in leaf N and P content produced marked variation in seagrass N:P ratios, which were lowest in Charlotte Harbor and highest in Florida Bay.

Rhizome carbohydrate content additionally displayed considerable variation across all

6

sampling years (Table1). Carbohydrate content was highest for Homosassa Bay and lowest for Anclote Keys. Within each region, carbohydrate content displayed higher coefficients of variation (0.31-0.71) relative to nutrient content (0.09-0.26).

3.3. Relationships between plant nutrient and carbohydrate content

All sampled regions displayed significant negative correlations between leaf nutrient content (%N and %P) and rhizome carbohydrate content. Intra-regional correlations (Spearman's rank) between nutrient and carbohydrate content were strongest for Charlotte Harbor (ρ=-0.73, $p<0.01$) and weakest for Tampa Bay (ρ=-0.28, $p<0.01$). Intermediate correlations were displayed for Homosassa Bay (ρ =-0.53, $p<0.01$), Anclote Keys (ρ =-.66, $p<0.01$), and Florida Bay (ρ =-0.46, $p<0.01$).Within each site, correlations were generally strongest for the nutrient which was in least supply for that respective region. Thus, regions with seagrass N:P ratios below 30 demonstrated higher correlation coefficients with %N as opposed to %P (with the exception of Anclote Keys). Conversely, regions with seagrass N:P ratios above 30 demonstrated highest correlations with %P. Linear regressions between carbohydrate and nutrient content revealed similar negative relationships for all regions (Fig. 1).

4. Discussion

Intra- and inter-regional variation in the leaf nutrient content of *T. testudinum* was detected during our sampling period, as previously documented in prior studies for seagrasses within these regions (Fourqurean and Cai, 2001). Between-region variation in leaf nutrient content was larger than within-region variation, generating significant differences in the nutrient content of *T. testudinum* across broad spatial scales. For example, leaf N:P ratios were low in Charlotte Harbor, indicating low nitrogen relative to phosphorus content. Conversely, Florida Bay displayed high N:P ratios, indicating high nitrogen relative to phosphorus content. These

large scale changes may be due to a number of attributes. Previous work has documented shifts

in the nutrient content of benthic plants, attributable to variation in the environmental availability

and/or supply rates of nitrogen and phosphorus (Fourqurean et al., 1992; Fourqurean and

Zieman, 2002). We suggest that similar variation in nutrient supply may be responsible for our

observed shifts in plant nutrient content. Changes in nutrient supply can result from either

changes in nutrient loading rates and/or shifts in a number of abiotic factors (i.e. sediment

mineralogy, sediment grain size, water clarity, or water depth) (Fourqurean and Zieman, 2002).

Such factors likely contributed to variation in plant nutrient content at both local and broad

spatial scales.

Relationships between the leaf nutrient and rhizome carbohydrate content of the seagrass

T. testudinum were detected during the course of a 3 year sampling period in Florida. While the

strength of these relationships displayed regional variation, all sampling sites demonstrated

significant negative correlations between leaf nutrient content (%N and %P) and rhizome

carbohydrate content within each region. Here, we hypothesize that within each region, nutrient

content may have influenced the carbohydrate storage reserves of this benthic plant because of

the process of reserve accumulation that has been described in terrestrial plants (Chapin et al.,

1990) . While the role of light availability certainly must be considered, we argue that the

relatively consistent depths and Secchi values within each region suggest that these nutrient-

carbohydrate relationships are not driven by large gradients in light availability. Terrestrial

studies have documented that nutrient-limited plants are constrained in their ability to produce

new biomass, thus carbon gain exceeds carbon demand, promoting the accumulation of storage

compounds (Chapin, 1980; Chapin et al., 1990; Wyka, 2000). Based upon our observations, we

hypothesize that a similar mechanism may operate for *T. testudinum*. Prior work with the

freshwater macrophyte, *Berula erecta* has experimentally demonstrated increased carbohydrate storage with nutrient limitation, and decreased carbohydrate storage under nutrient replete conditions (Puijalon et al., 2008). Our field observations for *T. testudinum* follow similar trends, and we suggest that these preliminary observations warrant further manipulative experimentation.

The strength of these nutrient-carbohydrate relationships displayed inter-regional variation, as Spearman's rank correlations were highest for the Charlotte Harbor region, and lowest for the Tampa Bay region. Such variation may be attributable to large scale, regional differences in a number of abiotic factors (i.e. light, temperature, and/or salinity), which may regulate the strength of the nutrient-carbohydrate relationship. Previous work has demonstrated a number of factors which can impact seagrass carbohydrate content: for example season (Vichkovitten et al., 2007), light availability (Burke et al., 1996; Zimmerman and Alberte, 1996; Lee and Dunton, 1997; Carlson et al., 2003; Collier et al., 2009), and grazing (Fourqurean et al., 2010). Large-scale regional variation in these factors may explain why the nutrient-carbohydrate relationships were relatively strong in some regions, while relatively weak in others.

The consistency of these negative relationships between nutrient and carbohydrate content within several geographically distinct regions suggests that seagrasses, such as *T. testudinum* with substantial storage organs, may accumulate reserves when some factor other than rates of photosynthetic carbon fixation limit plant biomass. We suggest that nutrient availability may need to be incorporated into the framework of factors that regulate the carbohydrate reserves of *T. testudinum*, and provide additional insight towards the storage dynamics of this benthic plant.

Acknowledgements

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Table 1: Abiotic measurements and seagrass leaf tissue chemistry (NSC = non-structural carbohydrate content) for each sampling region. Data from 1997-1999 were pooled. Values are means ± S.E.

Region	Depth (m)	Secchi Ratio	Temperature (°C)	Salinity	NSC (mg g^{-1} FM)	%C (% dry mass)	%N (% dry mass)	% P (% dry mass)	C:N	C:P	N:P
Homosassa Bay	1.66 ± 0.04	1.00 ± 0.01	25.5 ± 6.6	25.7 ± 4.8	40.7 ± 1.7	36.9 ± 0.2	2.39 ± 0.04	0.15 ± 0.01	18.3 ± 0.2	679.4 ± 12.3	37.5 ± 0.8
Anclote Keys	2.16 ± 0.06	0.65 ± 0.03	28.5 ± 3.8	29.7 ± 2.8	21.8 ± 1.6	35.7 ± 0.2	2.52 ± 0.03	0.21 ± 0.01	16.6 ± 0.2	466.1 ± 12.1	27.9 ± 0.6
Tampa Bay	1.07 ± 0.04	0.98 ± 0.01	30.6 ± 1.2	32.6 ± 0.8	38.9 ± 1.0	34.6 ± 0.2	2.46 ± 0.02	0.25 ± 0.01	16.6 ± 0.2	365.7 ± 5.1	22.2 ± 0.3
Charlotte Harbor	1.35 ± 0.06	0.79 ± 0.02	29.6 ± 2.4	17.0 ± 4.1	26.9 ± 1.9	37.9 ± 0.2	3.02 ± 0.04	0.70 ± 0.02	14.8 ± 0.2	148.4 ± 4.2	10.0 ± 0.2
Florida Bay	1.88 ± 0.07	1.00 ± 0.01	29.2 ± 2.1	36.7 ± 1.4	32.9 ± 1.2	35.7 ± 0.2	2.14 ± 0.02	0.09 ± 0.01	19.8 ± 0.2	1066.1 ± 23.3	53.5 ± 0.9

Figure Captions

Figure 1. Relationship between rhizome non-structural carbohydrate content (NSC) and leaf

nutrient content (%N or %P) for each region from 1997-1999. Lines represent significant linear

regressions. Mean elemental ratios for each region are indicated.