INTERACTIONS BETWEEN *THALASSIA TESTUDINUM* BANKS EX KOENIG AND *HALIMEDA INCRASSATA* (ELLIS) LAMOUROUX AND THEIR EFFECTS ON CARBON DYNAMICS IN A SHALLOW, TROPICAL LAGOON

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL
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To my mom and dad

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I thank my parents for always being supportive and helping me believe in my abilities and I thank my grandmother for inspiring me to be a stronger woman. I also thank all of the University of Virginia graduate students that inspired me to pursue graduate education. I appreciate logistical support provided by Morgan Edwards, Neil van Niekerk, Sky Notestein, Darlene Saindon, and Jessica Frost. I thank the staff of the Little Cayman Research Center for additional logistical support during the course of the study. I am grateful to the Cayman Islands Department of Environment and Marine Conservation Board for making this work possible. I also thank my advisory committee for guiding me through this experience and fostering my growth as a scientist.

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LIST OF ABBREVIATIONS

A-D	Anderson-Darling test for normality
B-F	Brown-Forsythe test for homoscedacity
$CaCO_3$	Calcium carbonate
CL	Confidence limit
CO_2	Carbon dioxide
CO_3^{2-}	Carbonate ion
DO	Dissolved oxygen
DW	Dry weight
H^+	Hydrogen ion
HHLT	High *Halimeda*, Low *Thalassia*
LHHT	Low *Halimeda*, High *Thalassia*
MHMT	Medium *Halimeda*, Medium *Thalassia*
OA	Ocean acidification
OM	Organic matter
SD	Standard deviation

Abstract of Thesis Presented to the Graduate School
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Ocean acidification poses a serious threat to a broad suite of calcifying organisms. Scleractinian corals and calcareous algae that occupy shallow, tropical waters around the globe are vulnerable to global changes in ocean chemistry because they already are subject to stressful and variable carbonate dynamics at the local scale. For example, net heterotrophy increases carbon dioxide concentrations, and pH varies with diurnal fluctuations in photosynthesis and respiration. Few researchers, however, have investigated the possibility that carbon dioxide consumption during photosynthesis by non-calcifying photoautotrophs, such as seagrasses, can ameliorate deleterious effects of ocean acidification on sympatric calcareous algae. Naturally occurring variations in the density of seagrasses and associated calcareous algae provide an ecologically relevant test of the hypothesis that diel fluctuations in water chemistry driven by cycles of photosynthesis and respiration within seagrass beds create microenvironments that enhance macroalgal calcification. In Grape Tree Bay off Little Cayman Island BWI, we quantified net production and characterized calcification for thalli of the calcareous green alga *Halimeda incrassata* growing in beds of *Thalassia testudinum* with varying

shoot densities. Results indicated that individual *H*. *incrassata* thalli were ~6% more calcified in dense seagrass beds. On an areal basis, however, far more calcium carbonate was produced by *H*. *incrassata* in areas where seagrasses were less dense due to higher rates of production. In addition, diel pH regimes in vegetated and unvegetated areas were not significantly different, suggesting a high degree of water exchange and mixing throughout the lagoon. These results suggest that, especially in well-mixed lagoons, carbonate production by calcareous algae may be more related to biotic interactions between seagrasses and calcareous algae than to seagrass-mediated changes in local water chemistry.

CHAPTER 1
INTRODUCTION

Acidification of the world's oceans generated by a global rise in carbon dioxide (CO_2) emissions represents a major ecological concern (Skirrow and Whitfield, 1975; Kleypas et al., 1999). As atmospheric CO_2 is absorbed by seawater, it alters the carbonate cycle primarily leading to lower concentrations of carbonate ions (CO_3^{2-}) and higher concentrations of hydrogen ions (H^+), which translates into lower pH values (McClendon, 1917, 1918; Guinotte and Fabry, 2008; Doney et al., 2012). These changes in concentrations of CO_3^{2-} and H^+ potentially interfere with sequestration of calcium carbonate ($CaCO_3$) by a broad suite of marine organisms that use this compound to form skeletons, shells, otoliths, statoliths and other key structures (Kleypas et al., 1999; Hoegh-Guldburg et al., 2007; Kroeker et al., 2009).

In shallow tropical waters, corals and calcareous algae, two key ecosystem components that sequester $CaCO_3$, are particularly vulnerable to an altered equilibrium because they already are subjected to other stresses mediated by a varying carbonate cycle. For example, diurnal fluctuations in seawater chemistry are driven by the relative intensities of photosynthesis and respiration, and heterotrophic conditions dominate, which means release of CO_2 through remineralization of organic matter exceeds consumption of CO_2 during photosynthesis (Andersson and Mackenzie, 2012). Other shallow-water organisms, however, are expected to be less affected and some may, in fact, benefit from changes in ocean chemistry driven by increased atmospheric CO_2. Seagrasses, for example, are likely to experience an increase in production, especially in those areas where dissolved inorganic carbon is presently limiting (e.g., Palacios and Zimmerman, 2007). Moreover, seagrasses, which consume CO_2 during photosynthesis,

may serve to mediate and possibly ameliorate the deleterious effects of ocean acidification on a large number of sympatric species (Semesi et al., 2009a, 2009b; Kleypas et al., 2011).

In shallow water, including tropical seas, seagrass beds represent a predominant source of production and serve also as a key structural habitat (Duarte et al., 2010). In fact, seagrass beds are among the most productive habitats on the planet, with global estimates of seagrass production on the order of 21–101 Tg C y^{-1} depending on estimates of seagrass areal coverage (Duarte et al., 2010). Thus, seagrasses are more productive than North American wetlands and undisturbed Amazonian rainforest (Duarte et al., 2010). Seagrasses can exist as extensive beds or a complex mosaic of patches, and they provide both refuge and a habitat for foraging used by myriad species, including a large number of commercially and recreationally important finfishes (Orth et al., 1984; Thayer et al., 1984; Virnstein and Howard, 1987). In addition, seagrasses sequester carbon, stabilize bottom sediments, dampen wave action, reduce turbulence, increase water clarity and reduce shoreline erosion (Duarte, 1995; Fourqurean, et al. 2012).

In the tropics, seagrasses often coexist with other ecologically important photoautotrophs (Littler and Littler, 1988, 1994; Dahlgren and Marr, 2004; Fong and Paul, 2011). Among the co-occurring species, representatives of the phylum Chlorophyta, i.e., green algae, are common (Littler, 1976; Littler and Littler, 1988, 1994). Calcareous green algae such as *Halimeda* spp. and *Penicillus* spp. are among the most cosmopolitan and well-studied species because they perform a number of important ecological functions. For example, photosynthetic production by *Halimeda incrassata* in

Florida Bay was 20% of that attributed to the dominant seagrass, *Thalassia testudinum* (Davis and Fourqurean, 2001). In addition, several species of *Halimeda* serve as a key food source and are, in fact, a preferred food of several coral reef fishes (Overholtzer and Motta, 1999; Mantyka & Bellwood, 2007). More importantly, however, *Halimeda* spp. and other calcareous algae play critical roles in the formation of $CaCO_3$ (Vroom et al., 2003; Nelson, 2009). In the Bahamas, for example, rates of $CaCO_3$ production by *Halimeda* spp. are nearly equal to rates estimated for coral reefs (Milliman & Droxler, 1996). In fact, green, calcifying algae can account for 35–40% of the carbonates generated in shallow, tropical marine waters, with corals and red, coralline algae accounting for an additional 50–55% (Lee and Carpenter, 2001). Furthermore, *Halimeda* spp. are known to be important producers of coarse-grained sediments in the Bahamas and elsewhere (Freile et al., 1995), and spalling of calcified plates is a primary mechanism by which sand is formed in tropical seas (Littler, 1976; Littler and Littler, 1988, 1994).

Seagrasses and calcified algae are known to compete for nutrients in oligotrophic waters, with competition reported to favor seagrasses (see Davis and Fourqurean, 2001). Few investigators, however, have studied potential positive interactions whereby seagrasses might promote the existence and co-occurrence of key calcareous algae by raising pH, which alters the stoichiometry of calcification by enhancing release of hydrogen ions formed as byproducts (Semesi et al., 2009a, 2009b). In this scenario, diel fluctuations in water chemistry within seagrass beds driven by cycles of photosynthesis and respiration are hypothesized to create microenvironments that are conducive to the calcification of algae (Semesi et al., 2009a, 2009b). Sites with varying densities of both

seagrasses and rhizophytic, calcareous algae provide opportunities to test this hypothesis *in situ*.

In this study, sites with varying densities of *T*. *testudinum* and *H*. *incrassata* were identified in Grape Tree Bay off Little Cayman Island. At these sites, production for *T*. *testudinum* and production and calcification for *H*. *incrassata* were measured to determine if significant interactions existed. Measures of key water quality parameters provided data to assess potential causes of variations in production or calcification. Thus, the calcification rate of *H. incrassata* in dense seagrass was hypothesized to be increased relative to sites with less seagrass due to a favorable pH regime.

CHAPTER 2
METHODS AND MATERIALS

Study Site

Grape Tree Bay is a shallow lagoon on the north coast of Little Cayman Island, BWI (Figure 2-1). A mixed seagrass and calcareous algal assemblage extends offshore for approximately 60–100 m where it is bounded by a fringing reef. The fringing reef delineates the seaward edge of Grape Tree Bay, which spans approximately 1.6 km of shoreline. Based on data from a National Oceanic and Atmospheric Administration Integrated Coral Reef Observation Network (ICON) station located just outside the fringing reef, ocean temperature ranged from 28.0 °C to 30.6 °C and salinity averaged 35.8‰ (range 33.0 to 36.1‰) during the period of this study.

Densities of Macrophytes and Site Selection

To select sites with differing densities of *T*. *testudinum* and *H*. *incrassata*, benthic vegetation in Grape Tree Bay was surveyed within a systematic grid. Forty points along the shoreline were marked with GPS waypoints, and these points, which were separated by 10 m, served as the origins for transects that ran offshore to the fringing reef. Along each transect, a 0.25-m^2 quadrat was placed at the 10-m mark and also at every successive 10-m mark. Thus, 6–10 quadrats were sampled along each transect depending on the distance between the shoreline and the fringing reef.

Within each quadrat, thalli of all algal taxa were counted. Subsequently, a 0.0625-m^2 subquadrat was thrown within each 0.25-m^2 quadrat. Within each subquadrat, shoots of all seagrass species were counted separately.

Sites for measurements of production for *T*. *testudinum* and production and calcification for *H*. *incrassata* were selected by comparing densities of shoots and thalli.

The goal was to identify sets of three replicate experimental sites that spanned the natural gradient in Grape Tree Bay. Three levels, termed treatments, were targeted, i.e., i) low density *Halimeda* combined with high density *Thalassia* (LHHT), ii) medium density *Halimeda* and *Thalassia* (MHMT), and iii) high density *Halimeda* combined with low density *Thalassia* (HHLT). At each experimental site, shoot and thalli counts were repeated to verify densities were appropriate.

Water Quality Measurements

During the course of the field experiment, a YSI 600R data sonde with a YSI 650 MDS data logger was deployed at each of the nine treatment sites and at an additional three unvegetated sites for at least 24 h. Temperature, salinity, dissolved oxygen, and pH were recorded at 30-min intervals throughout each 24-h period. Measurements were taken at a height of approximately 5 cm above the sediment to document conditions within the seagrass canopy, when seagrass was present.

Field Procedures and Laboratory Processing

Two methods were used to measure production for *T*. *testudinum* and *H*. *incrassata*. *Thalassia testudinum* production was measured by the leaf marking technique (Zieman, 1974), with a needle forced through all blades in a shoot just above their basal meristems and marked shoots allowed to grow in situ for 7 d. *Halimeda incrassata* production was measured using incorporation of Alizarin-S dye (Figure 2-2; Wefer, 1980; Multer, 1988; Davis & Fourqurean, 2001; Vroom et al., 2003). The dye stained existing tissue red, which allowed new (unstained) algal biomass to be distinguished from tissue present at the start of the 7-d, in situ growth period. When possible, 40 shoots and 25 thalli were marked within each experimental site; however, some sites did not contain 25 thalli, so all available thalli were marked.

After 7 d, marked macrophytes were harvested. *Halimeda incrassata* thalli were harvested by removing their basal holdfasts from the sediment. *Thalassia testudinum* shoots were harvested at the node where the short shoot meets the rhizome so that the entire sheath was retained. Individual algal thalli and seagrass shoots were placed in separate, labeled bags and frozen until processing.

Individual *T*. *testudinum* shoots were rinsed in freshwater, scraped with a razor blade to remove epiphytic material and briefly rinsed in freshwater again. For each blade with a hole, new and old growth were separated by cutting through the hole with a razor blade. Unmarked blades were considered new growth. Old and new materials were placed in separate borosilicate glass vials and dried at 50 °C to a constant weight.

Individual *Halimeda incrassata* thalli were rinsed with freshwater using the focused stream from a wash bottle to remove sand, debris, and epiphytic material. New or unstained plates and old, stained plates were separated, counted, placed into tared borosilicate glass vials, and dried at 50 °C to a constant weight.

In addition to production, calcification was quantified for *H*. *incrassata* to evaluate the influence of biogeochemical effects mediated by *T*. *testudinum*. Two methods were available to differentiate organic and inorganic content of *H*. *incrassata*: acidification and ashing. Three consecutive exposures of dried algal tissue to 5% hydrochloric acid removed the inorganic ($CaCO_3$) fraction of plates (Vroom et al., 2003). This method relied on acid penetrating all of the tissue, which could be problematic for large samples (S. Barry, pers. obs.). The alternative method subjected algal tissue to 500 °C for 3 h in a muffle furnace to remove the organic fraction of plates (Davis and Fourqurean, 2001). To test the methods, a known number of *H*. *incrassata* plates with a known dry weight

were ashed and a similar set of plates were acidified. Dry weights of the resulting material yielded estimates of both organic and inorganic carbon for each set of samples. Analyses of covariance (ANCOVAs) tested for significant relationships between weights of organic matter and $CaCO_3$ fractions and total dry weights, with method being a covariate. The ANCOVAs indicated a significant difference between the methods for both organic matter and $CaCO_3$ fractions (organic: $F_{1, 19} = 9.84$, $p = 0.005$; $CaCO_3$: $F_{1, 19} = 9.84$, $p = 0.005$). Linear regressions fitted to each set of measurements showed that acidification yielded higher estimates of $CaCO_3$ content and lower estimates of organic matter content (Figure 3-3), potentially due to loss of organic content, which has been reported previously (Roberts et al., 1973; Byers et al., 1978). Therefore, ashing was chosen as the method for this study.

After consistent dry weights were obtained, samples of *H*. *incrassata* plates were transferred into pre-weighed aluminum dishes, ashed for 3 h at 500 °C, and allowed to cool before being weighed to determine quantities of inorganic carbon, i.e., $CaCO_3$. For the purposes of this study, contributions from silicon and other trace elements that might have remained after ashing were considered negligible. Organic content was estimated by subtracting the inorganic fraction from the total, pre-ashing dry weight of a sample.

Metrics of Production and Calcification

Dry weights (DW) were used to calculate metrics that would elucidate interactions between *H*. *incrassata* and *T*. *testudinum*. Metrics characterized production for *T*. *testudinum* and *H*. *incrassata*, as well as calcification for *H*. *incrassata*.

Dry weights yielded measures of production for shoots of *T*. *testudinum* (mg DW $shoot^{-1}$ d^{-1}) and thalli of *H*. *incrassata* (mg DW $thallus^{-1}$ d^{-1}) directly, and multiplying these individual growth rates by the appropriate mean density generated estimates of

net areal production (mg DW m^{-2} d^{-1}). For *H*. *incrassata*, dry weights of thalli, organic tissue and $CaCO_3$ were measured separately; therefore, relative rates of production were calculated by standardizing increases in these weights to their initial values. Relative rates of production provided insights into growth performance.

Calcification for *H*. *incrassata* thalli was characterized by the ratio of $CaCO_3$ to organic matter because the photosynthetic activity of living, organic tissue is responsible for calcification of the thallus (Borowitzka and Larkum, 1976a, 1977; de Beer and Larkum, 2001). Estimates were calculated separately for entire thalli and new growth.

Statistical Analyses

Statistically significant differences in water chemistry at experimental and unvegetated sites were assessed with multivariate permutation analyses of variance (PERMANOVAs, Anderson et al., 2008). Analyses were based on range standardized mean water temperatures, salinities, dissolved oxygen concentrations and hydrogen ion concentrations calculated over 30-min intervals throughout the 24-h periods. Due to anomalies caused by two days of bad weather, data from one MHMT site and one LHHT site were excluded from the analysis. In total, three PERMANOVAs were performed, with the first examining differences among treatments (LHHT, MHHT, HHLT and unvegetated sand) for all environmental data. Two other analyses examined differences among treatments for i) hydrogen ion concentrations across full 24-h periods and ii) hydrogen ion concentrations during daytime periods in order to assess changes driven by photosynthesis.

Univariate analyses of variance (ANOVAs) were used to evaluate growth rates and data characterizing calcification. Normality was evaluated with Anderson-Darling tests, and homoscedasticity was evaluated with Brown-Forsythe tests. If necessary,

data were transformed to meet the assumptions. Areal production was analyzed with a one-way ANOVA with treatment considered a fixed effect. All other growth rates and calcification data were analyzed using nested ANOVAs with treatment as a fixed effect and sites nested within treatments. Ryan-Einot-Gabriel-Welsch Q multiple comparisons were employed to discern differences among treatments. For unbalanced ANOVAs, the Tukey-Kramer adjustment was applied to generate degrees of freedom for the post hoc tests.

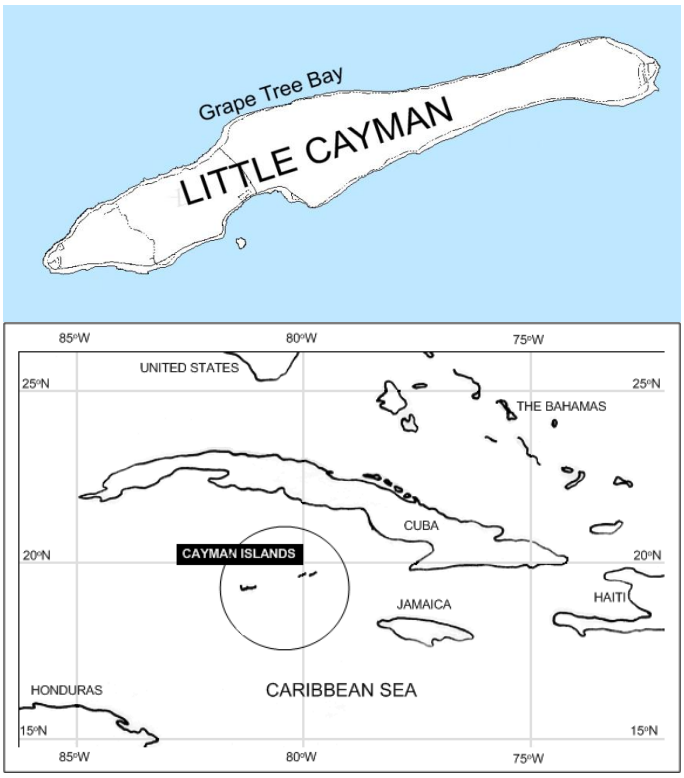

Figure 2-1. Location of Little Cayman Island and Grape Tree Bay.

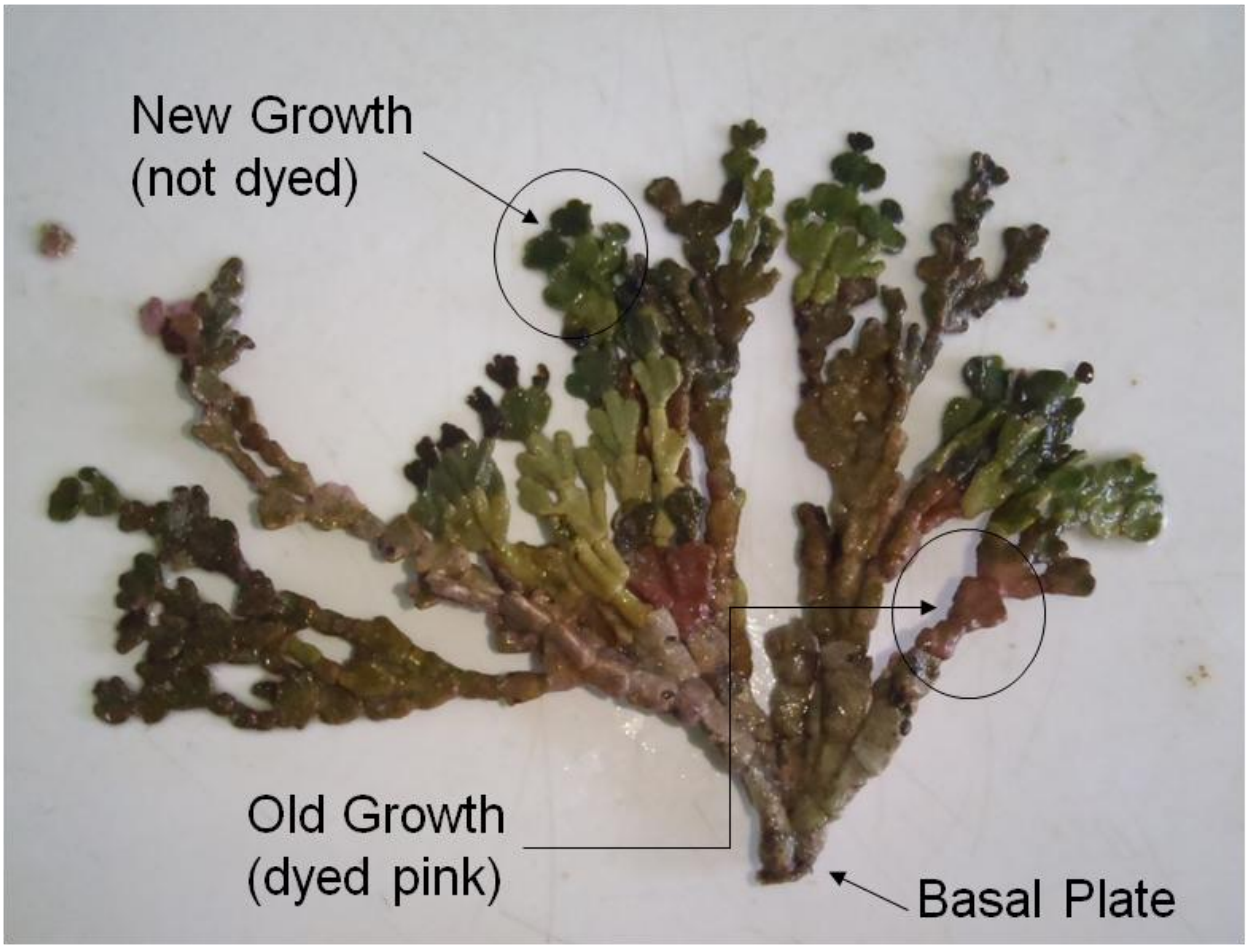

Figure 2-2. *Halimeda incrassata* morphological characteristics.

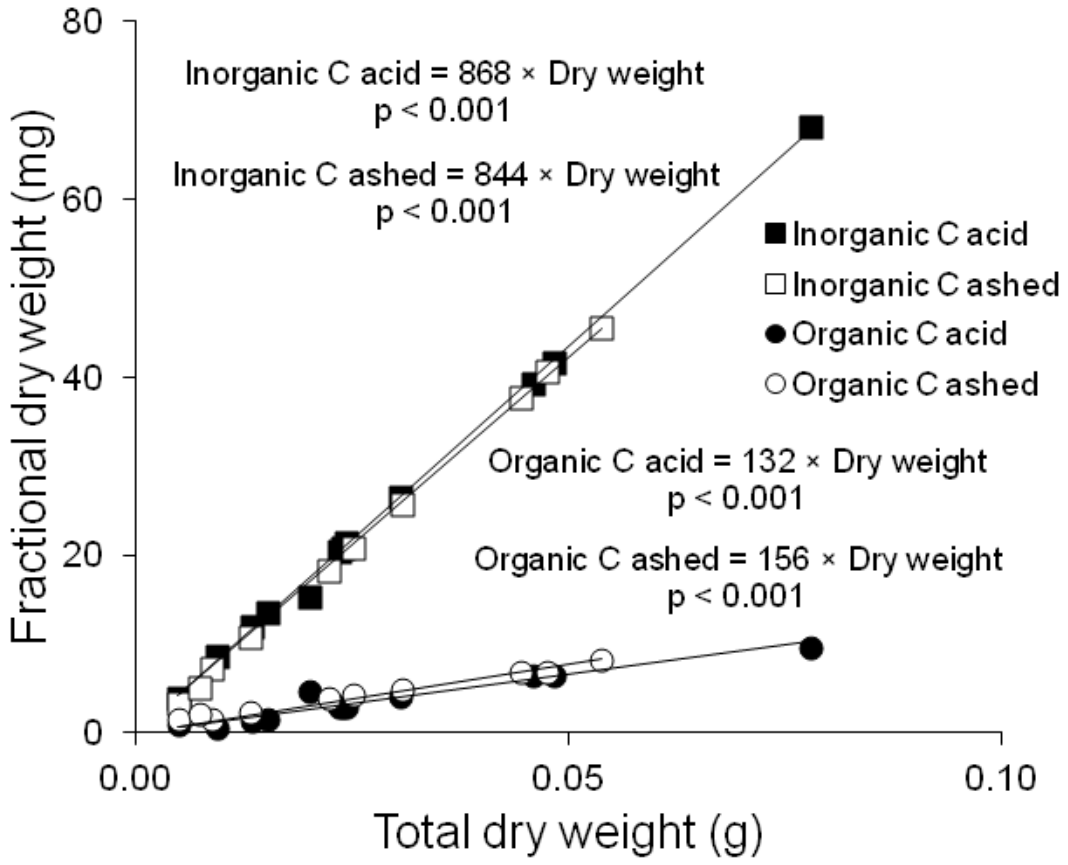

Figure 2-3. Regressions for inorganic and organic content versus total dry weight of *Halimeda incrassata* as determined by acidification and ashing.

CHAPTER 3
RESULTS

Densities of Macrophytes and Site Selection

The maximum shoot density of *T*. *testudinum* in Grape Tree Bay was greater than that of *H*. *incrassata* thalli by approximately an order of magnitude. The shoot density of *T*. *testudinum* ranged from 0 to 1900 shoots m^{-2} whereas the *H*. *incrassata* thallus density ranged from 0 to 246 thalli m^{-2}. Sites for measurements of productivity for *T*. *testudinum* and *H*. *incrassata* were selected to span the density gradient in Grape Tree Bay. A total of nine sites were chosen, with three replicate sites in each of three treatments (Low *Halimeda*, High *Thalassia* = LHHT; Medium *Halimeda*, Medium *Thalassia* = MHMT; and High *Halimeda*, Low *Thalassia* = HHLT; Figure 3-1).

Water Chemistry

PERMANOVAs indicated that the time series for all environmental variables and hydrogen ion concentrations did not differ significantly among treatments (Table 3-1). Thus, macrophytes at all sites were subjected to similar temporal variation in environmental conditions. Although not significantly different, patterns in DO and pH were of interest because they could potentially affect interactions between *T*. *testudinum* and *H*. *incrassata*.

The highest mean DO concentrations were recorded during the day for the LHHT treatment sites (Figure 3-2), which would be expected given photosynthesis by *T*. *testudinum*. Also, as expected, sites where seagrass was less dense (including unvegetated sites) exhibited less pronounced diel variations in DO concentrations (Figure 3-2).

Diel patterns for pH paralleled those of DO (Figure 3-3); increasing throughout the daylight hours and decreasing at night. The dense seagrass treatment (LHHT) exhibited a higher mean pH during the day and a slightly lower mean pH at night (Figure 3-3a). As a consequence, *H*. *incrassata* thalli within the LHHT treatment experienced, on average, slightly more variation in pH within a 24-h period than did thalli in other treatments. The mean pH values in the dense seagrass (LHHT) areas ranged from 7.68 at night to 8.15 during the daytime. These pH values corresponded to a mean $[H^+]$ of 21.17 nmol L^{-1} seawater at night and a mean of 7.22 nmol L^{-1} seawater during the day, a 98.3% difference over the course of approximately 12 h. In addition, pH for the LHHT treatment increased faster during the morning, which resulted in a slightly higher value than that in the other treatments by about 10:00 AM (Figure 3-3b). These patterns in pH were driven by metabolic activity.

Rates of Production

Rates of *T*. *testudinum* production (mg DW $shoot^{-1}$ d^{-1}) were homoscedastic and normal when log-transformed (Table 3-2). Production rates differed significantly among treatments and also among sites (Table 3-2). Variation among treatments was of greater biological interest, and post hoc, pairwise comparisons, with a Tukey-Kramer adjustment, showed that *T*. *testudinum* production increased with increasing shoot density (Table 3-3). Shoots in the treatment with the highest density (LHHT) were ~2.4 times more productive than shoots in treatment with the lowest density (Figure 3-4a).

For *H*. *incrassata*, rates of production (mg DW $thallus^{-1}$ d^{-1}) in terms of both organic material and $CaCO_3$ were homoscedastic and normal (Table 3-2) after log-transformation. Rates of production for organic and inorganic material differed among treatments (Table 3-2). The rate at which organic matter was produced also varied

significantly among sites within treatments (Table 3-2), but these results were not explored further. Post hoc, pair-wise comparisons showed that thalli produced organic matter and $CaCO_3$ at statistically equal rates in treatments with low and intermediate *T*. *testudinum* density (HHLT and MHMT), and thalli produced significantly less of both types of carbon d^{-1} in the treatment with the densest *T*. *testudinum* (LHHT). In fact, thalli in the LHHT treatment were roughly 3.5 times less productive than thalli in other treatments (Table 3-3; Figure 3-4b, c).

Growth rates for *H*. *incrassata*, whether standardized by total dry weight, organic matter or $CaCO_3$, were homoscedastic without transformation (Table 3-4). However, after repeated attempts at transforming data, normality could not be achieved. The untransformed data were analyzed, and results were interpreted with caution. Standardized growth rates based on total weights and $CaCO_3$ were significantly different among treatments, but rates based on organic matter were not statistically different (Table 3-4). Post hoc comparisons showed that mean standardized growth rates of thalli in dense seagrass (LHHT) were lower than rates recorded for other treatments in the case of total weight and $CaCO_3$ (Table 3-5). The mean standardized growth rate of thalli in dense seagrass treatments was 50% or 44% of that recorded for thalli in other treatments when expressed versus total weight or $CaCO_3$, respectively (Table 3-5).

When rates of production for individual shoots and thalli were scaled to 1 m^2 using the mean number of individuals m^{-2} in a given treatment, the resulting rates of areal production were homoscedastic and normal (Table 3-6). Areal production was significantly different among treatments for *T*. *testudinum* shoots, *H*. *incrassata* organic

matter and *H*. *incrassata* $CaCO_3$ content (Table 3-6). For *T*. *testudinum*, post hoc multiple comparisons showed sites with dense seagrass yielded significantly greater areal production and production decreased significantly with decreasing density (Figure 3-5a). Rates of areal production of organic matter and $CaCO_3$ for *H*. *incrassata* exhibited similar trends, with significant increases in production as density of *T*. *testudinum* decreased (Figure 3-5b, c). Mean rates of areal production for *T*. *testudinum* and *H*. *incrassata* spanned an order of magnitude (Table 3-7).

Calcification

Ratios of $CaCO_3$ to organic material ($CaCO_3$:OM) in the new growth of *H*. *incrassata* were normal and homoscedastic (A-D and B-F test $p > 0.05$) without transformation. These ratios were not significantly different among treatments, although there was significant variation among sites within treatments (Table 3-8). Thus, the $CaCO_3$ content of new plates varied among thalli but did not differ consistently among treatments.

Ratios of $CaCO_3$:OM for whole thalli were normal after log-transformation, but the data remained heteroscedastic so the results of the ANOVA were interpreted cautiously (Table 3-8). Ratios of $CaCO_3$:OM in whole thalli differed significantly among treatments and among sites within treatments (Table 3-8). Post hoc multiple comparisons indicated that thalli in the sites with the densest seagrass (LHHT) had higher $CaCO_3$:OM ratios than thalli in other treatments (Table 3-9), which indicated that relatively more calcification had occurred.

The proportions of whole thalli comprising $CaCO_3$ were normal and homoscedastic after arcsine-transformation (Table 3-10). Thalli from different treatments exhibited significant differences in their $CaCO_3$ content, and thalli from different sites within

treatments also differed significantly (Table 3-10). Post hoc multiple comparison tests, with Tukey-Kramer adjustments, showed that thalli in the dense seagrass treatment contained proportionately more $CaCO_3$ than thalli in other treatments. Thalli growing in dense seagrass were 5–6% more calcified than thalli growing in areas with sparse seagrass (Table 3-11).

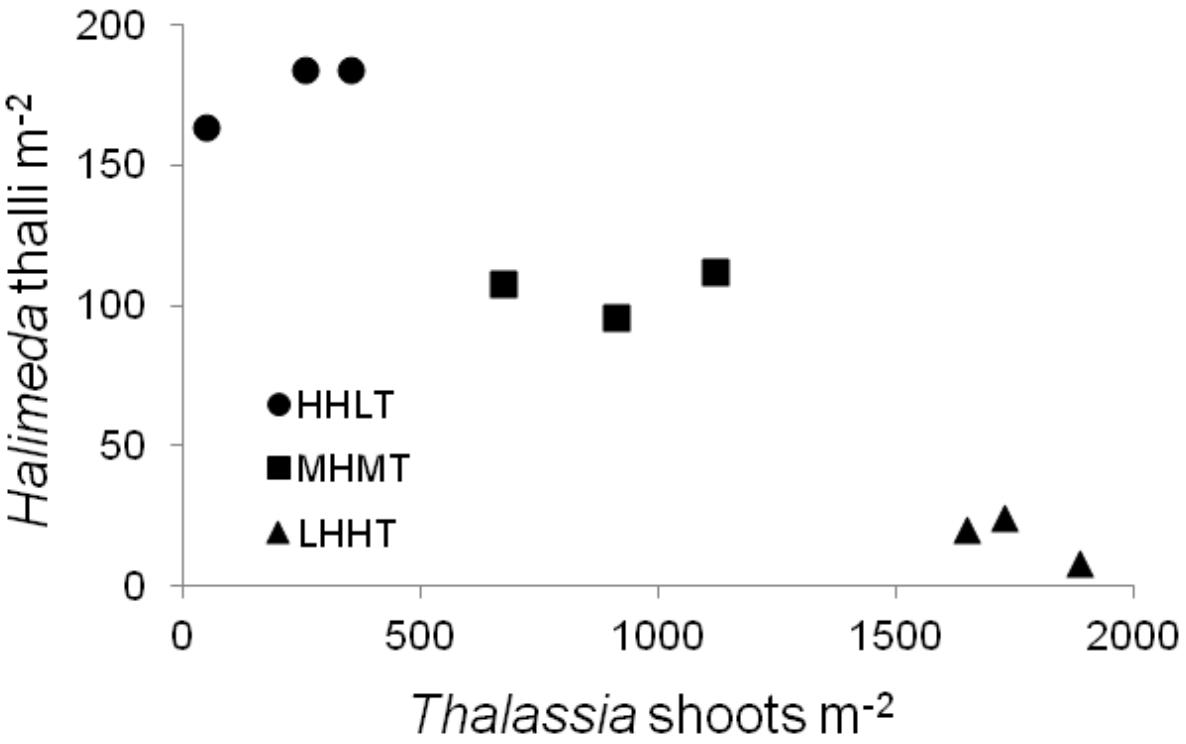

Figure 3-1. Plot of *Halimeda incrassata* thalli density against *Thalassia testudinum* shoot density for the sites chosen to represent treatments. HHLT = high density of *Halimeda* and low density of *Thalassia*; MHMT = medium density of *Halimeda* and medium density of *Thalassia*; LHHT = low density of *Halimeda* and high density of *Thalassia*

Table 3-1. Results of PERMANOVA analyses based on environmental data. [H+] = hydrogen ion concentrations

Parameter	Factor	df	SS	MS	Pseudo-F	p	Unique permutations
All environmental data	Treatment	3	528.3	176.1	1.66	0.068	918
	Error	6	637.9	106.3			
24 h [H^+]	Treatment	3	55.0	18.3	0.47	0.908	921
	Error	6	31.4	0.2			
Daytime [H^+]	Treatment	3	53.8	17.9	0.89	0.562	922
	Error	6	120.2	20.0			

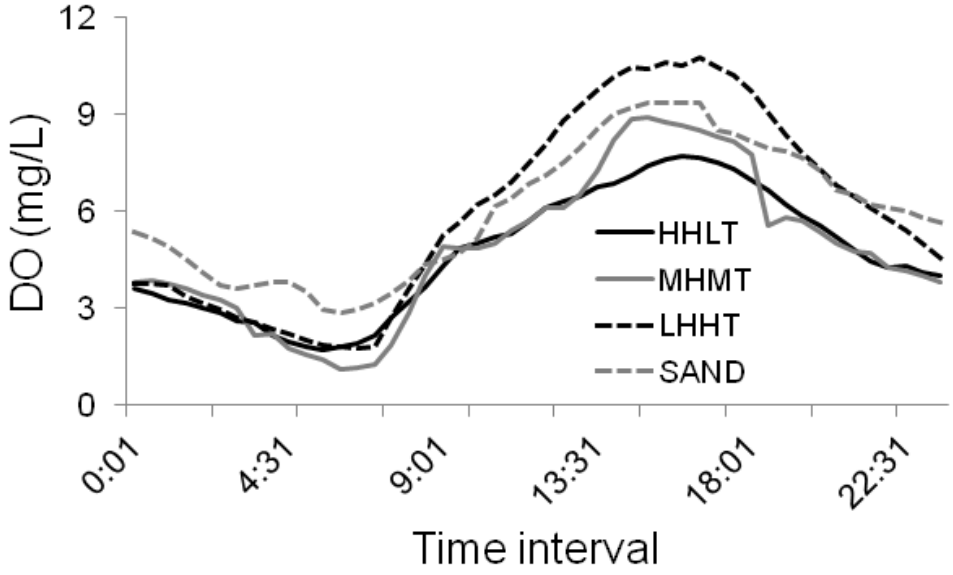

Figure 3-2. Mean dissolved oxygen concentrations (mg L^{-1}) among treatments on a 24-hour basis. HHLT = high density of *Halimeda* and low density of *Thalassia*; MHMT = medium density of *Halimeda* and medium density of *Thalassia*; LHHT = low density of *Halimeda* and high density of *Thalassia*; SAND = unvegetated sediment

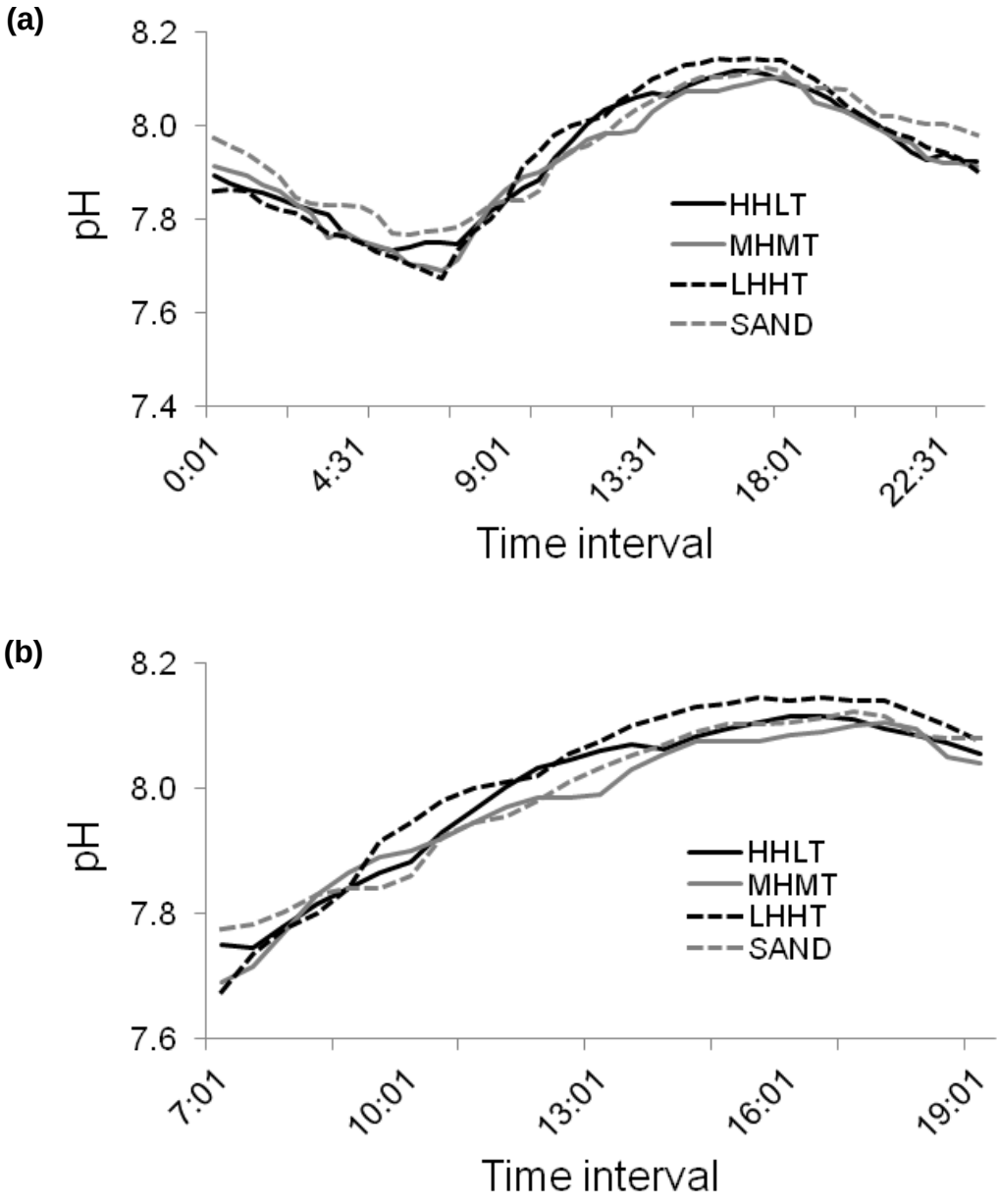

Figure 3-3**.** Mean pH levels by treatment during (a) a 24-h cycle and (b) daytime. HHLT = high density of *Halimeda* and low density of *Thalassia*; MHMT = medium density of *Halimeda* and medium density of *Thalassia*; LHHT = low density of *Halimeda* and high density of *Thalassia*; SAND = unvegetated sediment

Table 3-2. Results of ANOVAs based on rates of production for *Thalassia testudinum* and *Halimeda incrassata*. A-D p = p-value for Anderson-Darling test for normality; B-F p = p-value for Brown-Forsythe test for homoscedasticity; OM = organic matter, Trt = treatment

Species Metric	A-D p	B-F p	Factor	df	SS	MS	F	p
Thalassia testudinum								
mg DW shoot^{-1} d^{-1} (OM)	>0.25	0.67	Treatment	2	2.95	1.47	8.16	0.019
			Site(Trt)	6	1.08	0.18	8.96	< 0.001
			Error	331	6.68	0.02		
Halimeda incrassata								
mg DW thallus^{-1} d^{-1} (OM)	0.22	0.31	Treatment	2	2.73	1.36	7.15	0.026
			Site(Trt)	6	1.15	0.19	2.78	0.013
			Error	179	12.29	0.07		
mg DW thallus^{-1} d^{-1} ($CaCO_3$)	>0.25	0.06	Treatment	2	3.78	1.89	9.17	0.015
			Site(Trt)	6	1.24	0.21	1.76	0.110
			Error	179	21.00	0.12		

Table 3-3. Back-transformed mean rates of production for *Thalassia testudinum* shoots and *Halimeda incrassata* thalli. 95% CL = lower and upper 95% confidence limits; HHLT = high density of *Halimeda* and low density of *Thalassia*; MHMT = medium density of *Halimeda* and medium density of *Thalassia*; LHHT = low density of *Halimeda* and high density of *Thalassia*; OM = organic matter

Species	HHLT		MHMT		LHHT	
Metric	Mean	95% CL	Mean	95% CL	Mean	95% CL
Thalassia testudinum mg DW shoot^{-1} d^{-1} (OM)	0.86	0.74, 0.99	1.40	1.25, 1.55	2.09	1.89, 2.31
Halimeda incrassata mg DW thallus^{-1} d^{-1} (OM)	2.20	1.72, 2.71	2.46	2.01, 2.97	0.69	0.42, 1.02
mg DW thallus^{-1} d^{-1} (CaCO3)	3.19	2.41, 4.16	3.45	2.74, 4.29	0.91	0.53, 1.38

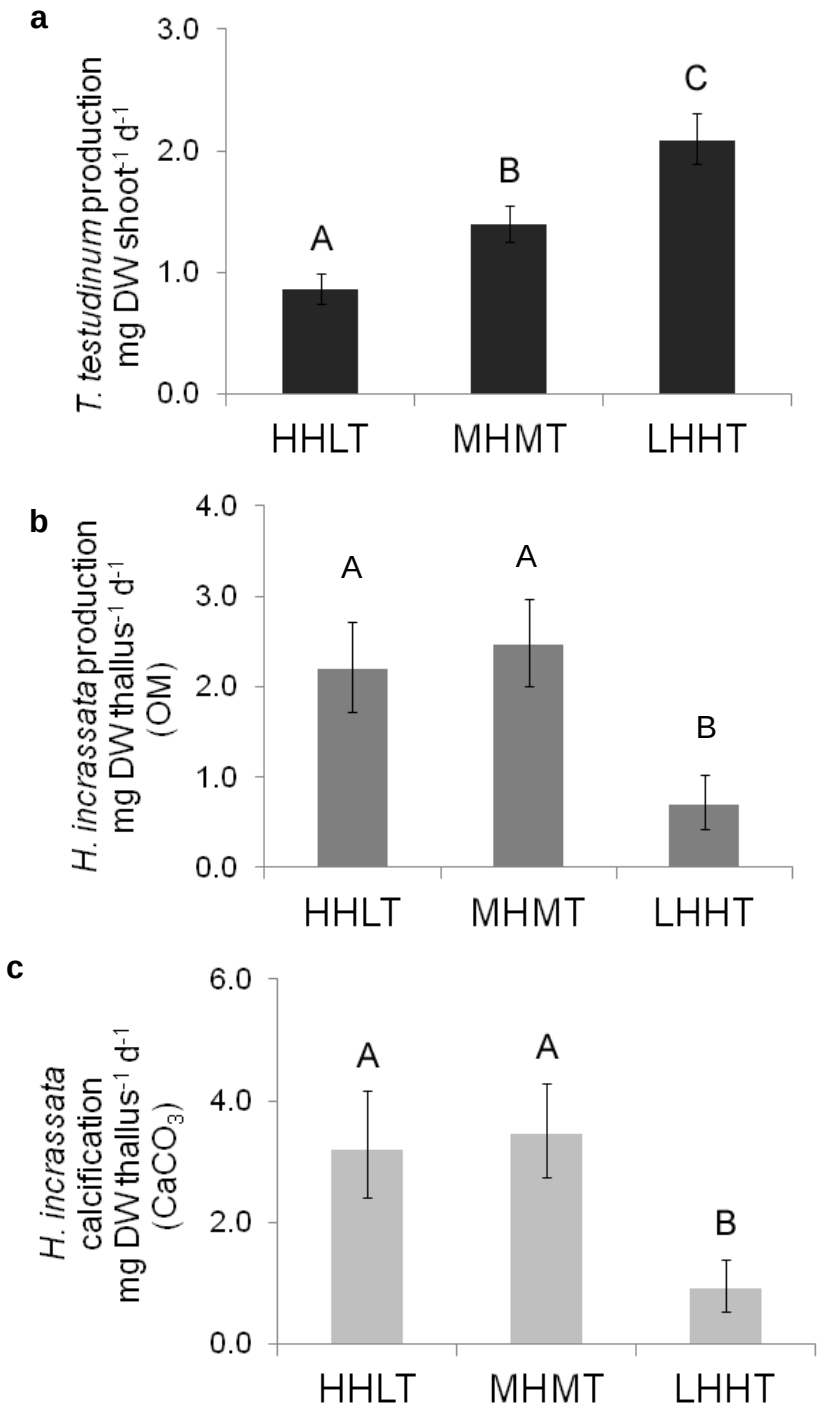

Figure 3-4. Back-transformed mean rates of production for a) *Thalassia testudinum* shoots and b) organic matter and c) $CaCO_3$ in thalli of *Halimeda incrassata*. Error bars indicate 95% confidence limits. Different capital letters above columns indicate statistically significant differences as determined by pairwise follow-up tests. Note that scales of y-axes differ among panels. HHLT = high density of *Halimeda* and low density of *Thalassia*; MHMT = medium density of *Halimeda* and medium density of *Thalassia*; LHHT = low density of *Halimeda* and high density of *Thalassia*

Table 3-4. Results of ANOVA based on rates of production for *Halimeda incrassata* standardized to initial sizes of thalli. A-D p = p-value for Anderson-Darling test for normality; B-F p = p-value for Brown-Forsythe test for homoscedasticity; OM = organic matter, Trt = treatment

Metric A-D p	B-F p	Factor	df	SS	MS	F	p
mg DW new mg DW old^{-1} d^{-1} (thallus)							
< 0.005	0.283	Treatment	2	3.79×10^{-4}	1.89×10^{-4}	6.64	0.030
		Site(Trt)	6	1.71×10^{-4}	2.85×10^{-5}	1.40	0.217
		Error	173	3.52×10^{-3}	2.04×10^{-5}		
mg DW new mg DW old^{-1} d^{-1} (thallus)							
< 0.005	0.113	Treatment	2	8.28×10^{-4}	4.14×10^{-4}	2.41	0.171
		Site(Trt)	6	1.03×10^{-3}	1.72×10^{-4}	2.05	0.061
		Error	173	1.50×10^{-2}	8.40×10^{-5}		
mg DW new mg DW old-^{1} d^{-1} (CaCO3)							
< 0.005	0.066	Treatment	2	2.62×10^{-4}	1.30×10^{-4}	9.52	0.014
		Site(Trt)	6	8.25×10^{-5}	1.37×10^{-5}	1.01	0.418
		Error	173	2.35×10^{-3}	1.36×10^{-5}		

Table 3-5. Mean rates of production for *Halimeda incrassata* standardized to initial sizes of thalli. SD = standard deviation; HHLT = high density of *Halimeda* and low density of *Thalassia*; MHMT = medium density of *Halimeda* and medium density of *Thalassia*; LHHT = low density of *Halimeda* and high density of *Thalassia*; OM = organic matter; SD = standard deviation

Metric	HHLT		MHMT		LHHT	
	Mean	SD	Mean	SD	Mean	SD
mg DW new mg DW old^{-1} d^{-1} (thallus)	0.00710	0.00477	0.00701	0.00450	0.00359	0.00417
mg DW new mg DW old^{-1} d^{-1} (OM)	0.01414	0.00909	0.01437	0.00848	0.00997	0.01113
mg DW new mg DW old^{-1} d^{-1} ($CaCO_3$)	0.00541	0.00391	0.00532	0.00376	0.00236	0.00131

Table 3-6. Results of ANOVAs based on rates of areal production for *Thalassia testudinum* and *Halimeda incrassata*. A-D p = p-value for Anderson-Darling test for normality; B-F p = p-value for Brown-Forsythe test for homoscedasticity; OM = organic matter, Trt=treatment

Species and Metric A-D p	B-F p	Factor	df	SS	MS	F	p
Thalassia testudinum mg DW m^{-2} d^{-1} (OM)							
>0.25	0.65	Trt	2	2.33×10^{7}	1.17×10^{7}	158.52	>0.25
		Error	6	4.42×10^{5}	7.36×10^{4}		
Halimeda incrassata mg DW m^{-2} d^{-1} (OM)							
0.14	0.74	Trt	2	4.01×10^{5}	2.00×10^{5}	311.31	0.14
		Error	6	3.86×10^{3}	6.43×10^{2}		
Halimeda incrassata mg DW m^{-2} d^{-1} (CaCO3)							
0.19	0.71	Trt	2	1.19×10^{6}	5.94×10^{5}	335.63	< 0.001
		Error	6	1.06×10^{4}	1.77×10^{3}		

Table 3-7. Mean areal rates of production for *Thalassia testudinum* and *Halimeda incrassata* standardized to initial sizes of thalli. SD = standard deviation; HHLT = high density of *Halimeda* and low density of *Thalassia*; MHMT = medium density of *Halimeda* and medium density of *Thalassia*; LHHT = low density of *Halimeda* and high density of *Thalassia*; OM = organic matter

Species and Metric	HHLT		MHMT		LHHT	
	Mean	SD	Mean	SD	Mean	SD
Thalassia testudinum mg DW m^{-2} d^{-1} (OM)	214.4	152.3	1379.1	343.0	4060.4	282.8
Halimeda incrassata mg DW m^{-2} d^{-1} (OM)	531.2	34.6	325.2	25.7	17.8	8.5
Halimeda incrassata mg DW m^{-2} d^{-1} (CaCO3)	915.0	59.6	505.7	40.0	26.2	12.6

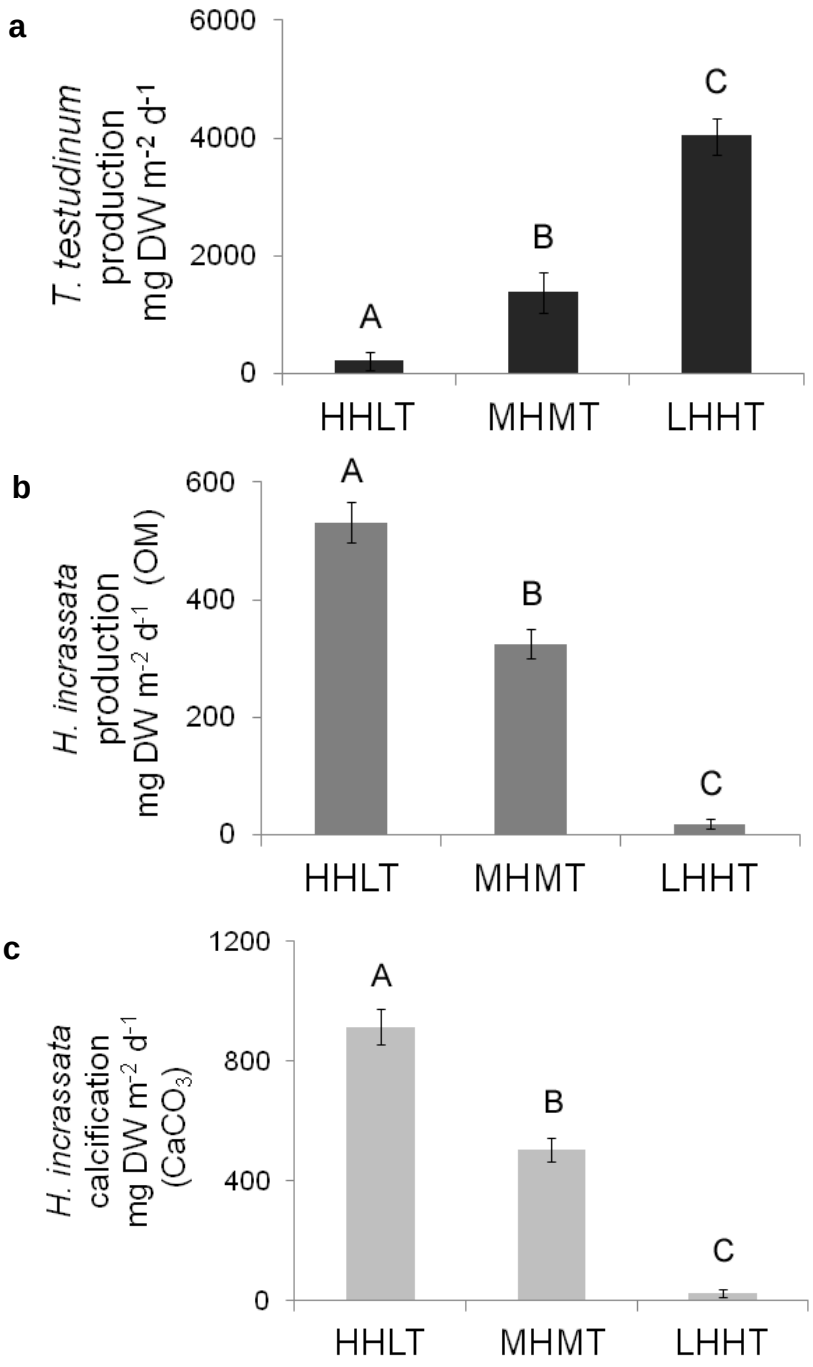

Figure 3-5. Mean areal rates of production for a) *Thalassia testudinum* shoots and b) organic matter and c) $CaCO_3$ in thalli of *Halimeda incrassata*. Error bars indicate ± 1 standard deviation. Different capital letters above columns indicate statistically significant differences as determined by pairwise follow-up tests. Note that scales of y-axes differ among panels. HHLT = high density of *Halimeda* and low density of *Thalassia*; MHMT = medium density of *Halimeda* and medium density of *Thalassia*; LHHT = low density of *Halimeda* and high density of *Thalassia*

Table 3-8. Results of ANOVAs based ratios of calcium carbonate content to organic matter in thalli of *Halimeda incrassata*. A-D p = p-value for Anderson-Darling test for normality; B-F p = p-value for Brown-Forsythe test for homoscedasticity; OM = organic matter, Trt = treatment

Metric	A-D p	B-F p	Factor	df	SS	MS	F	p
$CaCO_3$:OM for new growth	> 0.25	0.41	Treatment	2	0.46	0.23	0.34	0.723
			Site(Trt)	6	4.01	0.67	3.35	0.004
			Error	157	31.38	0.20		
$CaCO_3$:OM for thalli	0.21	< 0.01	Treatment	2	0.56	0.280	14.71	0.005
			Site(Trt)	6	0.11	0.020	3.79	0.001
			Error	180	0.90	0.005		

Table 3-9. Mean and back-transformed mean ratios of calcium carbonate content to organic matter in thalli of *Halimeda incrassata*. SD = standard deviation; 95% CL = upper and lower 95% confidence limits; HHLT = high density of *Halimeda* and low density of *Thalassia*; MHMT = medium density of *Halimeda* and medium density of *Thalassia*; LHHT = low density of *Halimeda* and high density of *Thalassia*; OM = organic matter

Metric	HHLT		MHMT		LHHT	
	Mean	SD or 95% CL	Mean	SD or 95% CL	Mean	SD or 95% CL
$CaCO_3$:OM for new growth	1.48	0.49	1.49	0.42	1.24	0.50
$CaCO_3$:OM for thalli	3.76	3.62, 3.91	4.00	3.82, 4.18	5.64	5.16, 6.16

Table 3-10. Results of an ANOVA based proportions of calcium carbonate in thalli of *Halimeda incrassata*. A-D p = p-value for Anderson-Darling test for normality; B-F p = p-value for Brown-Forsythe test for homoscedasticity; OM = organic matter

A-D p	B-F p	Factor	df	SS	MS	F	P
> 0.25	0.06	Treatment	2	0.15	0.070	12.18	0.008
		Site(Treatment)	6	0.04	0.010	4.16	< 0.001
		Error	180	0.27	0.001		

Table 3-11. Back-transformed mean proportions of calcium carbonate in thalli of *Halimeda incrassata*. 95% CL = upper and lower 95% confidence limits; HHLT = high density of *Halimeda* and low density of *Thalassia*; MHMT = medium density of *Halimeda* and medium density of *Thalassia*; LHHT = low density of *Halimeda* and high density of *Thalassia*; OM = organic matter

HHLT		MHMT		LHHT	
Mean	95% CL	Mean	95% CL	Mean	95% CL
0.79	0.78, 0.80	0.80	0.79, 0.81	0.85	0.84, 0.86

CHAPTER 4
DISCUSSION

Predicting the potential impact of ocean acidification on organisms that rely on $CaCO_3$ requires an understanding of their responses to local variations in factors affecting the carbonate cycle and physiological carbon dynamics. For example, seawater chemistry in shallow, tropical lagoons tends to vary across 24-h periods because rates of photosynthesis and respiration vary. Furthermore, seagrasses and sympatric macroalgae interact ecologically in positive and negative ways. By measuring *in situ* rates of production and calcification at sites with different densities of *T*. *testudinum* and *H*. *incrassata*, this study contributes insights into the relative importance of biotic and abiotic factors at the local scale.

Biotic interactions between *T. testudinum* and *H. incrassata* include facilitation and competition. *Halimeda incrassata* and other rhizophytic algae can successfully colonize and persist in unstable, nutrient-poor sediments due to their ability to anchor themselves and more efficiently garner scarce nutrients (Hillis-Colinvaux, 1980; Williams, 1981; Demes et al., 2010). Thus, rhizophytic algae can facilitate colonization and growth of *T*. *testudinum* and other seagrasses by stabilizing sediments, increasing the accumulation of nutrients in the sediment as thalli decompose, and reducing transfer of nutrients into the water column by protecting sediments from turbulence (McRoy and McMillan 1977; Orth, 1977; Williams, 1984a, 1990). After colonization and establishment by seagrass, calcifying macroalgae often decrease in abundance because seagrasses tend to be superior competitors for space, light or nutrients. In fact, *T*. *testudinum* represents a particularly strong competitor, and it dominates many tropical seagrass beds via exploitative competition (Zieman and Wetzel, 1980; Williams, 1987, 1990). Furthermore,

T. *testudinum* has been shown to compete for nitrogen with *H*. *incrassata* when present at densities of 400–800 shoots m^{-2} (Davis and Fourqurean, 2001), and *T*. *testudinum* densities as low as 200 shoots m^{-2} were associated with a decline in the abundance of rhizophytic algae (Williams, 1990). In the present study, no significant negative effect on *H*. *incrassata* production was observed at seagrass densities up to 1312 shoots m^{-2} (i.e., HHLT and MHMT treatments). However, rates of production were reduced at seagrass densities at or above 1650 shoots m^{-2} (i.e., LHHT treatment), suggesting that some level of competition (*sensu* Davis and Fourqurean, 2001) occurs at high seagrass densities in Grape Tree Bay. Given light regimes in clear, shallow, tropical waters, such competition is likely to be for nutrients rather than light (Davis and Fourqurean, 2001). Therefore, biotic interactions, i.e., competition, could affect the dynamics of $CaCO_3$ production by *H*. *incrassata* at scales similar to Grape Tree Bay.

As thalli grow, *Halimeda* spp. produce both organic matter and $CaCO_3$, where production of $CaCO_3$ is driven by photosynthesis in the thallus. Jensen et al. (1985) reported that 77% of the variation in calcification of *H. copiosa, H, cryptica, H. discoidea, and H. lacrimosa* could be explained by variation in the rate of photosynthesis. In addition, net carbonate accretion by *Halimeda* spp. does not occur in the dark (Borowitzka and Larkum, 1976a, 1976b, 1976c, 1977; de Beer and Larkum, 2001), which further demonstrates the link between calcification and photosynthesis. Thus, ratios of $CaCO_3$ to organic matter (OM) yield insights into the dynamics of calcification.

When only new growth of *H*. *incrassata* was considered, $CaCO_3$:OM ratios were not significantly different regardless of the density of *T*. *testudinum* surrounding the

algae, with the range of ratios across densities of seagrass being 0.25. In contrast, the maximum difference among $CaCO_3$:OM ratios for whole thalli, i.e., ratios including older plates, was 1.88, which is 7 times the range observed for new growth, suggesting that continued $CaCO_3$ accumulation varied significantly among sites with different densities of seagrass. In fact, the highest $CaCO_3$:OM ratio was recorded for *H*. *incrassata* growing among the highest density of *T*. *testudinum*. In combination, these results indicate that new *H*. *incrassata* plates, i.e., those less than 7 d old, are produced with a relatively constant $CaCO_3$:OM ratio and $CaCO_3$ content continues to increase in older plates, especially for thalli in dense seagrass (LHHT). Previously, van Tussenbroek and van Dijk (2007) found that mature, basal plates of *H*. *incrassata* were heavier than newly produced plates, and other studies have documented an increase in $CaCO_3$ content as plates age (Borowitzka and Larkum, 1976a, 1977; Multer, 1988). This is the first study to report that accumulation of $CaCO_3$ may depend on the density of surrounding seagrasses. Competition for nutrients represents a potential influence on calcification if thalli amid dense seagrass continue to photosynthesize and produce $CaCO_3$ in existing tissues without the nutrients required to synthesize new living tissue.

Some investigators have reported abiotic influences on calcification, with seagrasses or fleshy macroalgae creating seawater chemistry favorable to algal and coral calcification (Semesi et al., 2009a, b; Anthony et al., 2011; Kleypas et al., 2011). These results were obtained under naturally (Kleypas et al., 2011) or artificially (Semesi et al., 2009a, b; Anthony et al., 2009) low mixing conditions (high water residence time). Findings here suggest that abiotic effects may not be very significant in well-mixed conditions, because (1) the pH regime in the seagrass canopy did not differ significantly

from unvegetated areas in Grape Tree Bay and (2) growth rates for *H. incrassata* in dense *T*. *testudinum* beds were significantly lower, which further indicates that biotic interactions, e.g., competition, between these macrophytes could represent a key factor.

Collectively, the results presented here suggest that enhancement of calcification will depend on water residence time (Anthony et al., 2011; Kleypas et al., 2011) and ecological processes, such as competition (Davis and Fourqurean, 2001) and succession (Williams, 1990), that affect both the abundances of macrophytes and their ability to garner resources (van Tussenbroek and van Dijk, 2007).

Further research combining manipulative and mensurative experiments is needed to elucidate the outcomes of positive and negative interactions between seagrasses, like *T*. *testudinum*, and rhizophytic, calcareous algae, like *H*. *incrassata*, in oceans that are becoming increasingly acidic. Data on local hydrodynamics and diel variations in seawater chemistry also will be important in furthering our understanding of biotic and abiotic influences on calcium carbonate production.

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BIOGRAPHICAL SKETCH

Savanna Barry grew up in central Virginia and attended Patrick Henry High School, where she graduated as Valedictorian in 2006. She studied biology at the University of Virginia and held multiple internships at the Anheuser-Busch Coastal Research Center in Oyster, VA. She graduated from UVA with honors in 2010 entered the University of Florida Fisheries and Aquatic Sciences Master of Science program in the fall of the same year. Her graduate work was completed in Little Cayman, BWI and she plans to enroll in a Doctor of Philosophy program at the University of Florida in the fall of 2012.