INTRODUCTION

The coral reefs of Florida, like much of the Caribbean, have lost significant amounts of coral cover over the past two decades (e.g. Dustan and Halas 1987; Porter and Meier 1994). Since this loss has been accompanied by an increase in fleshy macroalgae, which are generally considered to be nutrient-limited on coral reefs and a symptom of elevated nutrients, there is widespread belief that this transition resulted from anthropogenic nutrification of Florida Keys coastal water (eg. Ward 1990; Hallock et al. 1993). The population of Florida and the development of the Florida Keys increased dramatically since the turn of the century and anthropogenic nutrients have reportedly been detected in coastal waters (Lapointe et al. 1990, Lapointe and Clark 1992, Lapointe 1997). These nutrient sources were suggested as a major cause of the shift in the structure and function observed on Florida reefs. However, there is little evidence to suggest that nutrient enrichment has occurred at the scale necessary to produce the transition in community structure that has been observed on Florida Keys reefs. Recent changes in our understanding of benthic nutrient dynamics and recognition of alternate nutrient sources available to benthic algae indicate that nutrient enrichment may not represent a necessary ingredient for macroalgal blooms, because natural sources of benthic nutrients may provide these algae with an ample nutrient supply. The work described here investigated one alternative, benthic nutrient source, detritus, and the physical entrapment, remineralization, and importance of this nutrient source to the growth of two dominant types of macroalgae occurring on Florida reefs, *Dictyota* spp. and *Halimeda* spp.

Algal Phase Shifts

Coral reefs throughout the Caribbean and the Florida Keys have undergone algal phase shifts in recent decades, characterized by an increased abundance of macroalgae and epilithic algae (turf) and a simultaneous decrease in living coral and crustose coralline algae (Hughes 1994). In tropical marine systems, most of the primary productivity is associated with the benthos. An increase in macroalgae increases the potential for competitive interactions between algae and other organisms that occupy the substrate. Direct competition between algae and coral has been observed as some forms of benthic algae have been demonstrated to overgrow and smother coral tissue (reviewed by McCook et al. 2001), and increasing algal cover has been shown to limit coral recruitment (Edmunds and Carpenter 2001). Stress and abrasion of coral tissue caused by contact with macroalgae has also been associated with reduced growth rates (Miller and Hay 1996, Lirman 2001, decreased fecundity (Tanner 1995), disease transmission (Nugues unpublished data) and increased susceptibility to disease (McCook et al. 2001). Therefore, an improved understanding of the processes responsible for algal phase shifts is critical for management strategies aimed at reversing these algal-dominated states and promoting coral health and the recovery of reefs.

Despite recent efforts by the scientific community to understand these processes, controversy remains over the major causes of the transition to algal dominance. The most dramatic and sudden changes in community composition have been associated with top-down perturbations and severe reductions in herbivory. These occurred on coral reefs throughout the Caribbean and Western Atlantic as a result of the 1983 catastrophic die-off of the sea urchin *Diadema antillarum* (Lessios 1988), an important grazer capable of maintaining short epilithic algal turfs (Carpenter and Williams 1993). However, because coral reef macroalgae are

generally considered nutrient-limited and their appearance on reefs is often associated with elevated nutrients, anthropogenic nutrient enrichment has been implicated, by a few researchers, as a major cause for this transition (Lapointe et al. 1990, Lapointe and Clark 1992, Lapointe 1997, Lapointe 2001) .

With the population of Florida experiencing an increase from <500,000 at the turn of the century to over 14 million at present time, and with over 40% of Florida's population living in southern Florida, the use of coastal waters and influence of this population increase on coastal water quality has become a central concern for the scientific community and management. Anthropogenic nutrients have been shown to enter coastal waters from canals and waterways, marinas, terrestrial runoff, and contaminated groundwater seepage both near-shore as well as offshore (Lapointe et al. 1990; Paul et al. 1995). However, while nutrient levels are elevated in inshore areas there is little evidence that these nutrients are reaching offshore reefs at the scale necessary to produce the observed changes in community structure, and the total annual input from anthropogenic sources represents a small fraction of natural nutrient inputs to the south Florida region (Szmant and Forrester 1996). Upwelling of new nitrogen from beneath the Gulf Stream, frontal eddies and internal waves have been shown to serve as a "nutrient pump" for the outer shelf of the southeastern U.S. (Lee et al. 1991; 1994, Leichter et al. 1996; 1998) and additional nutrients are entering the reef tract from the Florida Bay and the West Florida Shelf through passages in the middle and lower Keys (Szmant and Forrester 1996). Despite the contribution from these various sources, average water column nutrient concentrations occurring on the Florida Keys reef tract remain below threshold values suggested to result in or support persistent macroalgal blooms on coral reefs (Bell 1991; Lapointe 1997). So, the question

remains – how have nutrient-limited macroalgae been able to bloom and dominate the reefs of the Florida Keys if ambient nutrient concentrations have not increased?

A Changing Paradigm

Until recently, most arguments for nutrient limitation were based on nutrient concentrations of water samples taken from the water column overlying coral reefs. The nutrient concentrations of this water are typically low - <1.0 μM for dissolved inorganic nitrogen and < 0.5 μM soluble reactive phosphate (eg. Szmant and Forrester 1996; Koop et al. 2001), yet coral reefs are among the most productive and diverse marine ecosystems on earth with rates of net productivity in excess of 1000 g dry wt m^{-2} $year^{-1}$ (Hatcher 1988). While water column productivity is limited at these concentrations, the high rates of coral reef primary productivity described here are associated with the benthos, where nutrient sources and availability are different than those in the water column. Therefore, studies that have assessed nutrient limitation by examining ratios of dissolved inorganic nitrogen: phosphate (DIN:PO_4) in seawater from the water column and total nitrogen:total phosphorus (TN:TP) of algal tissue (Atkinson 1988) are inadequate as they have failed to account for benthic nutrient sources. Analyses based on algal tissue TN:TP are also unreliable due to interspecific differences in nutrient storage capacity, different requirements for growth (Fong et al. 1994), and seasonal patterns in growth rate and storage capacity within and between algal species (Rosenberg and Ramus 1981,1982).

In contrast to elemental comparisons which, in most cases, suggest nitrogen or phosphorus limitation, numerous nutrient enrichment and caging experiments have failed to demonstrate nutrient-limitation of benthic macroalgae and indicate that top-down control (herbivory) is more important than bottom-up control (nutrients) in maintaining low standing

crops of algae on coral reefs (e.g. Miller et al. 1999; Koop et. al 2001; Thacker et al. 2001; McClanahan et al. 2002; Belliveau et al. 2002; Szmant unpublished data). This discrepancy may be a result of our failure to recognize the benthic sources of nutrients available to coral reef algae and simulate these sources and natural conditions experimentally.

Only in recent decades have sources of benthic nutrients been identified and their importance to coral reef algae been demonstrated. The remineralization of detritus in sediment patches and reef cavities (Andrews and Muller 1983; Williams et al. 1985; Capone et al. 1992; Boucher et al. 1994), macrofaunal excretion (Meyer and Schultz 1985; Williams and Carpenter 1988), and groundwater seeps (D'Elia et al. 1981; Lewis 1987) can contribute to elevated nutrient concentrations at the sediment-water interface. The potential for the accumulation of nutrients is determined by the thickness of the boundary layer and the rate of mixing into the water column (Larned and Stimson 1996), which is influenced by the benthic community. Numerous authors have reported nutrient enrichment occurring within the thalli of algae (eg. Lapointe and O'Connell 1989; Lavery and McComb 1991; Larned and Stimson 1996; Larned 1998). Larned and Stimson (1996) and Larned (1998) recognized that not all algae are capable of meeting their nutrient requirements through the utilization of water column nutrients and determined that microenvironment nutrient enrichment, within thalli, is necessary for the growth of several species of macroalgae.

In addition to influencing the mixing and accumulation of dissolved nutrients, it is likely that benthic algae can promote the formation of nutrient-enriched microzones by trapping particulate organic matter. Previously, Carpenter and Williams (1993) described a positive correlation between epilithic algal community (EAC) canopy height and boundary layer thickness, and recent work showed that sediment load and detritus distribution are positively

correlated with the biomass of EACs on the windward side of a reef in the Great Barrier Reef (Purcell and Bellwood 2001). Therefore, the entrapment and remineralization of detritus among thalli likely contribute to the elevated nutrient concentrations observed within algal mats and represent a mechanism of localized nutrient cycling.

Detritus

The majority of coral reef detritus is generated in situ rather than being derived from terrestrial sources (Alongi 1988). While sources of coral reef detritus are numerous and variable, depending on the type and location on the reef, algal debris is a major component of the detrital pool (Lewis 1977). Primary production may enter the detrital pool both directly or indirectly. Algal grazing is intense on coral reefs, but assimilation efficiencies are only between 35-45% for macro-invertebrates (Klumpp and Pulfrich 1989) and 20-70% for reef fishes (Bruggemenn et al. 1994; Galetto and Bellwood 1994). Therefore, much primary production enters the food web as detritus in the form of fecal material and algal fragments generated through processes of mechanical erosion (Hatcher 1983; Alongi 1988; Hansen et al. 1992; Arias-Gonzalez et al. 1997; Purcell and Bellwood 2001). Since algal detritus is more readily available to consumers than vascular plant material (Atkinson and Smith 1984), detritus on coral reefs tends to be utilized and recycled more quickly and efficiently than in other coastal or shallow water ecosystems (Hatcher 1983).

Using models, Johnson et al. (1995) predicted that a shift in community structure to an algal-dominated system, like that observed on Florida Keys reefs, would result in an increase in the amount of benthic primary production transferred to the detritus pool. An increase in the size of the detritus pool would likely result in increased rates of remineralization and flux of

dissolved nutrient from the benthos, creating conditions favorable for microzone nutrient enrichment and the persistence of macroalgal blooms. While the impacts of recent phase shifts on coral reef community structure and coral recruitment are well documented (e.g. Carpenter 1990; Tanner 1995), a detailed understanding of how these alterations of the benthic community structure have impacted nutrient dynamics and nutrient availability to the dominant macroalgae on these reefs is lacking.

In this study I investigated a proposed positive feedback mechanism through which macroalgae are able to promote and support their own growth by trapping particulate organic matter and remineralized nutrients. The goals of the study were: (1) to quantify and compare detritus pools occurring within the dominant algal/substrate types found on Florida reefs - *Halimeda opuntia*, *Dictyota* spp. and turf algae. (2) to quantify dissolved nutrient pools associated with these microhabitats, since dissolved nutrients are a product of the remineralization and use of detrital material, and (3) to conduct experiments with two types of macroalgae, *Halimeda tuna* and *Dictyota* spp., to determine if benefits of detrital nutrient sources and the microenvironment accumulation can be demonstrated for these algae. I hypothesized that: (1) the structural forms of macroalgae, *Halimeda opuntia* and *Dictyota* spp., are capable of trapping detrital material (2) internal remineralization of particulate organic matter, within the thalli of these algae, creates nutrient-enriched microenvironments, and (3) the concentrations of dissolved nutrients occurring as a result of this internal remineralization and accumulation are important for growth of these macroalgae.

METHODS

The Study Area

The Florida Keys reef tract is located offshore from a line of barrier islands of Pleistocene origin that extend from the city of Miami to Key West. The reef tract is oriented from NNE to SSW at its northern extent in the upper keys and gradually curves to an E-W orientation in the lower keys. It is bounded on the offshore side by the Florida Current, a swift current that frequently meanders close to shore or spins off eddies that come inshore over the reefs of the outer shelf. There are two types of coral reefs in the Florida reef tract: smaller Holocene patch reefs that formed on the offshore side of Hawk Channel, a 10 m deep channel that runs parallel to and 2-3 km offshore from the barrier islands, and larger bank reefs that form the offshore coral reef barrier 8-9 km offshore (Marzelak et al. 1977; Jaap 1984). The latter are relic Pleistocene reefs with varying amounts of Holocene growth on them. Patch reef waters can be relatively clear (10 to 20 m visibility), but are generally much more turbid, (especially during windy periods) than those on the barrier reefs which are predominantly bathed in clear Florida Current waters.

Located at the latitudinal limits of reef growth on the eastern coast of North America, Florida Keys reefs display characteristics of high latitude reefs, including low coral cover, reduced coral growth rates, and increased cover by algae (Jaap 1984; Dustan and Halas 1987; Glynn et al. 1989). The 1983 mass mortality of *D. antillarum* marked the beginning of a period of rapid decline in coral cover and increase in algal dominance that continues today. Populations of *D. antillarum* have failed to recover since their die-off in 1983 (Chiappone et al. 2002) and the substrate currently is dominated by thick algal turf and macroalgae (Chiappone and Sullivan

1994, Lirman and Biber 2000). Recent surveys of benthic community composition on reefs of the upper Keys indicate that the macroalgal community is dominated, in terms of percent cover and biomass, by *Dictyota* spp. and *Halimeda spp*. respectively (Szmant and Mason, in prep). All of the work described in this paper was conducted on the shallow fore-reef (2 -7 meters) of Little Grecian Reef, an offshore bank reef located near Key Largo, FL (25˚07.098' N; 80˚18.042' W; Figure 1). The substrate composition of this reef is characteristic of high-relief offshore reefs in the Florida Keys and, at the time this study was conducted, percent cover was dominated by three algal types - *Halimeda* (12%), *Dictyota* (33%) and crustose coralline algae (CCA)/turf (28%) (Figure 2).

Nutrient and Detritus Surveys

Sampling

The nutrient concentrations of near-bottom water, and the distribution and abundance of particulate matter trapped by the dominant algal types on Key Largo reefs, *Halimeda opuntia*, *Dictyota* spp., and algal turf were examined. These substrate types were sampled along fifteen, 10 meter long transects haphazardly placed at various depths along the fore-reef and reef crest of the study site. Two patches of each of the three substrate types (*Halimeda*, *Dictyota* and CCA/turf) were sampled along each transect. The total sample size equaled 90 samples (30 samples of each substrate type). For each of these samples the water depth, and canopy height of the algae were measured, and near-bottom water was collected from two centimeters above the substrate within each of the targeted algal patches. Lastly, all algae and particulate matter was collected from each sample patch, sorted and analyzed as described below.

Collection and Analysis of Water Samples

Prior to sampling or disturbing the substrate, water samples were carefully collected from the substrate-water interface of the targeted patches. Sampling was accomplished using acrylic tube, with a diameter of 3.5 cm in diameter, and containing a sampling port designed for insertion of a 60 ml syringe. The sampling syringe extended through the top of the tube and was held in a fixed position, two centimeters above the substrate. Water drawn into the syringe during sampling was replaced by near-bottom water from outside of the sampling tube through two openings near the base (Figure 3). Water samples were drawn slowly to minimize turbulence and mixing of water inside the tube caused by the entry of replacement water. Two ambient (water column) water samples were collected in acid-washed bottles from haphazardly selected positions along each transect at a distance approximately 50 centimeters above the substrate. Water samples collected in syringes were transferred to clean, acid-washed 50 ml centrifuge tubes upon returning to the boat. All samples were kept on ice in the dark until we returned from the field (<2 hours) and at that time were frozen. Frozen samples were shipped to the Center for Marine Science at the University of North Carolina at Wilmington (UNCW) for analysis. Inorganic nutrients (ammonia, nitrate, and phosphate) were determined using a Bran Luebbe AutoAnalyzer 3. TOC and TDN were measured using a Shimadzu TOC-5050A.

Collection of Detritus and Algae

After the water samples were collected, a wire brush was used to clear algae and sediment from the substrate surrounding the bottom-water sampling device. This created a buffer zone around each patch of substrate, and allowed collection of algae and particulate matter from a known surface area of the algal patch while minimizing contamination from the

surrounding substrate. Once a buffer zone was established, the sampling device was removed and the canopy height of the algal sample was measured to the nearest centimeter. The algal biomass was scraped from the substrate using a paint scraper and all algae and particulate matter present were collected using a suction device equipped with in-line mesh filter bags (after Purcell 1996). The mesh bags containing the sample were removed from the suction apparatus while underwater, and transferred to pre-labeled zip-lock bags. All samples were stored on ice and in the dark until they could be processed in the laboratory.

Processing and Analysis of Samples

Upon returning to the laboratory, the contents of the mesh bags were removed and rinsed, with filtered seawater, through a series of sieves to separate the samples into the following fractions: Algae (> 1 mm); Coarse Particulate Matter (500 um – 1 mm); Fine Particulate Matter (200 – 500 um). Macroscopic animals (> 1 mm), and large inorganic particles (shell fragments, stones etc…) were removed from the algal fraction during processing. The solid fractions were collected in aluminum weigh boats, dried at 60°C for 48 hours, weighed, and shipped to UNCW for analysis. Dried samples were ground to a fine powder using a mortar and pestle, and sub-samples were analyzed for total nitrogen and total carbon using a Carlo Erba ThermoQuest NC2100 Sediment Analyzer. The organic carbon content was determined by measuring the ash-free dry weight (AFDW) of the samples. To determine AFDW, sub-samples were weighed, ashed at 550°C for 24 hours, and reweighed. The resulting weight (ash) was subtracted from the original sub-sample weight. Total phosphorus content was determined by ashing samples at 550°C with $MgNO_3$, dissolving the ash in HCl, and determining the phosphate concentration of the resulting solution using a Bran Luebbe AutoAnalyzer 3 (after Ruttenberg 1992).

Statistical Analysis

All data analyses were performed using JMP Version 4.0.3. The response of detrital biomass to algal biomass, algal canopy height, and water depth of associated algae was analyzed for each of the algal types using multiple regression analysis (α=0.05). Single-factor ANOVA was used to determine differences in nutrient content of algal tissue and associated detritus among the three algal types. Single-factor ANOVA was also used to analyze the relationship between algal tissue nutrient content and the nutrient content of the associated detritus for each algal type. N and P content data were arcsine transformed prior to analyses. This transformation normalized distributions (determined by Shapiro-Wilk W Test) and homogenized the variance of these data. Tukey's HSD tests were used for unplanned comparisons (α=0.05).

Log_{10} transformations were used to normalize the distribution and homogenize the variance of water sample, nutrient concentration data. Once assumptions were met, single-factor ANOVA was used to determine differences in nutrient content (factor = sample type). Tukey's HSD tests were once again used for unplanned comparisons (α=0.05).

Algal Growth Experiment

The Growth Chambers

Cylindrical growth chambers were constructed using PVC rings (3" diameter x 1" thickness (top) and 2" thickness (bottom)) to allow 1 inch for insertion into PVC flanges. The walls, top and bottom of the chambers consisted of 1mm mesh, nylon screen. Prior to attaching

the mesh to the PVC rings, strips of ½ inch Velcro tape were sewn to either end of the mesh. The mesh was glued to the outer surface of the PVC rings using a glue gun, leaving the final 0.25 inches of the mesh detached. The Velcro allowed the mesh sides to seal closed but serve as a door that could be opened allowing the insertion and removal of algae fragments. The base of each chamber, assigned to a benthic treatment, was inserted into a 3"x 4" PVC closet flange. Flanges were securely attached to the substrate using galvanized nails, prior to attachment of the chambers (Figure 4).

The Algae

Fragments of *Dictyota* spp. and *Halimeda tuna,* that were similar in size and apparent condition, were collected from the fore reef of Little Grecian Reef. Specimens were removed from the substrate by hand, placed in zip-lock bags while underwater and returned to the laboratory in coolers filled with seawater. Once in the laboratory, the collected algae were rinsed with seawater, gently cleaned of epiphytes using forceps, and sorted into experimental units of similar weight. Individual experimental units were spun in a salad spinner to remove excess water and immediately weighed using an Ohaus Adventurer-AR3130 digital balance. Samples then were assigned randomly to treatments, placed in a numbered growth chamber, and stored in coolers of seawater while the others samples were prepared.

Field Deployment and Sample Processing

Chambers were transported, in coolers, to the study site and installed, according to the treatment designation, by attaching them to flanges as described above. Chambers were left in the field for five days (120 hrs.), collected and returned to the laboratory in coolers of seawater.

Once in the laboratory, the algae were removed carefully from the chambers using forceps, rinsed with seawater, cleaned of epiphytes, spun in a salad spinner and weighed as described above. Samples were then dried at 60°C for 48 hours, packaged in aluminum foil and transported to the Center for Marine Science at UNCW for elemental analysis. Total nitrogen and total carbon were determined using a Carlo Erba ThermoQuest NC2100 Sediment Analyzer, as described for detritus samples.

The Treatments

Treatments were created by manipulating the substrate, enclosed by each anchored PVC flange, immediately preceding the attachment of the chambers. Five treatments were created (Figure 5). These were:

1. Bare Substrate (Bare) – created by attaching a flange to naturally bare substrate ['bare' included micro-algal film but not CCA or turf]
2. Barrier (Barrier)- created by placing a flange as to enclose a clump of algae, removing the algae and inserting a growth chamber with a PVC cap attached to the bottom to serve as a barrier between the substrate and the overlying chamber
3. Algae Removed (Remove)– created by attaching a flange as to enclose a clump of algae. The algae was then removed as for treatment 2, leaving behind particulate matter that had accumulated below the algal canopy.
4. Fertilized (Fert.) – created by placing a flange over bare substrate and inserting a mesh bag containing 5 grams of Osmocote® commercially available fertilizer prior to attaching the growth chamber.

5. Water Column (Float) – created by tethering a chamber, with an attached float, to the substrate using a nail and a wire lead, allowing the chamber to float approximately 50 cm above the substrate

Each treatment was replicated 5 times for each algal type, during each run of the experiment and the experiment was conducted three times (n=15). The experiment was deployed as a randomized block design, with each combination of treatment and algae type replicated once per block.

Water Sampling and Analysis

Water samples were collected from 25 of the 50 growth chambers (5 samples from each treatment) 24 hours after deployment of the experiment in order to estimate differences in nutrient concentrations between, and the effectiveness of, the various treatments. Samples were collected using 60 ml syringes fitted with needles, allowing samples to be drawn through the mesh, from inside the chambers, thereby minimizing the disturbance of the algae and treatment conditions. Full syringes were returned to the boat and the water was transferred to 50 ml tubes, and kept on ice and in the dark until they could be frozen. They were later returned to the Center for Marine Science in Wilmington, NC for analysis. Inorganic nutrients, TOC and TDN were analyzed as above.

Statistical Analysis

Randomized Block ANOVAs were used to determine differences among treatments in algal growth and nutrient content of treatment water for each of the algal types examined in this

study. Data from the three separate trials of the experiment were combined and blocks containing missing data were excluded from the analysis.

RESULTS

Nutrient and Detritus Surveys

Detritus Distribution

The mean biomass of detritus associated with benthic algal communities (CCA/turf = 28.9 g organic C m^{-2}; *Dictyota* = 36.4 g organic C m^{-2}; *Halimeda* = 33.01 g organic C m^{-2}) did not differ significantly among the algal types examined in this study (Single-factor ANOVA: $F_{0.05(1),3,92} = 2.1052$; $p = 0.1276$; see Figure 6a). Multiple regression analyses were used to examine the response in detrital biomass to algal biomass (AFDW), canopy height, and water depth for each of the three algal types. Results from these tests indicated algal biomass (AFDW) and depth were significant predictors of detrital biomass occurring within CCA/turf communities (Whole Model: $p < 0.0001$, $R^2 = 0.593$; Algal Biomass: $p < 0.0001$, $R^2 = 0.3723$; Canopy Height: $p = 0.5834$; Depth: $p = 0.0004$, $R^2 = 0.1062$). Significant relationships between these regressors and detrital biomass were not observed for the other two algal types (*Dictyota* - Whole Model: $p = 0.4089$, $R^2 = 0.096$; *Halimeda* – Whole Model: $p = 0.6019$, $R^2 = 0.073$; Table 1).

Nutrient Content of Algal Tissue and Detritus

Differences in the nutrient content of detritus were observed among algal types for total N and N:P (Single-factor ANOVA: total N - $F_{0.05(1),2,90} = 3.4739$; $p = 0.0352$ and N:P - $F_{0.05(1),2,90} = 3.0243$; $p = 0.0536$) but not for total P ($F_{0.05(1),2,90} = 0.6644$; $p = 0.5171$; Figure 6). Detritus associated with *Halimeda* had a significantly higher N content and the N:P ratio of detritus

associated with *Dictyota* was lower than the ratio of detritus associated with CCA/turf algae. Differences also were observed among algal types in the nutrient content of algal tissue. The mean N content of *Halimeda* tissue (3.80%) was significantly greater than that of both CCA/turf and *Dictyota* (Mean N: 2.42 and 2.25% respectively; Single-factor ANOVA: $F_{0.05(1),2,86} = 38.3178$; $p < 0.0001$; Figure 6). Similarly, the P content of *Halimeda* (0.22%) was greater than that observed in the tissue of the other algae (CCA/turf: 0.21%; *Dictyota*: 0.16%) but was only significantly different from the composition of *Dictyota* (Single-factor ANOVA: $F_{0.05(1),2,86} = 4.0406$; $p = 0.0210$; Figure 6). While differences were observed in the nutrient content of individual elements, no difference in elemental ratios (N:P) was observed among algal types (Single-factor ANOVA: $F_{0.05(1),2,86} = 2.1627$; $p = 0.1212$; Figure 6).

Comparison of the nutrient content of the three algae with the content of detritus associated with each indicated that the two components were significantly different in total N, total P and N:P for all three algal types (Figure 7). While N in algal tissue was significantly greater than that observed in the detritus (Single-factor ANOVA: CCA/turf; *Dictyota*; *Halimeda*: $p < 0.0001$), the P in the algal tissue was significantly less than the associated detritus (Single-factor ANOVA: CCA/turf: $p = 0.0236$; *Dictyota*: $p < 0.0001$; *Halimeda*: $p < 0.0001$). Likewise, the N:P of detritus was consistently higher than the associated algae (Single-factor ANOVA: CCA/turf:; *Dictyota*; *Halimeda*: $p < 0.0001$).

Nutrient Content of Water Samples

Differences were observed among associated algal types and/or sample locations (bottom water versus water column) for all of the nutrients measured in this study (Single-factor ANOVA: PO_4 ($F_{0.05(1), 3, 114} = 15.249$; $p = <0.0001$); NO_3 ($F_{0.05(1), 3, 114} = 7.711$; $p = <0.0001$); NH_4

($F_{0.05(1),\,3,\,114} = 2.993$; $p = 0.0338$; Figure 8); DOC ($F_{0.05(1),\,3,\,114} = 3.074$; $p = 0.0305$); DON ($F_{0.05(1),\,3,\,114} = 3.012$; $p = 0.0335$); Figure 9). Mean PO_4 concentrations associated with the three algal types (CCA/turf = 0.162 μM; *Dictyota* = 0.167 μM ; *Halimeda* = 0.181 μM) were higher than that measured in the water column (Ambient = 0.139 μM) samples. Differences were also observed among the algal types. The mean concentration of PO_4 in water associated with *Halimeda* were significantly greater than water associated with the CCA/turf substrate (Tukey HSD ($\alpha = 0.05$); Figure 8a).

The mean NO_3 concentration in the water column samples was significantly lower than the mean concentrations in bottom water associated with the three algal types (CCA/turf = 0.371 μM; *Dictyota* = 0.459 μM; *Halimeda* = 0.553 μM; Ambient = 0.269). However, significant differences were not observed among the three algal types examined in this study (Tukey HSD ($\alpha = 0.05$); Figure 8b).

Similar differences were observed among mean NH_4 concentrations (CCA/turf = 0.340 μM; *Dictyota* = 0.518 μM; *Halimeda* = 0.479 μM; Ambient = 0.294). However, the only significant difference observed was between *Halimeda* and Ambient concentrations (Pairwise comparisons were made using Tukey HSD; $\alpha = 0.05$; Figure 8c).

The trends observed for inorganic nutrients were similar to those observed for dissolved organic nitrogen (DON) but were quite different from those observed for dissolved organic carbon (DOC)(Figure 9). Mean DON concentrations (CCA/turf = 6.152 μM; *Dictyota* = 6.655 μM; *Halimeda* = 7.008 μM; Ambient = 5.615) were consistently higher for the three algal types than for the water column samples. However, as with NH4, the only significant difference was between *Halimeda* and Ambient (Tukey HSD ($\alpha = 0.05$); Figure 9a). The mean, Ambient DOC concentration (121.2 μM) was actually significantly higher than that observed for the CCA/turf

substrate (101.4 μM), and similar to the concentrations observed for the other two algal types (*Dictyota* = 121.4 μM; *Halimeda* = 122.3 μM; Tukey HSD (α = 0.05); Figure 9b).

Algal Growth Experiment

Algal Growth

The growth of *Halimeda tuna* and *Dictyota* spp. responded differently to the treatment conditions used in this experiment (Figure 10). While significant differences were not observed in the percent change (wet weight) of *Halimeda* among treatments (Randomized Block ANOVA: $F_{0.05(2),4,44} = 0.486$; $p > 0.50$), the growth of *Dictyota* varied significantly in response to the treatments, and was enhanced by nutrient enrichment (Randomized Block ANOVA: $F_{0.05(2),4,52} = 3.515$; $0.05 > p > 0.02$; Figure 9). The mean growth rates of *Halimeda* were consistently lower than those observed in *Dictyota* for all treatments. The mean growth rates of *Halimeda* ranged from 2.63%

day $^{-1}$ in the fertilizer (Fertilized) treatment to 3.56% day $^{-1}$ in the algal removal (Remove) treatment, while *Dictyota* growth rates varied from 3.93% day $^{-1}$ in the bare substrate (Bare) treatment to 10.49% day $^{-1}$ in the water column (Float) treatment (Figure 10).

Nutrient Analysis

Analysis of the inorganic nutrient concentrations of water collected from within the experimental growth chambers 24 hours after installation of the experiment, indicated that significant differences existed among treatments for two of the three nutrients measured (Figure 11). While PO_4 did not differ significantly among treatments (Randomized Block ANOVA: $F_{0.05(2),4,40} = 1.312$; $p > 0.50$), significant differences were observed for both NO_3 (Randomized

Block ANOVA: $F_{0.05(2),4,40} = 7.146$; $p < 0.001$) and NH_4 (Randomized Block ANOVA: $F_{0.05(2),4,40} = 6.674$; $p > 0.001$). In both cases, the highest concentrations were observed in the fertilizer treatments (Mean: $NO_3 = 0.52$ μM; $NH_4 = 0.69$ μM; see Figure 11).

DISCUSSION

The results of this study support my original hypotheses that: (1) the structural forms of macroalgae, *Halimeda opuntia* and *Dictyota* spp., are capable of trapping detrital material (2) internal remineralization of particulate organic matter, within the thalli of these algae, creates nutrient-enriched microenvironments, and (3) the concentrations of dissolved nutrients occurring as a result of this internal remineralization and accumulation are important for growth of these macroalgae. While differences in the standing stock of detritus were not observed among the algae examined, the data presented here demonstrate that all three algal types – *Halimeda opuntia*, *Dictyota spp*. and turf algae – are capable of trapping detrital material. The depleted nutrient content of this material suggests that significant remineralization had occurred and may represent a major source of dissolved nutrients. Elevated concentrations of dissolved nutrients measured within the thalli of *H. opuntia* and *Dictyota* spp., suggest that the morphology of these algae impede the mixing of dissolved nutrients, released within their thalli, into the water column, thereby promoting the formation of nutrient-enriched microenvironments. Differences in the growth rate of *Dictyota* spp., among experimental treatments, indicate it is likely that this alga benefits from microenvironment nutrient enrichment. The failure to observe a treatment effect on the growth rate of *H. tuna* suggests that this alga is not N-limited. While it may be P-limited, the benefits that *H. tuna* receives from microenvironment nutrient enrichment are likely

less than for *Dictyota spp*. The nutrient status of these algae may be related to their morphology and differences in their ability to accumulate detrital material and impede the flux of dissolved nutrients, released from this material, into the bulk water.

Detritus Standing Stock

The standing stock of particulate organic matter associated with the algae in this study was similar to values reported in other studies but did not differ among the algal types examined here. Purcell and Bellwood (2001) reported values of detrital biomass associated with epilithic algal communities (EACs) on the windward side of Lizard Island, (GBR) Australia. In this study they measured approximately 5-30 g $C_{org} \cdot m^{-2}$ with considerable variation among reef zones. Schaffelke (1999) reported much lower biomass values for particulate matter associated with the thalli of *Sargassum* (0.2-1.2 g $C_{org} \cdot m^{-2}$), but these estimates do not include material trapped below the canopy of this alga. The biomass of particulate matter associated with the algae in this study ranged from 28.9 (CCA/turf) to 36.4 (*Dictyota*) g $C_{org} \cdot m^{-2}$. While these values are comparable, they are higher than those reported by Purcell and Bellwood (2001). In addition, the authors of this study defined "detritus" as all particulate material greater than 60 μm in diameter and therefore their measurements included a much broader size range of particles than was included in samples in the present study. If the samples in this study had included particles in the size range from 60-200 μm it is likely that a significantly larger standing stock of detritus would have been observed in the present study. This observation is consistent with elevated rates of detritus formation and greater flow of organic carbon through the detrital pool that have been predicted to result from the transition to algal dominance (Johnson et al. 1995).

While the values of detrital biomass measured in this study suggest that *H. opuntia*, *Dictyota* spp., and turf algae do not differ in their ability to trap and retain detrital material (Figure 6), I believe that the failure to observe a difference is a result of two factors: (1) the size fraction of particulate matter that was collected, and (2) differences in meiofaunal communities and thus processing rates of the organic material trapped by these algae.

The sampling device used in the collection of detrital samples contained a 200 μm mesh screen. As a result, an important, smaller size class of particles and one that likely contained more highly processed organic material (eg. feces of fish and meiofauna) was absent from the analysis. Several samples of *H. opuntia* were observed to contain an abundance of fine particles that were readily suspended in sample water. The presence of highly processed organic material in *H. opuntia* clumps is consistent with the diverse and abundant meiofaunal communities that are reported to reside within the thalli of these algae. Rader (2001) identified organisms belonging to 67 different families, representing 12 phyla, in 24 samples of *Halimeda* spp. collected from Florida Keys reefs and observed an average of 19.9 (s.d. $\pm$ 17.0) individuals$\cdot g^{-1}$ and 4.3 (s.d. $\pm$ 3.7) taxa$\cdot g^{-1}$ of *H. opuntia* (dry wt). A variety of detritivores were present among the observed taxa, including bivalved mollusks, sedentary polycheates, copepods, bryozoans, holothuroids, ophiuroids, nematodes, and sipunculids. With the abundance of detritivores and diversity of trophic categories represented among these infaunal organisms, it is likely that clumps of *Halimeda* spp. are functioning as micro-communities capable of rapidly recycling organic carbon and nutrients. It is unlikely that *Dictyota* spp. and algal turf provide equivalent habitat, in terms of available interstitial space, stability and protection, and therefore are not capable of supporting the diverse assemblage of organisms found within *H. opuntia*. As a result, the rates of consumption and remineralization of detritus are likely different among these algal

types and may compensate for differences in the ability of these algae to trap and accumulate detrital particles.

The rapid consumption of organic matter on coral reefs has been suggested as an explanation for differences in nutrient content commonly observed between sediment collected in sediment traps and adjacent surface sediments. The organic content of particulate matter collected in settlement traps is generally one order of magnitude higher than the surrounding surface sediments (Johnstone et al. 1990). Therefore the production of ammonia and organic nitrogen resulting from heterotrophic consumption of organic matter likely represents an important source of dissolved nutrients for the adjacent and overlying algae (Meyer and Schultz 1985, Gray 1985).

Nutrient Content of the Detritus

Evidence that the particulate matter sampled in this study had undergone processes of remineralization, is found in comparing the nutrient content of algae and associated detritus. Since the majority of coral reef detritus is autochthonous and is derived primarily from macroalgae (Lewis 1977, Alongi 1988), newly formed detritus would be expected to have a similar nutrient content as the dominant algae occurring on the reef. In this study significant differences were observed between the nutrient content of algae and that of the detritus (Figure 7). Detritus was significantly depleted in nitrogen and enriched in phosphorus relative to the alga from which it was collected. Koop and Larkum (1987) determined that only 60% of nitrogen from reef plant tissue is retained in the system and deposited to the benthos, suggesting that the balance is leached into the water prior to or during sedimentation (Linley and Koop

1986). A similar difference was observed in this study. Detritus associated with the three algal types studied here contained 64.0, 56.0, and 45.8% of the nitrogen content of the associated algae, CCA, *Dictyota* and *Halimeda* respectively.

The nitrogen that is not lost to the water column, while detrital particles are in suspension, or consumed and transformed by benthic meiofauna, is likely rapidly remineralized through microbial processes. It has been documented that diverse microfloral assemblages are often associated with detritus (Delong et al. 1993) and that these assemblages create stratified zones and steep microgradients that favor rapid and efficient remineralization of N (Wetzel 1993). Rates of organic matter decomposition, nitrification, N_2 fixation and denitrification that are depressed in the water column are often enhanced on detritus as organic particles often develop strong and persistent O_2 gradients that support anaerobic, microaerophilic and aerobic microbial consortia (Paerl and Pinckney 1996). This microzonal stratification of metabolically diverse microbes creates niches capable of supporting biological transformations not feasible in surrounding, O_2-rich waters such as found on coral reefs. The establishment of these microzones on the surface of detrital particles have been traced to the colonization by O_2 tolerant heterotrophic bacteria. O_2 consumption by these bacteria is capable of exceeding diffusional replacement and leads to O_2–depleted and anoxic microenvironments that are then colonized by anaerobic or metabolically flexible bacteria (Paerl and Pinckney 1996). While ammonia is a commonly released metabolite under anaerobic conditions, the main form of inorganic nitrogen released from detritus in NO_3. Based on this observation, Paerl and Pinckney (1996) described the following scenario for N cycling on detrital particles: (a) NH_4 is produced by N_2 fixation and ammonification of organic matter, (b) NH_4 is assimilated or (c) undergoes nitrification to NO_2/NO_3 by bacteria at the periphery of detrital particles.

Dissolved Nutrient Concentrations

The elevated concentrations of NH_4, NO_3, DON and PO_4 measured in interstitial water collected from within algal clumps in this study (Figures 8;9) suggest that the microbial remineralization and consumption of detrital material are likely occurring within these algal communities. From previous studies it is known that the dissolved nutrients occurring in this water represent the result of complex nutrient dynamics and remineralization processes. While the detritus likely represents the primary source of dissolved nutrients in interstitial water, rates of remineralization and flux of these nutrients from detrital sources were not determined in this study. Therefore the concentrations reported here represent the net accumulation of dissolved nutrients within the interstitial water of these algal clumps. The contribution of other benthic nutrient sources (eg. groundwater and macrofaunal excretion) were not determined and the rate and quantity of dissolved nutrients lost to the water column, and through uptake by the associated micro- and macroalgae are not known. In addition, not all of the nutrient molecules derived from detrital sources necessarily enter the dissolved nutrient pool within algal thalli. A significant quantity of N may be released within the boundary layer surrounding the algal tissue as epiphytic microbial communities associated with these algae remineralize particulate nutrients on the surfaces of thalli. These molecules may be taken up quickly and efficiently without entering the dissolved nutrient pool (Schaffelke 1999).

While rates of mineralization were not measured in this study, the net accumulation of dissolved nutrients within clumps of macroalgae indicates that these rates were high enough to exceed losses to the water column and uptake by algae. Rates of NH_4 uptake by macroalgal beds have been reported to range from 0-5 μmol g dw^{-1} h^{-1} (Tyler 2003). While rates of NO_3 and urea

(DON) uptake can be inconsequential when NH_4 is readily available, the uptake of NO_3 and urea has been shown to increase in importance as NH_4 availability decreases (Naldi and Wheeler 2002; Tyler 2003). Therefore, while it may not be surprising that the production of NO_3^- and DON exceed losses and rates of uptake, especially since NO_3 is the primary nutrient resulting from the remineralization of organic material, the accumulation of NH_4 is surprising and suggests that high rates of ammonification are occurring with these algae. While P does not undergo biological transformation in the way that N does, the elevated concentrations of PO_4 observed in interstitial water are not surprising. Entsch et al. (1983) identified particulate organic matter as a favorable source for the net accumulation of P in interstitial water of the surface layer of sediments and concluded that this accumulation should favor high rates of uptake by benthic algae.

Despite the inability to determine the relative contribution of detrital resources to the dissolved nutrient pools within these algae, it is likely that the accumulation of detritus is serving as an important nutrient source for these algae. Schaffelke (1999) provided evidence that *Sargassum* spp. utilize nutrients derived from the layer of particulate matter deposited on their thalli. In this study, she recorded growth rates of *Sargassum* as much as 180% higher when particulate matter was present on algal thalli than when these particles were removed, and measured activity of the enzyme alkaline phosphatase in particulate matter collected from the algal surface, providing evidence for the remineralization of this material. She hypothesized that the uptake of nutrients released from particulate matter at the surface of algal thalli would be taken up more efficiently than nutrients from the water column, because remineralization near the thalli surface would create steep diffusion gradients within and across the diffusive boundary layer on the thallus surface. In addition, other studies have demonstrated that nutrient-enriched

microenvironments, with similar concentrations to those observed here (Table 2), are critical for the sustained growth of several species of macroalgae (Lavery and McComb 1991, Larned and Stimson 1997).

Algal Growth Experiments

The results of this study provide evidence that some macroalgae benefit from internal, microenvironment nutrient enrichment. When concentrations of NH_4 and NO_3, similar to those observed within the thalli of algal clumps, were re-created through artificial nutrient enrichment (Figure 11), the growth rate of *Dictyota* spp. was stimulated (Figures 9,10). This was not the case for H. tuna, and the growth rates of both algae were highest in the water column treatments. The different responses observed between algae may be related to differences in growth form and physiology.

It has been proposed that the uptake of nutrients by coral reef algae is limited by diffusion through the boundary layers between the water and the algal tissue, a condition referred to as mass transfer limitation. The mass transfer limitation of, and nutrient uptake by, coral reef algae is determined by nutrient concentration and water velocity (Atkinson 1988, Baird and Atkinson 1997). At low nutrient concentrations and low water velocities, algae are likely to be mass-transfer limited and uptake rates increase with increasing nutrient concentration until a physiological uptake maximum is reached. As water velocity increases, the boundary layers between the water and algal tissue are reduced, and rates of diffusion across this boundary layer, and uptake by the algae, increase (Figure 12).

In this study, differences in dissolved nutrient concentrations, among the benthic treatments, and the elevated flow assumed to occur in water column treatments, were used to

investigate nutrient limitation of growth, and the importance of microenvironment nutrient enrichment for two types of macroalgae common on Florida reefs. Due to the frangible nature of *H. opuntia* (the *Halimeda* species examined in detritus and nutrient surveys) it was not used in the following growth experiment. Instead, *H. tuna* – a calcified green alga, with an upright more two-dimensional growth form, and *Dictyota* spp. (examined in the surveys above) – a fleshy, branching brown alga that forms dense mats and clumps - were used in the experiments (Figure 13).

The response of *H. tuna* to the treatments used in this study differed from that of *Dictyota* spp. Growth rates of *H. tuna* were not enhanced in nutrient enrichment treatments, when NH_4 and NO_3 concentrations were elevated, but were higher in water column treatments. This suggests that *H. tuna* is not N-limited at ambient concentrations. The growth of *H. tuna* may be P-limited since PO_4 concentrations remained unchanged in fertilizer treatments and the elevated flow experienced by the algae in water column treatments appeared to stimulate growth. In a study conducted in the Bahamas, Littler et al. (1988) provided evidence that the photosynthesis and growth in *H. tuna* are P-limited. However, P-limited growth is unlikely in the present study since the concentrations of PO_4 measured in water samples during this study were well above the 0.1 μM threshold value suggested to sustain algal blooms on coral reefs (Lapointe et al. 1993).

An alternative explanation is the growth of *H. tuna* may have been limited by other nutrients or environmental conditions that were constant among the benthic treatments. *H. tuna* is a calcified alga and thus requires inorganic C and Ca^{++}. Growth may have been controlled by the mass transfer limitation of these molecules, which would explain the slightly elevated growth rates observed among algae when maintained in a higher water velocity environment present in the water column treatments.

The growth of *Dictyota* spp. was stimulated by nutrient enrichment in fertilizer treatments, suggesting that the growth of this alga is N-limited at ambient concentrations of NH_4^+ and NO_3^-. It is likely that this alga benefits from microenvironment nutrient enrichment occurring within its thalli. However, nutrient uptake and the growth rate of this alga may still be N-limited at the DIN concentrations occurring naturally within its thalli, since higher growth rates were observed when this alga was exposed to elevated water velocity in the water column treatments.

Mean growth rates, of both *H. tuna* and *Dictyota spp*., were positive for all treatments in this study and were similar to growth rates previously reported for these algae. In similar nutrient enrichment experiments, Kuffner and Paul (2001) observed growth rates ranging from approximate 4-8% day^{-1} for *Dictyota bartayresiana* and Fong et al. (2003) reported changes of approximately 2-6% day^{-1} for *Dictyota cervicornis*. Average growth rates of *H. tuna,* measured at a similar depth and time of year as this study, at another location in the Florida Keys were approximately 1.5% day^{-1}(Vroom et al. 2003). This rate is similar but slightly lower than the 2.63-3.56% day^{-1} change we observed during this study.

Despite evidence to suggest that *Dictyota* spp. was N-limited at ambient nutrient concentrations, the nutrient content of algae sampled in this study did not suggest that the algae were nutrient-limited in their natural, attached state. Mean N, P and N:P ratios of the algae in this study (Figure 7) were within the range typically reported for benthic marine macroalgae (Atkinson and Smith 1983). Therefore, N-limitation of *Dictyota* spp. observed in the growth experiment likely resulted from the manipulation of algal specimens and N-limitation may be uncommon among attached algae with access to internal detrital nutrient sources.

While this study only examined growth in two species of algae, the differences in the nutrient status and growth response of *H. tuna* and *Dictyota* spp. may be related to the morphology of the algae.

H. tuna has an upright, coarsely branching, two dimensional growth form (Figure 13(b)) while *Dictyota* spp. has a dense, finely branching form (Figure 13(c)) that is likely more effective at detrital material and impeding the mixing of benthic nutrients into the water column. Microenvironment nutrient enrichment is more likely within *Dictyota* spp. and therefore the physiology of this alga may be adapted to facilitate the uptake of nutrient molecules in low flow, high nutrient environments. Evidence to support this is suggested by the elevated growth rate observed in *Dictyota* spp. (a branching, foliose genera) and lack of response by *H. tuna*. (an erect, two-dimensional species) to nutrient enrichment in fertilizer treatments. Others have hypothesized that the growth form of an alga is related to its ability to utilize different nutrient sources. It previously has been suggested that algae with upright thalli and open-branching growth forms may have a more direct relationship between tissue nutrient content and water column nutrient content than other growth forms (Fong et al. 2001). In the same study (Fong et al. 2001) suggested that mat-forming algae (eg. *Dictyota* spp.) may deplete dissolved nutrients within the mat and rely on strong currents to penetrate mats and replenish these nutrients. The results of this study suggest that this is not the case. Instead it appears as though detritus trapped within these algae serves as an internal nutrient source and the remineralization of this material actually results in nutrient-enrichment of this interstitial water.

CONCLUSIONS

The elevated concentrations of inorganic nutrients and organic nitrogen measured within the thalli of *H. opuntia* and *Dictyota* spp. demonstrate the ability of these algae to accumulate nutrients and establish nutrient-enriched microenvironments. While there are various factors contributing to the success and dominance of these algae on the Florida Reefs, for example, structural and chemical defenses (Hay and Fenical 1988) and their ability to propagate asexually (Walters 2002), other macroalgae with similar characteristics have not enjoyed similar success. I

propose that, in addition to the above characteristics, the morphology and ability of these algae to trap organic matter, provide stable habitat for meiofauna, and thus establish nutrient-enriched microenvironments within their thalli may be a critical difference and important to the growth and success of these species. It is likely that the growth forms of algae are related to their nutrient status, their ability to utilize benthic versus water column nutrient sources, and as a result may influence competitive interactions and algal community characteristics.

It has become evident from this and previous studies that have investigated and observed nutrient enrichment within algal mats and thalli that there exists a need to exercise caution when interpreting and designing algal growth experiments. The detachment and manipulation of algae inevitably creates an artificial scenario that is not representative of the natural nutrient environment and status of the algae. Future studies should acknowledge the potential for nutrient accumulation within algae, recognize the delicate, and complex networks of interactions and pathways that occur within, and use extreme care in designing experiments that do not disrupt or that attempt to recreate the structure and function of these micro-communities.