IMPACTS OF SPONGE PRODUCED DISSOLVED INORGANIC NITROGEN ON
CARIBBEAN CORAL REEF SEAWEED COMMUNITIES

Nyssa Joy Silbiger

A thesis submitted to the faculty of the University of North Carolina at Chapel Hill in partial
fulfillment of the requirements for the degree of Master of Science in the Department of
Marine Sciences

Chapel Hill
2009

Approved by

Advisor: Niels Lindquist

Reader: John Bruno

Reader: Chris Martens

Reader: Charles H. Peterson

ABSTRACT

NYSSA SILBIGER: Impacts of sponge produced dissolved inorganic nitrogen on Caribbean
coral reef seaweed communities.
(Under the direction of Niels Lindquist)

Sponges are known to excrete copious amounts of remineralized nitrogen, but it is
unknown how this may potentially facilitate seaweed proliferation on Caribbean coral reefs.
We used the unusually low $\delta^{15}N$ value of sponge-produced nitrate as a natural tracer to assess
whether seaweeds utilize sponge nitrate on Conch Reef in FL. Over summer and fall
seasons, we examined C and N tissue characteristics of 4 seaweed species (*Dictyota
pulchella*, *D. menstrualis*, *Halimeda tuna* and *Amphiroa beauvoisii*) naturally growing in the
excurrent flow of 4 sponge species (*Agelas schmidti*, *Niphates digitalis*, *Verongula gigantea*
and *Xestospongia muta*) versus control seaweeds growing away from the sponges. An
additional experiment transplanted seaweeds into the excurrent plume of *X. muta* with
appropriate controls. We found that *Dictyota* spp., the most abundant seaweeds on Conch
Reef, utilized sponge effluent more efficiently than the other seaweeds. If sponges are
promoting changes in seaweed biomass or community composition, this may have significant
impacts on coral reef health, as seaweeds can be harmful to corals. This information will help
managers better understand the implications of sponge-seaweed interactions for coral reef
degradation and recovery.

To Matthew W. Beard whom I know has been by my side throughout this whole experience

and whose passion for coral reefs will always be remembered.

TABLE OF CONTENTS

LIST OF TABLES

Table

LIST OF FIGURES

Figure

LIST OF SYMBOLS AND ABBREVIATIONS

$\delta^{13}C$: Expression of the isotopic composition of carbon in a sample, relative to the standard Pee Dee Belemnite. It is calculated according to the following:

$$\delta^N X = [(R\text{sample} - R\text{std})/R\text{std} - 1] \times 1000 \text{ in units of parts per thousand (‰).}$$

Where $^N X$ is the coefficient of the heavy isotope (i.e. ^{13}C), and R is the ratio of the heavy to light isotope in both the standard and sample (i.e. $^{13}C/^{12}C$)

$\delta^{15}N$: Expression of the isotopic composition of nitrogen, analogous to the above equation, but where $R = (^{15}N/^{14}N)$ and the standard is atmospheric N_2

‰: Units in which δ values are expressed

DIN: Dissolved inorganic nitrogen, including nitrate, nitrite, and ammonium

DOM: Dissolved organic matter

LMA: Low microbial abundance (*sensu* Hentschel et al. 2006), referring to species of sponge with microbial communities of similar composition and density to the water column

HMA: High microbial abundance, referring to species of sponges containing large internal microbial communities

NH_4^+: Ammonium

NO_3^-: Nitrate

POM: Particulate organic matter

INTRODUCTION

Caribbean coral reefs have been undergoing a phase-shift from coral-dominated to seaweed and sponge-dominated reefs (Done 1992, Hughes 1994, Aronson et al. 2002). The effects of seaweed proliferation on Caribbean coral reefs have been extensively investigated (e.g., McCook et al. 2001, McManus & Polsenberg 2004), but how an increasing sponge population may affect coral reef communities is not well understood. While sponges are important for "gluing" the reef together (Wulff 1984) as well as filtering significant amounts of seawater (Reiswig 1971), their increasing biomass may also pose potential threats to reef health. Although there is a long history of research on sponge biology and physiology (e.g., Reiswig 1971, Hentschel et al. 2006, McMurray et al. 2008, Wulff 2008), little is known about how their ability to excrete copious amounts of dissolved inorganic nitrogen (DIN) impacts coral reef communities (Reiswig 1981, Corredor et al. 1988, Diaz & Ward 1997, Southwell et al. 2008b). Here we examine a potential positive interaction between sponge-produced nitrogen (i.e., DIN) and seaweeds on Caribbean coral reefs. This facilitation may stimulate coral reef degradation on Caribbean reefs by increasing the abundance of seaweeds, such as *Dictyota* spp., that can be harmful to the recovery of coral reefs (Kuffner et al. 2006, Titlyanov et al. 2007).

Sponges are recognized as substantial sources of DIN to coral reef communities (Reiswig 1981, Corredor et al. 1988, Diaz & Ward 1997, Southwell et al. 2008b). Studies have found that remineralized nitrogen from both encrusting and non-encrusting sponges

may contribute significantly to productivity on Caribbean reefs. For example, Corredor et al. (1988) demonstrated that the encrusting liver sponge *Chondrilla nucla* potentially contributed 50 – 120% of the nitrate (NO_3^-) required to sustain productivity on a reef in southwest Puerto Rico. Additionally, Southwell et al. (2008b) reported that the non-encrusting sponge community from our study site on Conch Reef, a coral reef habitat approximately 4 miles east of Tavernier Key, Florida was responsible for net NO_3^- and ammonium (NH_4^+) fluxes of 480 ± 93 $\mu mol\ m^{-2}\ h^{-1}$ and 57 ± 73 $\mu mol\ m^{-2}\ h^{-1}$, respectively.

The NO_3^- and/or NH_4^+ concentration excreted from sponges is dependent on the presence of microbial communities in the sponge's ectosome. Many coral reef sponges, once called bacteriosponges, host large, diverse microbial communities (Reiswig 1974). These more recently termed high microbial abundance (HMA) sponges have microbial concentrations of $\geq 10^8$ cell gram^{-1} (Hentschel et al. 2006), and are known to excrete large quantities of NO_3^- (Corredor et al. 1988, Diaz & Ward 1997, Southwell et al. 2008b). In contrast, low microbial abundance (LMA) sponges, with microbial concentrations of $\leq 10^6$ cell gram^{-1} (Hentschel et al. 2006), excrete only NH_4^+ (Southwell et al. 2008b). Understanding the impacts of HMA sponges on Caribbean coral reefs is extremely important due to their often overwhelming biomass (HMA ~3.3 L m^{-2}, LMA ~0.3 L m^{-2}; Southwell et al. 2008b) and their large capacity to produce DIN (Corredor et al. 1988, Diaz & Ward 1997, Southwell et al. 2008b). Because seaweeds and other primary producers often have distinct preferences for either NO_3^- or NH_4^+ (Bracken & Stachowicz 2006), the preponderance of NO_3^- in the large quantities of DIN released from HMA sponges on Caribbean coral reefs may have significant implications for seaweed community composition and, consequently, seaweed-coral interactions. Because non-encrusting sponges at Conch Reef are known to

produce relatively low $\delta^{15}N$ NO_3^- (-19.4 to + 1.8‰) compared to the ambient NO_3^- in reef water surrounding the sponges (4.2 ± 0.8‰) (Southwell et al. 2008a), it is possible to track the movement of sponge-produced NO_3^- into surrounding primary producers at this site.

It is likely that upwelling events, which are a common feature on Conch Reef in the summer (Leichter et al. 1996), and anthropogenic sources of nutrients may contribute to macroalgal proliferation. Pulsed NO_3^- due to upwelling events has the ability to increase ambient water NO_3^- by 1.0 – 4.0 µmol L^{-1} (Leichter et al. 2003). Anthropogenic additions of only 1 µmol L^{-1} DIN and 0.1 µmol L^{-1} phosphate has been proposed to initiate and sustain a macroalgal bloom (Lapointe 1997). Thus, Lapointe (1999) attributed the coral-algal phase shift to anthropogenic eutrophication. However, while near-shore coral reef communities may likely experience anthropogenic eutrophication, the idea that this form of eutrophication strongly impacts the outer-reef tract in the Florida Keys has been challenged (Hughes et al. 1999, Szmant & Forrester 1996). Because anthropogenic eutrophication is unlikely on Conch Reef, it will not be addressed in this study. The exceptionally high inputs of DIN from sponges and upwelling events to Florida coral reef waters may contribute to seaweed proliferation as well as changes in seaweed community structure, with negative impacts on overall coral health (Nugues et al. 2004, Foster et al. 2008) and recruitment (Lirman 2001, Kuffner et al. 2006).

On Conch Reef, *Dictyota menstrualis* and *D. pulchella* percent cover has increased substantially over the last fifteen years (Beach et al. 2006), while subsequently *Halimeda tuna* and *H. opuntia* percent cover has declined (Beach et al. 2003). Although epiphytization and the release of allelopathic chemicals by *Dictyota* spp. may contribute to the percent cover decline in *Halimeda* spp. (Beach et al. 2003), this does not explain the cause of the problem:

the rapid rise in *Dictyota* spp. on Conch Reef. Sponges have increased dramatically on Caribbean reefs (Aronson et al. 2002, Lopez-Victoria & Zea 2004, Ward-Paige et al. 2005, Norstrom et al. 2009), and it is possible that consequent increases of sponge-produced DIN may be aiding the proliferation of *Dictyota* spp. and other seaweeds at these sites.

Here we investigate *in situ* the potential positive interaction between sponge-produced DIN and seaweeds on coral reefs by tracing the unusually low $\delta^{15}N$ NO_3^- values from HMA sponge effluent. To address the potential effects of upwelled DIN on Conch Reef we also examined the relative importance of sponge produced NO_3^- versus NO_3^- in sub-thermocline waters that are episodically delivered to Conch Reef by tidal bore transport, particularly during the summer months when water column stratification is strongest. In contrast to the ^{15}N-depleted NO_3^- released by HMA sponges, upwelled NO_3^- has a high $\delta^{15}N$ value, typically ~4 - 4.5‰ (Leichter et al. 2007) and can also be traced. We hypothesized that 1) seaweeds are able to utilize sponge-produced DIN, 2) seaweeds will respond to upwelling events during the summer months by having a higher $\delta^{15}N$ value than the fall, and 3) seaweed species will differentially respond to sponge DIN. Specifically, we hypothesize that the abundance of *Dictyota* spp. may be partially due to its increased ability to effectively utilize DIN due to its high surface area to volume ratio (Littler & Littler 1980, Steneck & Dethier 1994, Fong et al. 2003). This research will provide crucial information regarding the roles of sponges on coral reefs. The results will help inform coral reef managers about sponge-seaweed interactions that potentially act as impediments to the recovery of corals on Caribbean reefs.

MATERIALS AND METHODS

Study Site

This study was conducted in 2007 and 2008 on Conch Reef (24° 57.439 N; 80° 26.829 W), a large barrier reef located on the outer Florida Keys reef tract (Fig. 1). Conch Reef is a no-take, no public entry research sanctuary where the Aquarius Reef Based Observatory is located. Conch Reef is reported to frequently receive nutrient rich sub-thermocline waters from the Florida Straits during episodic tidal bores (Leichter et al. 1996). These upwelling events occur most frequently and with the greatest intensity during the summer months (Leichter et al. 1996, Leichter et al. 2003). In recent years, Conch Reef, like other Florida Keys reefs, has experienced a significant proliferation of seaweeds, specifically *Dictyota* spp., (Chiappone & Sullivan 1997, Walters & Beach 2000, Beach et al. 2003), possibly due in part to increased inputs of nutrients (Lapointe 1997, Leichter et al. 2007).

Study Organisms

Seaweeds used in this study included some of those most abundant on Conch Reef: (1) the erect calcareous red *Amphiroa beauvoisii*, (2) siphonous green *Halimeda tuna*, and (3) the mat-forming, fleshy brown *Dictyota menstrualis* and *Dictyota pulchella*. We focused on three HMA sponge species, *Agelas schmidti*, *Verongula gigantea* and *Xestospongia muta*, and one LMA sponge species, *Niphates digitalis*, which comparatively has relatively few associated microorganisms (Weisz et al. 2007). These HMA species are known to excrete large quantities of DIN (Southwell et al. 2008b), predominantly as NO_3^-, whereas the LMA species releases only NH_4^+. The re-mineralized NO_3^- excreted by all of these HMA sponges is known to have unusually low $\delta^{15}N$ values (Southwell et al. 2008a).

Natural Experiment

We measured $\delta^{15}N$ and $\delta^{13}C$ values, C:N ratios and percent tissue C and N content for *Amphiroa beauvoisii*, *Dictyota menstrualis*, *Dictyota pulchella* and *Halimeda tuna* growing adjacent to the excurrent jets of *Agelas schmidti*, and in the oscular chamber of *Niphates digitalis*, *Vergongula gigantea* and *Xestospongia muta*. Seaweeds adjacent to, but not growing within, *A. schmidti* were selected because *A. schmidti* exhibits "backward" pumping with ambient water drawn into large oscular-like openings which is then expelled through small openings near the base of the sponge. Due to their close proximity to sponge effluent, the selected seaweeds should receive nearly constant exposure to the DIN released by the sponges. We also collected and analyzed corresponding seaweeds of the same species growing ~1 m from the sponges as a control. A total of 91 pairs of seaweeds (Table 1) were analyzed for basic C and N characteristics using mass spectrometry and elemental analysis. Seaweed samples were collected in July 2007 and July, September and October 2008. Collections were made using SCUBA between 15 and 22 m depth near the Aquarius Reef Based Observatory. Individual seaweeds were transported on ice to the shore lab. On shore, the seaweeds were cleaned of epibionts and frozen for later mass spectral and elemental analysis.

Seaweed Transplant Experiments

Seaweed Transplant Experiments

To more rigorously investigate possible links between sponge excreted DIN and seaweed C and N characteristics, *Dictyota menstrualis* and *Halimeda tuna* found growing

away from sponges were transplanted into the large oscular cavity of *Xestospongia muta* for a period of 23 days from September 17, 2008 to October 10, 2008. Seaweeds were collected by SCUBA from 16-18 m depth and transplanted into *X. muta* individuals at 16-20 m depth. Multiple individuals of these two species were collected simultaneously and brought to the surface in plastic bags. These seaweeds were placed in a large cooler of Conch Reef seawater and returned to the shore lab. Twenty large thalli of each species were pulled from the larger collection for use in the transplant experiment.

Ten thalli of each species were designated as "treatment" samples for transplant into the oscular cavity of *Xestospongia muta*. Ten additional thalli were designated as "control" samples for transplant to an area ~1 m away from each of the 10 *X. muta* individuals used in this experiment. Individual seaweeds were split in half; one piece was immediately frozen for later elemental and mass spectral analysis (t = 0) while the other, each approximately 4 grams wet weight, was transplanted into the field. Transplanted seaweeds were individually placed in "mini-cages" constructed of PVC rings (7.6 cm diameter and 2.5 cm height) covered with clear nylon netting (0.25 cm^2 mesh opening) which allowed high water flow while excluding large herbivores. Seaweeds were submerged in their cages in aerated Conch Reef seawater and held overnight prior to deployment. We were unable to compare growth rates of seaweeds in *X. muta* treatments and controls due to a series of severe storm events.

One *Dictyota menstrualis* and one *Halimeda tuna* (in separate mini-cages) were placed side-by-side in the oscular cavity of 10 large *Xestospongia muta* individuals. Two lengths of a malleable plastic coated aluminum wire were inserted at a 90° angle through the body of each treatment sponge near the upper edge of the oscular cavity such that the wires crossed at the center of the osculum. The two mini-cages were attached to these wires using

cable ties and were oriented vertically within the sponge. Ten control cages housing one *D. menstrualis* and one *H. tuna* mini-cage in a similar orientation as the treatment mini-cages were placed 1-2m from each treatment sponge (see fig. 2). Each control cage was tied to a stand that elevated the cages 20 cm off the bottom, placing the control seaweeds at a height above the bottom similar to that of the seaweeds inside the *X. muta* oscula. No mesh covered the top of the sponge or the large control cages to eliminate possible fouling and shading effects.   After 23 days all treatment and control seaweeds were recovered, removed from the mini-cages, cleaned of any epibionts, and frozen for later mass spectral analysis (t= final).

Mass Spectral and Elemental Analysis

Methods for mass spectral and elemental analysis were modeled after Weisz et al. (2007). All seaweed samples were dried in a Savant freeze dryer for 12 hours and then homogenized using a ceramic mortar and pestle and stored in glass scintillation vials. Before further processing, samples were placed in an oven at 80°C for one hour to ensure dryness. The mass of the homogenized samples was next determined using tared, combusted silver boats (5 x 9 mm; Costech Analytical, Valencia, CA, USA) on a Cahn C-30 Microbalance. The samples were then exposed to HCl vapor in a desiccator overnight to remove inorganic carbon. Samples were placed in the drying oven again (80°C for one hour) to remove residual acid. Tissue carbon and nitrogen content and stable isotopic composition were determined using a Carlo Erba NA 1500 elemental analyzer equipped with autosampler interfaced via a Finnigan ConFlo II™ to a Finnigan MAT 252 isotope ratio mass spectrometer. Isotope values were calculated as: $\delta^N X = [(R\text{sample} - R\text{std})/R\text{std} - 1] \times 1000$ in units of parts per thousand (‰). Where $^N X$ is the coefficient of the heavy isotope (i.e. ^{15}N

or ^{13}C), and R is the ratio of the heavy to light isotope in both the standard and sample (i.e. ^{15}N/^{14}N or ^{13}C/^{12}C). The standard for nitrogen is atmospheric N_2 (0.0‰) and the standard for carbon is Pee Dee Belemnite (0.0‰). Samples were compared to a pure acetanilide standard with a known isotopic composition. The standard deviation of isotope measurements of the instrument was equal to or better than 0.3‰ and 0.2‰ for δ^{15}N and δ^{13}C, respectively. The elemental analyzer output also yielded the mass of C and N in the sample, thereby allowing determination of percent tissue C and N content and C:N ratios of the seaweeds.

Statistical Analyses

Paired t-tests were used to identify statistically significant differences in the C and N characteristics (δ^{15}N, δ^{13}C, C:N and tissue C and N content) between seaweeds directly exposed to sponge excurrent flow and paired seaweeds growing ~1 m away from sponges. For comparison with 6 or fewer replicates, the Wilcoxon signed rank test was used. Because *Dictyota menstrualis* and *Dictyota puchella* often grew tangled together and can be difficult to distinguish underwater, these two species were pooled and hereafter referred to as *Dictyota* spp. for the natural experiment. We used Mann-Whitney tests to assess seasonal differences between summer (July) and fall (September and October) within seaweed species. These comparisons included all three seaweeds collected away from sponges, and *Dictyota* spp. found in the oscular chamber of *Niphates digitalis, Verongula gigantea* and *Xestospongia muta*. For comparisons among seaweed species by season we used ANOVAs when all three seaweed species were compared, Student's t-tests when only *Dictyota* spp. and *H. tuna* where compared, and Mann-Whitney tests when the sample size of any variable was small (n ≤ 6). No significant difference was found between seaweeds collected in July 2007 and July 2008,

9

and between those collected in September and October 2008; therefore, the inter-year July data were pooled and the September and October 2008 data were pooled for the seasonal analyses.

For the seaweed transplant experiment, we identified statistically significant differences in the mass spectral data for seaweeds transplanted into *Xestospongia muta* oscula for 23 days. This analysis was conducted by taking the differences between the t_{final} and t_0 data and comparing the between treatment differences of $\delta^{15}N$, $\delta^{13}C$, C:N ratio, tissue C and N content using Student's t-tests. This analysis was used for both seaweed species. All statistical analyses were conducted using the JMP$^{®}$ 7.0.1 software package (SAS Institute, Cary, NC, U.S.A).

RESULTS

Natural Experiment

Among the 12 sponge-seaweed pairings, only *Dictyota* spp. in the oscular chamber of *Verongula gigantea* (Fig. 3c) and *Xestospongia muta* (Fig. 3d) had significantly lower $\delta^{15}N$ values than the control seaweeds collected away from the sponges (~1.4‰ lower; , $t_8 = -5.91$, $p \leq 0.001$ and $t_{12} = -9.72$, $p \leq 0.001$, respectively; Fig. 3) All three seaweed species collected from the oscular chamber of *Niphates digitalis* had lower, but non-significant, $\delta^{15}N$ values of up to 0.3, 0.2 and 0.9‰ for *Amphiroa beauvoisii, Dictyota* spp., and *Halimeda tuna*, respectively (Fig. 3b). However, the p-value for the comparison with *A. beauvoisii* was marginally significant ($t_7 = -2.07$, $p = 0.08$), and in the case of *H. tuna* only two plants were found growing in the oscular chamber of *N. digitalis*. *A. beauvoisii* consistently exposed to the excurrent flow of *V. gigantea* (Fig. 3c) and *X. muta* (Fig. 3d) also had relatively large, but non-significant, declines in $\delta^{15}N$ values (~0.5‰, n = 4 and 2, respectively) compared to control seaweeds. The $\delta^{13}C$ value of *Dictyota* spp. attached inside the oscualr chamber of *Niphates digitalis* was slightly higher than *Dictyota* spp. found away from sponges (p = 0.052; Fig. 4b). None of the other 11 sponge-seaweed pairings showed any significant differences in $\delta^{13}C$ values (Fig. 4), but there was a significant difference among seaweed species (*A. beauvoisii*: ~8‰ heavier than *Dictyota* spp. and *H. tuna*; $F_{2, 178} = 222.74$, $p < 0.001$).

Dictyota spp. C:N ratios were significantly lower by 10, 29 and 17% for *Agelas schmidti* ($t_9 = -3.13$, $p = 0.01$)*, Verongula gigantea* ($t_8 = -2.45$, $p = 0.04$) and *Xestospongia muta* ($t_{12} = -4.61$, $p < 0.001$), respectively, when exposed to the excurrent flow of these HMA

sponges (Fig. 3e,g,h). *Dictyota* spp. trended toward a lower C:N ratio when growing in the oscular chamber of *Niphates digitalis* (Fig 3f). Tissue N values for *Dictyota* spp. were increased significantly when exposed to the excurrent flow of *A. schmidti* (20%; $t_9 = 2.77$, p = 0.02; Fig. 3i) and *V. gigantea* (43%; $t_8 = 4.83$, p = 0.001; Fig. 3k), while showing non-significant increases of 21 and 11% when exposed to *N. digitalis* and *X. muta* excurrent waters, respectively (Fig. 3j,l). *Amphiroa beauvoisii* and *Halimeda tuna* showed no significant changes in their C:N ratios or percent tissue N content between plants in the excurrent flow of sponges and away from sponges. None of the 12 sponge-seaweed pairings showed any significant differences in the tissue carbon values (Fig. 4), but any slight changes in the tissue carbon content between the paired seaweeds could have contributed to changes in C:N ratios.

Seasonal Effects:

Control Seaweeds: Seaweeds away from sponges

  The δ^{15}N value of *Dictyota* spp. growing away from the direct influence of sponge excurrent flow was significantly lower by 0.52‰ in July than in the months of September and October ($Z_{39} = -4.06$, p < 0.001; Fig. 5a). *Halimeda tuna* and *Amphiroa beauvoisii* did not show statistically significant changes in δ^{15}N values between summer and fall (Fig. 5a). There were no seasonal differences in the δ^{13}C values for *Dictyota* spp. or *A. beauvoisii*, but *H. tuna* showed a strong seasonal trend (~1‰ lighter in the fall; $z_{24} = -1.95$, p = 0.051). The C:N ratio for *Dictyota* spp. was significantly higher in summer than fall (21.8 vs. 17.9; $z_{39} = 3.34$, p < 0.001; Fig. 5c), with tissue N content increasing by 22% over the same period ($z_{39} = 1.80$, p = 0.07, Fig. 5e). *H. tuna* showed no significant seasonal difference in C:N ratio or

percent tissue N content when growing away from sponge excurrent flow (Fig. 5c,e). *A. beauvoisii* showed opposite seasonal trends in C:N ratio and percent tissue N content compared to *Dictyota* spp. (Fig. 5c,e), but the low number of July replicates for *A. beauvoisii* (n = 2) make these comparisons less accurate. There were no significant seasonal differences in the tissue C content within each seaweed species ($p \geq 0.34$).

Among *Dictyota* spp., *Amphiroa beauvoisii*, and *Halimeda tuna* growing away from sponges, large statistically significant differences in C:N ratios and percent tissue N values were observed in both summer and fall (Fig. 5c,e). C:N ratios of *Dictyota* spp. and *H. tuna* were ~58 and ~78% lower, respectively, than the C:N ratio of *A. beauvoisii* in the fall ($F_{2,67} = 81.85$, $p < 0.001$). With only 2 summer samples for *A. beauvoisii*, a similar rigorous comparison could not be made for summer. *H. tuna* had a significantly lower C:N ratio in both the summer (53%; $z_{17} = -3.19$, $p = 0.001$) and fall (48%; $t_{46} = -14.21$, $p < 0.001$) than *Dictyota* spp. *Dictyota* spp. and *H. tuna* had similar levels of percent tissue N content (1.02 and 1.06%, respectively) in the fall, while *A. beauvoisii* had a significantly lower percent tissue N content (0.31%; $F_{2,67} = 36.06$, $p < 0.001$). There was no significant difference in the tissue N content between *Dictyota* spp. and *H. tuna* in summer or fall. The significant difference in the C:N ratio is due to *H. tuna* having a significantly lower tissue C content than *Dictyota* spp. (summer 30%: $z_{17} = -2.08$, $p = 0.04$; fall 43%: $t_{46} = -6.27$, $p < 0.001$), which is expected because *H. tuna* is a calcareous species and *Dictyota* spp. is not. During the summer, *Dictyota* spp. had a significantly lower $\delta^{15}N$ value than *H. tuna* (0.73‰ lower; $Z_{17} = -2.54$, $p = 0.01$; Fig. 5a), but not in the fall.

Treatment seaweeds: Dictyota spp. i*n Sponge Excurrent Flow*

The mean $\delta^{15}N$ value of *Dictyota* spp. inhabiting the oscular cavity of *Niphates digitalis* was a significant 1.0‰ higher ($z_7 = 2.37$, $p = 0.02$) in the fall than in summer (Fig. 5b). This seasonal pattern mirrored that of *Dictyota* spp. growing away from sponges (Fig. 5a). In contrast, the $\delta^{15}N$ values of *Dictyota* spp. found in the oscular chambers of *Verongula gigantea* and *Xestospongia muta* did not change seasonally (Fig. 5b). The $\delta^{13}C$ value of *Dictyota* spp. found in *X. muta* oscula did change seasonally though (~2‰ lighter in the fall; $z_{11} = -2.56$, $p = 0.01$). *Dictyota* spp. growing inside the oscular chamber of *X. muta* always had a lower $\delta^{15}N$ value than *Halimeda tuna* growing in *X. muta* oscula (summer difference 1.67‰; $z_{11} = -2.86$, $p = 0.004$; fall difference 1.50‰; $z_{13} = -3.00$, $p < 0.001$).

C:N ratios for *Dictyota* spp. inside the oscular cavities of *Niphates digitalis*, *Verongula gigantea* and *Xestospongia muta* were 9, 24, and 21% lower, respectively, in fall than in summer, but the decline was only statistically significant for *Dictyota* spp. in the osculum of *X. muta* ($z_{11} = -2.41$, $p = 0.02$; Fig. 5d). These decreases in C:N ratios moving from summer to fall were accompanied by 38, 3, and 30% increases in the percent tissue N content for *Dictyota* spp. growing in the oscular cavities of *N. digitalis*, *V. gigantea* and *X. muta*, respectively. The increase for *Dictyota* spp. attached inside *X. muta* oscula was significant ($z_{11} = 1.98$, $p = 0.05$; Fig. 5f) and showed a strong trend in *N. digitalis* ($z_6 = 1.88$, $p = 0.06$; Fig. 5f). There were no seasonal differences in the tissue C content for any of the seaweed species. Low replication ($n = 2$) for *Dictyota* spp. collected from *V. gigantea* in July prevented a rigorous statistical evaluation of the season change associated with this sponge.

Seaweed Transplant Experiment:

14

After the 23-d experimental period, we recovered 8 pairs of *Dictyota menstrualis* and 8 pairs of *Halimeda tuna* deployed in the oscula of *Xestospongia muta* individuals and adjacent reef substrate. Since all test seaweeds were taken from a single pooled collection for each species, the t_0 data points for each N and C comparison within each seaweed species were the same. The δ^{15}N value of *D. menstrualis* from the sponge treatment was a significant 1.0‰ lower than *D. menstrualis* transplanted to the adjacent substrate (t_{14} = -9.31, $p < 0.0001$, Fig. 6a). The C:N ratio of *D. menstrualis* in the oscular chamber of *X. muta* dropped 30% (t_{14} = -2.18, $p = 0.05$, Fig. 6c), while the percent tissue N content increased 23%, which was not identified as a significant increase ($t_{14} = 1.23$, $p = 0.23$, Fig. 6e). *H. tuna* directly exposed to *X. muta* effluent had a δ^{15}N value a significant 0.53‰ lower than that of the paired seaweeds transplanted to the adjacent substrate ($t_{14} = 2.30$, $p = 0.04$; Fig. 6b). The C:N ratio and percent tissue N content of *H. tuna* did not change (Fig. 6d,f). *D. menstrualis* and *H. tuna* had identical δ^{15}N values at t_0, and by t_{final} the δ^{15}N value of the control *D. menstrualis* and *H. tuna* had not changed significantly. *D. menstrualis* transplanted in the oscular chamber of *X. muta* had a much lower δ^{15}N value than *H. tuna* (0.42 vs. 0.87‰: t_{14} = -2.61, $p = 0.02$). Neither the δ^{13}C value, nor the tissue C content significantly changed between the treatments and controls for either seaweed.

DISCUSSION

Implications for Sponge DIN on Conch Reef

Seaweeds that utilize sponge DIN

Previous research has demonstrated that HMA sponges excrete large quantities of ^{15}N-depleted NO_3^- (Southwell et al. 2008a). Southwell and colleagues proposed that sponge-produced NO_3^- on coral reefs may facilitate seaweed proliferation. Here we address this hypothesis and confirm that seaweeds on Conch Reef are in fact utilizing NO_3^- excreted from HMA sponges. Further, we suggest that the primary source of DIN taken up by primary producers on Conch Reef is autochthonous in origin, emerging from the effluent of a thriving sponge population rather than DIN advected onto the reef from outside sources, such as upwelling or anthropogenic sources. Finally, we also demonstrated that *Dictyota* spp. are the most efficient seaweeds at utilizing sponge effluent on Conch Reef.

Using the ^{15}N-depleted NO_3^- values from sponge effluent as a natural tracer, we found a strong positive interaction between *Dictyota* spp. and HMA sponge-produced NO_3^- on Conch Reef. The lower δ^{15}N values, C:N ratios, and higher tissue N values for *Dictyota* spp. found inside *Xestospongia muta* and *Verongula gigantea*, compared to control seaweeds (Fig. 3,6), suggests that *Dictyota* spp. are capable of utilizing the ^{15}N-depleted NO_3^- produced by these sponge species. *Dictyota* spp. immediately adjacent to the excurrent jets of the "backward" pumping sponge *Agelas schmidti* did not have significantly different δ^{15}N values compared to control seaweeds. They did however, have significantly lower C:N ratios and significantly higher tissue N values than seaweeds away from this sponge species. Because the NH_4^+ and NO_3^- concentration and the δ^{15}N value of NO_3^- in *A. schmidti* effluent have not been measured, it is possible that this sponge's effluent has a δ^{15}N value

16

substantially greater than those reported for other HMA sponges. *Dictyota* spp. near the excurrent jets of this sponge could therefore have a higher $\delta^{15}N$ value than *Dictyota* spp. inside the oscular chamber of the other HMA sponges. Alternatively, the seawater surrounding seaweeds immediately adjacent to *A. schmidti* is likely mixing with ambient water. Thus, seaweeds next to and away from *A. schmidti* would therefore most likely have similar isotopic values.

The C:N ratio and tissue N data suggest that even though *Dictyota* spp. near *A. schmidti* do not have lower $\delta^{15}N$ values, there is an increased concentration of DIN in the water immediately adjacent to the sponge. *Halimeda tuna* appeared not to utilize sponge produced NO_3^- as efficiently as *Dictyota* spp. (Figs. 3,6), and the sample size of *Amphiroa beauvoisii* was too small to rigorously determine the degree to which this seaweed might be utilizing NO_3^- from HMA sponges. These data suggest a strong positive interaction between *Dictyota* spp. and HMA sponges.

Southwell et al. (2008a) found that NO_3^- in the excurrent plume of *Xestospongia muta* (sponge produced NO_3^- plus ambient NO_3^-) had a $\delta^{15}N$ value of ~2.5‰. While this $\delta^{15}N$ value is ~2‰ lower than that of the ambient NO_3^- away from sponges, this sponge's excurrent plume has a higher $\delta^{15}N$ value than the *Dictyota* spp. attached inside the oscula of this sponge species. Because there is a much higher concentration of NO_3^- inside the sponge oscula than in the ambient water (~1.8 μmol L^{-1} vs. ~0.84 μmol L^{-1} (Southwell et al. 2008a)), *Dictyota* spp. maximally exposed to *X. muta* effluent are likely preferentially selecting the isotopically lighter NO_3^- (i.e. $^{14}NO_3^-$), as the concentration of NO_3^- increases well beyond the physiological demands of the seaweed and the relative abundance of $^{15}NO_3^-$ declines. Many primary producers are known to discriminate against ^{15}N-enriched DIN during uptake and

assimilation processes (Fourqurean et al. 2005, Bracken & Stachowicz 2007, Umezawa et al. 2007). *Dictyota* spp. may be especially efficient at capitalizing on the increased DIN pool in HMA sponge effluent and the higher ambient concentrations produced in reef waters by the sponges. Average NH_4^+ and NO_3^- concentrations from DIN measurements in near bottom waters (<0.5 m above the reef) at Conch Reef were 0.43 μmol L^{-1} and 0.48 μmol L^{-1}, respectively (Lindquist unpublished data). While levels of NH_4^+ at more inshore sites were often higher than at our site, the sponge-rich regions of Conch Reef had the highest mean NO_3^- concentrations, even higher than deep offshore sites during low upwelling activity (Lindquist unpublished data). Sponges are increasing on Caribbean reefs (Aronson et al. 2002, Lopez-Victoria & Zea 2004, Ward-Paige et al. 2005, Norstrom et al. 2009), and consequently the flux of sponge remineralized DIN to the reefs will increase as well.

Sponges as an important nutrient source on Conch Reef

The average $\delta^{15}N$ value of *Dictyota* spp., *Halimeda tuna* and *Amphiroa beauvoisii* away from sponges was a low 1.49‰, 1.56‰, and 1.27‰, respectively (Table 2). Because the seaweed species collected on Conch Reef displayed a low $\delta^{15}N$ value, it is likely that sponges play an important, and possibly at times dominant, role in the nutrient dynamics for primary producers on Conch Reef. Conch Reef lies between two areas with ^{15}N-enriched DIN in the water column: NO_3^- (4-5‰) from sub-thermocline water that is seasonally upwelled onto Conch Reef (Leichter et al. 2003) and anthropogenic DIN mostly in the form of NH_4^+ (> 4‰) (Lapointe 1997). An increased $\delta^{15}N$ value in response to increased exposure to anthropogenic DIN is often seen in near-shore seaweeds growing where sewage output is high (Umezawa et al. 2002, Lapointe et al. 2004, Todd 2008). Given that none of the

seaweeds tested in this study had high $\delta^{15}N$ values suggest that these seaweeds were probably not receiving substantial outputs of DIN from either of these sources during our study period. Also, because upwelled NO_3^- is not a consistent source of nutrients year-round, particularly at the shallower depths involved in this study (<22 m), it probably is not the most important source of DIN for Conch Reef primary producers. Further, Szmant & Forrester (1996) examined the inshore to offshore gradient in water column and sediment nitrogen, phosphate, and chlorophyll a in the Florida Keys. They found that water column concentrations of N and chlorophyll a in the upper keys were much higher inshore than offshore and that phosphorus was often higher offshore. They suggested that no anthropogenic nutrients are transported to offshore reefs in the upper Florida Keys and that nutrients in the outer-reef tract probably come from autochthonous sources. This study suggests that sponges may be the main nitrogen source for Conch Reef primary producers.

Reported $\delta^{15}N$ values for *Dictyota* spp. vary dramatically (Table 2). For example, the $\delta^{15}N$ values of *Dictyota barayresiana* fluctuated dramatically even on small spatial scales on a Puerto Rican reef (Todd 2008). Values ranged from ~0‰ on a deep reef drop off to ~8‰ near the Puerto Rican mainland, with seaweeds on adjacent patch reef systems varying by 1‰. The highly variable $\delta^{15}N$ values of *Dictyota* spp. suggests that these seaweeds may be readily able to utilize whatever form of nitrogen is most available and may contribute to their predominance on many coral reefs.

Here we demonstrate that *Dictyota* spp. are able to utilize sponge NO_3^- and that sponges are an important source of NO_3^- on Conch Reef. However, the low $\delta^{15}N$ values of Conch Reef seaweeds may be due to other factors in addition to responses to sponge effluent. For example, N-fixation, the process of converting inert atmospheric di-nitrogen into NH_4^+ or

NO_3^-, may also explain why these seaweeds have atypically low $\delta^{15}N$ values at our study site. However, ambient seawater at our site was not truly oligiotrophic and N-fixation is unlikely to be a strong contributor. At the Water Quality Monitoring Network at the Florida International University Southeastern Research Center (SERC-FIU WQMN) Conch Reef site (~1.3 mi inshore and shallower from our site) the average benthic DIN:SRP ratio of POM in the water column from 1995-2007 was 32.81 ± 4.69 SE (SERC-FIU WQMN). Nitrogen fixation is only energetically favorable when the DIN:SRP ratio is less than Redfield (N:P = 16). Additionally, an ambient DIN concentration of ~0.9 μmol L^{-1} in the benthos (Lindquist unpublished data) on our Conch Reef site is just under what Lapointe (1997) suggests would cause a macroalgal bloom. This implies that there is a sufficient concentration of DIN on Conch Reef and N-fixation would therefore be an unfavorable process. Even if N-fixation were energetically favorable on Conch Reef there is no evidence to suggest that *Dictyota* spp. host diazotrophs.

Several studies suggest that seaweeds on coral reefs have low $\delta^{15}N$ values because of N-fixation (e.g. France et al. 1998, Todd 2008), but none of these studies consider sponge effluent as a possible explanation for seaweeds having a low $\delta^{15}N$ value. HMA sponge effluent has been overlooked as a possible source of low $\delta^{15}N$ NO_3^- on coral reefs and consequently many studies that suggest primary producers have low $\delta^{15}N$ values because of N-fixation may be unfounded.

Seasonal Trends in Seaweed N and C Tissue Chemistry

The results from the seasonal analysis revealed that *Dictyota* spp. found away from sponges and inside the oscular chamber of *Niphates digitalis* displayed a higher $\delta^{15}N$ value in

the fall than in the summer (Fig. 5). Neither *Halimeda tuna* nor *Amphiroa beauvoisii* showed any significant seasonal trends. *A. beauvoisii* showed strong trends (p = 0.07) toward a higher C:N ratio and lower tissue N content in the fall, but the very small number of replicates for July (n = 2) made the results of the seasonal comparison for this species less reliable. Because *Dictyota* spp. appear to be capitalizing extensively on available NO_3^- and *N. digitalis* does not produce NO_3^- (Southwell et al. 2008b), it is reasonable to propose that those *Dictyota* spp. seaweeds growing in the oscular chamber of *N. digitalis* simply picked up available ambient NO_3^- being pumped through the sponge. These seaweeds showed a seasonal pattern similar to seaweeds growing away from sponges.

The $\delta^{15}N$ values of *Dictyota* spp. growing in the oscula of the HMA sponges *Verongula gigantea* and *Xestospongia muta* did not shift seasonally probably because low $\delta^{15}N$ sponge NO_3^- dominated the DIN pool in both summer and fall. Because the $\delta^{15}N$ values of the *Dictyota* spp. did not change seasonally inside the HMA sponges in 2008, it implies that sponge-produced DIN was a more important DIN source than seasonally variable sub-thermocline NO_3^- on our reef site.

The results for *Dictyota* spp. away from sponges are interesting because *Dictyota* spp. at ~20 m on Conch Reef displayed a seasonal trend in $\delta^{15}N$ values opposite of what one would predict based on the results from Leichter et al. (2003). Leicther et al. (2003) reported that the green alga *Codium isthmocladum*, which they found from a depth of 9-34 m along the Florida Keys reef tract, had whole tissue $\delta^{15}N$ values between 2.5 and 5.5‰. The highest values were at the deeper sites more frequently exposed to upwelled sub-thermocline waters which have high concentrations of relatively high $\delta^{15}N$ NO_3^-. They further report that at the 33 m sampling station at Conch Reef the $\delta^{15}N$ value of *C. isthmocladum* was significantly

21

higher (by ~2‰) during the summer upwelling season than at other times of the year. The fact that the $\delta^{15}N$ values of *Dictyota* spp. at 20 m on Conch Reef showed a pattern of seasonal change opposite that of *C. isthmocladum* at 33 m further indicates that upwelled NO_3^- was not the primary source of DIN for *Dictyota* spp. on our Conch Reef site.

We have four possible hypotheses to explain why seasonal changes in the $\delta^{15}N$ values of the *Dictyota* spp. were opposite those of *C. isthmocladum*: (1) the sponge physiology may be changing seasonally, (2) there was a greater abundance of sponges at the shallow Conch Reef site (<22 m) compared to the deep 33 m site on Conch Reef used by Leichter et al. (2003) for their seasonal comparison, (3) different seasonal rates of denitrification in reef sediments and/or dead coral heads may affect the $\delta^{15}N$ value in the ambient water near the benthos and, (4) physiological processes in the seaweed may be controlling the response.

HMA sponges on Conch Reef may be changing from largely NO_3^- to NH_4^+ excretion moving from summer to fall as Bayer et al. (2008) described for the Mediterranean sponge *Aplysina aerophoba*. If this is a general characteristic of HMA sponges, then HMA sponges on Conch Reef may seasonally alter nitrification rates with presently unknown effects on the $\delta^{15}N$ value of the excreted DIN. Although, because some HMA sponges, including *Xestospongia muta*, nitrify virtually all the NH_4^+ before it exits the sponge, no net change in the $\delta^{15}N$ of the DIN pool in the sponge excurrent plume would be expected to occur. N metabolism and N fractionation within HMA sponges is not well understood (Hentschel et al. 2006), thus it is unrealistic to make a definitive statement about how DIN $\delta^{15}N$ values shift as metabolism changes. Further, if sponge feeding rates and metabolism slow substantially moving from the summer to fall months, this change could possibly reduce the overall size of the DIN pool in the near-bottom waters, and if nitrification rates also drop, increase the

cumulative $\delta^{15}N$ value of the DIN pool. Reiswig (1971) discovered that 3 Caribbean sponge species reduce their pumping rates at cooler winter temperatures and in more turbid waters following large storm events. The author found that the HMA sponge *Verongula gigantea* decreased its pumping velocity by 35% from summer to winter. If pumping rate is coupled with the rate of organic matter (OM) uptake and the rate at which OM is remineralized and nitrified, then reduced sponge pumping rates moving from summer to fall or after periods of high seas could affect the concentration of sponge-produced DIN in the ambient water.

Secondly, differences in the sponge biomass at our Conch Reef site (<22 m) versus the 33 m site that Leichter et al. (2003) worked may explain the seasonal disparity in $\delta^{15}N$ values between *Dictyota* spp. and *Codium isthmocladum*. Leichter and colleagues' 33 m site, where *C. isthmocladum* samples were collected for a seasonal analysis, lies off the outer edge of Conch Reef and structurally consisted of mostly coral rubble and sand. Few sponges, if any, are within 50-100 m of the site (pers. comm. J. Leichter). It is possible that the high concentration of sponge derived DIN at our shallower site over-powered the upwelled NO_3^- that was able to reach shallower water. Leichter et al. (2003) measured twice as many "degree cooling hours" (hours at site <25°C), which represents the intrusion of cool sub-thermocline water, on average at the 33 m site than our 22 m site. Because there were very few sponges at the 33 m site, the upwelled ^{15}N-enriched NO_3^- was probably the most readily available DIN source to *C. isthmocladum*.

Denitrification can play a role in the isotopic signature of ambient NO_3^- near the benthos. In sediments, the process of denitrification converts pore water NO_3^- into N_2 gas, which is inert. Nitrate having the lighter isotope (i.e. ^{14}N) is thermodynamically preferred in this microbial mediated reaction, thereby leaving the heavier isotope (i.e. ^{15}N) in the residual

23

NO$_3^-$ pool, which may then diffuse back into the water column. This process, while most common in eutrophied systems, also occurs in shallow sediments (Corredor & Capone 1985) and dead coral heads (Seitzinger & D'Elia 1985) in coral reef environments. Alongi et al. (2008) discovered a seasonal and spatial trend in denitrification rates in sediments in the Great Barrier Reef. While Alongi et al. (2008) found that denitrification rates varied significantly by site in the Great Barrier Reef, it seemed as though denitrification rates were consistently the lowest during July (Austral winter). Fractionation of the available NO$_3^-$ pool near shallow sediments or in dead coral heads (which are numerous around Conch Reef, Silbiger pers. obs.) may respond to fluctuating denitrification rates and therefore ^{15}N-enriched NO$_3^-$ may diffuse out of these sources and become available to benthic primary producers (Alongi et al. 2008).

Finally, it is important to consider physiological processes of primary producers when interpreting stable isotope data. Fourqurean et al. (2005) suggested that when the DIN pool is relatively large, primary producers can discriminate against ^{15}N-enriched DIN. As the DIN pool decreases, primary producers become less discriminatory and, consequently, take up proportionally more DIN with the ^{15}N isotope and thereby have a higher δ^{15}N value. Because a change in the concentration of the DIN pool seemed to affect the δ^{15}N value of *Dictyota* spp. in and away from sponges, a possible interpretation for this seasonal shift may be a response to an increased DIN pool resulting from seasonal upwelling (Leichter et al. 2003). Increased growth rates can also alter the N isotopic composition of primary producers. The δ^{15}N values typically increase with increasing growth rate due to high N needs and less discrimination against ^{15}NH$_4^+$ and ^{15}NO$_3^-$ (Fourqurean et al. 2005). If changing growth rate were the cause of the seasonal shift in the N isotopic composition of

Dictyota spp., it would suggest that *Dictyota* spp. are growing faster in the fall than the summer. This would be contrary to the findings of Lirman & Biber (2000) and to the lower C:N ratio and high tissue N content for *Dictyota* spp. in the fall (Fig. 5c,e). During periods of increased growth rate in primary producers, tissue N content decreases because stored internal N pools are mobilized to meet growth demands as photosynthetic activity ramps up. During periods of slower growth, N uptake internal availability can surpass that need to meet growth potential and consequently, the plant tissue will have a higher percent of total N. Testing these hypotheses would be interesting to enhance our understanding of the seasonal dynamics of Conch Reef primary producers.

Implications for Sponges versus Other Biotic Controls of Seaweeds

    Dictyota seaweeds have been spreading and increasing in abundance throughout the Caribbean since the mid-1980's (Carpenter 1985, Levitan 1988, Morrison 1988, Shulman & Robertson 1996, McClanahan & Muthiga 1998). One potential driver of algal abundance is the intensity of herbivory (e.g., Lewis & Wainwright 1985, Burkepile & Hay 2008). For example, *Dictyota* spp. responded dramatically to the mass mortality of *Diadema antillarum* in 1983 by immediately increasing in biomass by almost 3000% on some Caribbean reefs (Levitan 1988). However, *Dictyota* spp. and other genera such as *Halimeda* produce noxious secondary metabolites and are well defended against other many herbivores, such as fishes (Hay 1981, Norris & Fenical 1982, Paul & Hay 1986). Lirman and Biber (2000) compared herbivorous fish abundance to macroalgae percent cover on multiple Florida reefs and found no relationship between herbivorous fish abundance and percent cover of *Dictyota* spp., suggesting that herbivorous fish exert little control over *Dictyota* spp. populations. In

contrast to fish, the urchin *D. antillarum* will eat many seaweeds that are chemically unpalatable to fish, including *Dictyota* spp. (Carpenter 1985, Levitan 1988, Morrison 1988, Shulman & Robertson 1996, McClanahan & Muthiga 1998). Thus it was the die-off of *D. antillatum* that effectively removed a substantial portion of the top-down control over many species of coral reef seaweeds, including *Dictyota* spp., that were historically rare on Caribbean coral reefs (Steneck 1983, Hay 1984, Carpenter 1985, Littler et al. 1987, Shulman & Robertson 1996).

Beach et al. (2003) reported that *Dictyota* spp. percent cover has been increasing on Conch Reef over the last 15 years while over the same period of time *Halimeda tuna* percent cover has decreased. Beach and colleagues attributed the decline of *H. tuna* to epiphitization and allelopathy by *Dictyota* spp., but did not offer an explanation as to why *Dictyota* spp. were still blooming on Conch Reef 20 years after the *Diadema antillarum* die-off. Given that our study showed that *Dictyota* spp. utilize and benefit from sponge NO_3^- additions to reef waters, potentially more so than other seaweed species, and that other studies report sponge biomass is increasing on Caribbean reefs (Aronson et al. 2002, Lopez-Victoria & Zea 2004, Ward-Paige et al. 2005, Norstrom et al. 2009), the large sponge population on Conch Reef (Southwell et al. 2008b) is likely facilitating bottom up stimulation of seaweed growth, specifically *Dictyota* spp. Results of a herbivore-exclusion/nutrient addition experiment by Thacker et al. (2001) support the general premise that *Dictyota* seaweeds are particularly opportunistic when nutrient levels are increased. They found that removal of herbivores had the strongest effect on seaweed and cyanobacteria cover, but only *Dictyota* spp. showed a significant positive growth from the nutrient additions.

Changes in the composition and abundance of chemically defended seaweeds on Caribbean coral reefs may broaden the range of potential negative interactions on coral reefs and pose challenges for reef restoration and conservation. For example, increased seaweed abundance can stress corals by various mechanisms, including (1) shading, especially that of newly recruited corals (Box & Mumby 2007); (2) physical abrasion (River & Edmunds 2001, Box & Mumby 2007, Titlyanov et al. 2007); (3) reducing fecundity (Foster et al. 2008); (4) allelopathic interactions (Kuffner et al. 2006); and (5) the release of excess photosynthate that is hypothesized to stimulate the excessive growth of coral epizootics (Nugues et al. 2004) and alter microbial communities in surface mucus layers (Smith et al. 2006).

The absence of *Diadema antillarum* and the injections of substantial quantities of remineralized DIN from the thriving sponge population on Conch Reef likely interact strongly to sustain and enhance macroalgal dominance on Conch Reef, particularly *Dictyota* spp. Because many reefs throughout the Caribbean have followed a similar trajectory of decline and phase shift from coral dominance to seaweed-sponge dominance, coral reef management needs to consider the effects of copious on-site production and release of DIN from sponge communities. These DIN-mediated sponge-seaweed interactions on degraded coral reefs potentially act as a serious impediment to the recovery of reefs throughout the Caribbean basin.

Table 1. Number of seaweed samples collected per sponge species.

Seaweed Species	Sponge Species	No. of samples collected
Amphiroa beauvoisii	*Agelas schmidti*	10
	Niphates digitalis	8
	Verongula gigantea	4
	Xestospongia muta	2
Dictyota spp.	*A. schmidti*	10
	N. digitalis	9
	V. gigantea	9
	X. muta	13
Halimeda tuna	*A. schmidti*	6
	N. digitalis	2
	V. gigantea	3
	X. muta	15

Table 2: δ^{15}N values for *Dictyota* spp. and *Halimeda* spp.

Seaweeds	δ^{15}N value	Location	Region	Citation
Dictyota bartayresiana	0 ± 0.08	Puerto Rico mid-shelf	Caribbean	Todd 2008
D. bartayresiana	3.48 ± 0.09	Puerto Rico inshore	Caribbean	Todd 2008
D. cervicornus	~2	Looe Key Back Reef	Florida Keys	Lapointe et al. 2004
D. menstrualis	~5.5	Looe Key Fore Reef	Oceanside	Lapointe et al. 2004
D. pulchella	~6	Looe Key Back Reef	Florida Keys	Lapointe et al. 2004
D. pulchella	~1	Looe Key Fore Reef	Oceanside	Lapointe et al. 2004
Dictyota sp.	1.9 ± 0.11	Belize	Caribbean	Abed-Navandi & Dworshak 2005
Dictyota sp.	~1.6	Curaco reef	Caribbean	de la Moriniere et al. 2003
Dictyota sp.	3.6 ± 1.7	Corsica	Mediterranean	Lepoint et al. 2000
Dictyota sp.	2.1 ± 0.1	Todoroki river reef	Indo-Pacific	Umezawa et al. 2002
Dictyota sp.	4.7 ± 0.1	Maezato reef	Indo-Pacific	Umezawa et al. 2002
Dictyota sp.	3.9 ± 0.5	Shiraho reef	Indo-Pacific	Umezawa et al. 2002
Dictyota sp.	2.8 ± 0.4	Nagura Bay	Indo-Pacific	Umezawa et al. 2002
Dictyota sp.	3.1 ± 0.7	Kabira Reef	Indo-Pacific	Umezawa et al. 2002
Dictyota sp.	1.9 ± 0.3	Hirano Reef	Indo-Pacific	Umezawa et al. 2002
Dictyota sp.	2.4 ± 0.4	Kuura Bay	Indo-Pacific	Umezawa et al. 2002
Dictyota spp	1.49 ± 0.06	Conch Reef	Oceanside / Florida Keys	This study
Halimeda goreaui	~5	Looe Key Fore Reef	Oceanside	Lapointe et al. 2004
H. incrassata	~4	Bird Island	Florida Bay	Lapointe et al. 2004
H. monile	~6	Bird Island	Florida Bay	Lapointe et al. 2004
H. opuntia	~4	Looe Key Fore Reef	Florida Keys	Lapointe et al. 2004

Table 2: Con't

Seaweeds	$\delta^{15}N$ value	Location	Region	Citation
		Reef	Oceanside	Lapointe et al. 2004
		Looe Key Back	Florida Keys	
H. opuntia	~6.2	Reef	Oceanside	Lapointe et al. 2004
H. opuntia	~6	Bird Island	Florida Bay	Lapointe et al. 2004
H. opuntia	~8	Content Keys	Florida Bay	Lapointe et al. 2004
H. tuna	1.3 ± 0.3	Corsica	Mediterranean	Lepointe et al. 2000
H. tuna	5.0 ± 0.7	Stagone di Marsala	Mediterranean	Vizzini et al. 2002
			Florida Keys	
H. tuna	1.56 ± 0.11	Conch Reef	Oceanside	This study
Halimeda sp.	~1.6	Curaco reef	Caribbean	de la Moriniere et al. 2003
Halimeda sp.	~0.5	Curaco bay	Caribbean	de la Moriniere et al. 2003
Halimeda sp.	2	Palau	Indo-Pacific	Yamamuro et al. 1995

Figure 1: Map of field site. Conch Reef, represented by the star, is ~4 miles east of Tavernier Key, Florida.

Figure 2: Seaweed Transplant Experiment Set-up a) Treatment Sponge: *Xestospongia muta* individual with two mini-cages. b) Control Cage: Large cages (0.3 x 0.3 x 0.3 m, width, length, height, respectively), covered with wide-mesh Vexar (1.27 cm^2) to exclude large herbivores from accessing the smaller mini cages except through the top. Mini-cages contained either a *Dictyota menstrualis* or *Halimeda tuna* individual.

Figure 3: Natural Experiment: δ^{15}N, C/N ratio, and total nitrogen (%) values for *Amphiroa beauvoisii, Dictyota* spp. and *Halimeda tuna* found immediately adjacent to small excurrent jets of the HMA sponge *Agelas schmidti* and inside the oscular chamber of the HMA sponges *Verongula gigantea* and *Xestospongia muta* and the LMA sponge *Niphates digitalis* (black bars). Gray bars show mean values for the seaweeds collected approximately 1 m away from each sponge. Numbers inside the bars of panels a, b, c, and d indicated sample sizes for all panels within columns 1-4, respectively. Each column groups data for one sponge species and the rows group δ^{15}N (‰), C/N ratio, and total nitrogen (%) values. Values are means ± 1SE. P-values are from paired t-tests or a Wilcoxin signed rank tests for comparisons with less than six replicates.

Figure 4: Natural Experiment: δ^{13}C and total organic carbon (%) values for *Amphiroa beauvoisii, Dictyota* spp. and *Halimeda tuna* found immediately adjacent to small excurrent jets of the HMA sponge *Agelas schmidti* and inside the oscular chamber of the HMA sponges *Verongula gigantea* and *Xestospongia muta* and the LMA sponge *Niphates digitalis* (black bars). Gray bars show mean values for the seaweeds collected approximately 1 m away from each sponge. Numbers inside the bars of panels a, b, c, and d indicate sample sizes for all panels within columns 1-4, respectively. Each column groups data for one sponge species and the rows group δ^{13}C (‰) and total organic carbon (%) values. Values are means ± 1SE. P-values are from paired t-tests or a Wilcoxin signed rank tests for comparisons with less than six replicates.

Figure 5: Seasonal Effects: black bars are for Summer (July 2007 and 2008) and gray bars are for Fall (September and October 2008). Panels a, c, and e are the δ^{15}N (‰), C/N ratio, and total nitrogen (%) values for *Dictyota* spp.*, Amphiroa beauvoisii* and *Halimeda tuna* found approximately 1 m away from sponges. Panels b, d and f are δ^{15}N (‰), C/N ratio, and total nitrogen (%) values, respectively, for *Dictyota* spp. found inside the oscular chamber of the HMA sponges *Verongula gigantea* and *Xestospongia muta* and the LMA sponge *Niphates digitalis*. Values are means ± 1SE. P-values are from Mann-Whitney tests.

Figure 6: Seaweed Transplant Experiment: δ^{15}N (‰), C/N ratio and total nitrogen (%) values for *Dictyota mensturalis* (left column) and *Halimeda tuna* (right column) placed inside the oscular chamber of *Xestospongia muta* and 1 m away from each sponge (control). Black and gray bars show data for t = 0 and t = 23 d (final), respectively. Values are means ± 1SE.

Numbers inside the bars in panels a and b indicate sample sizes for all panels within rows 1-2, respectively. P-values are from taking the t_{final} - t_0 datum for replicate seaweeds in the *X. muta* oscula versus next to the sponge. The within treatment differences between t_{final} and t_0 were compared using Student's t-tests.
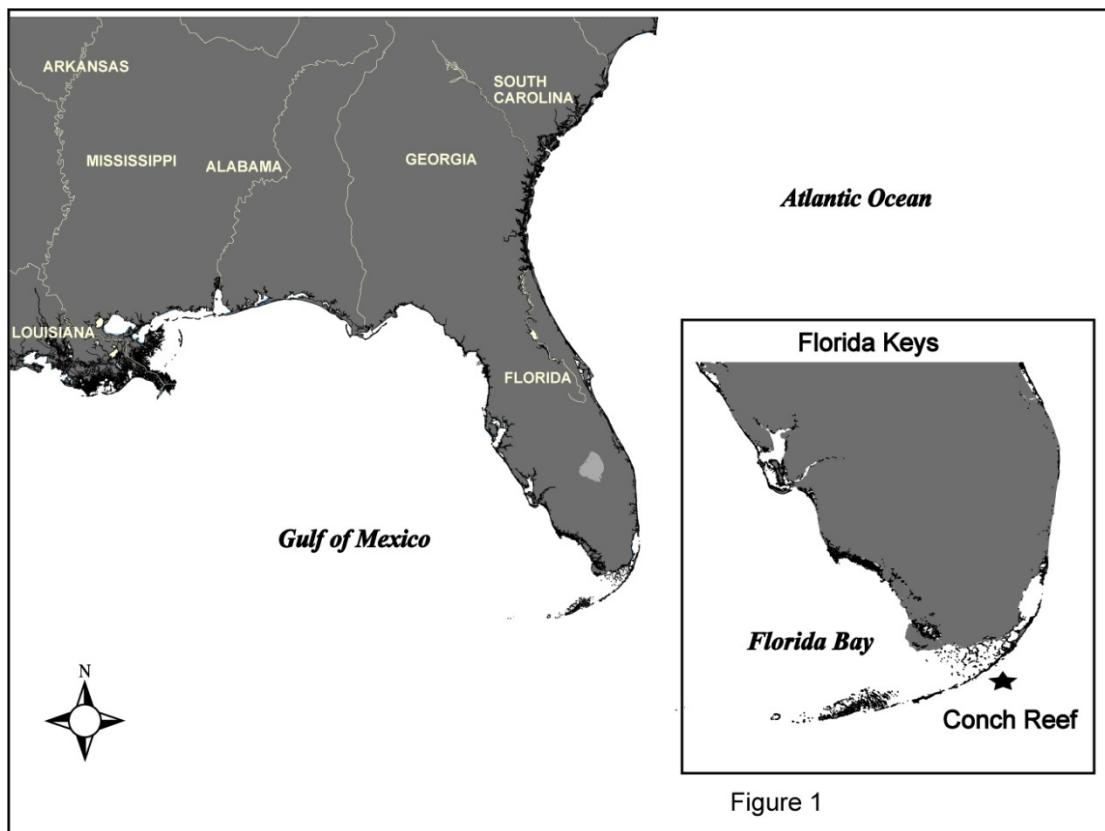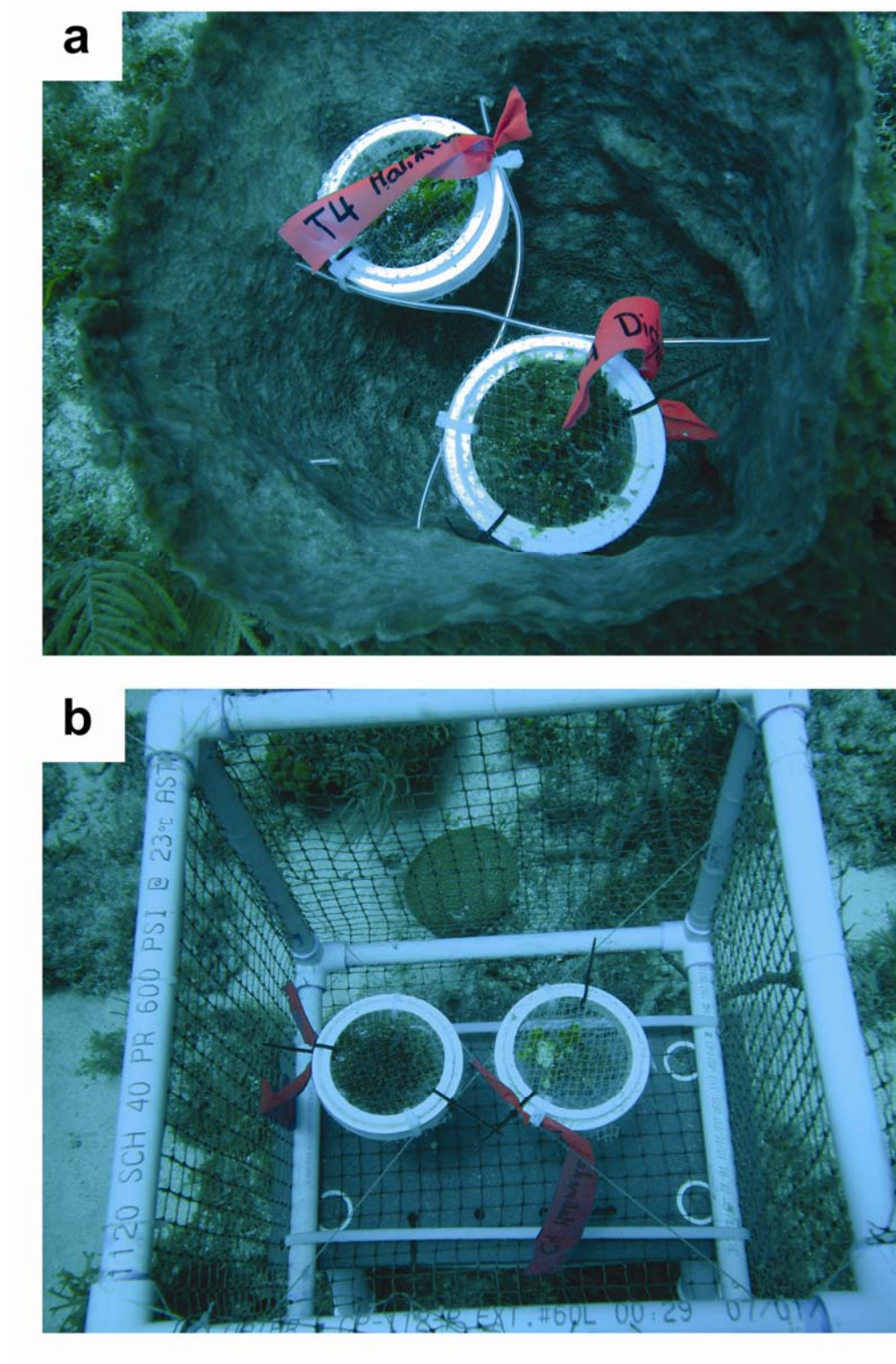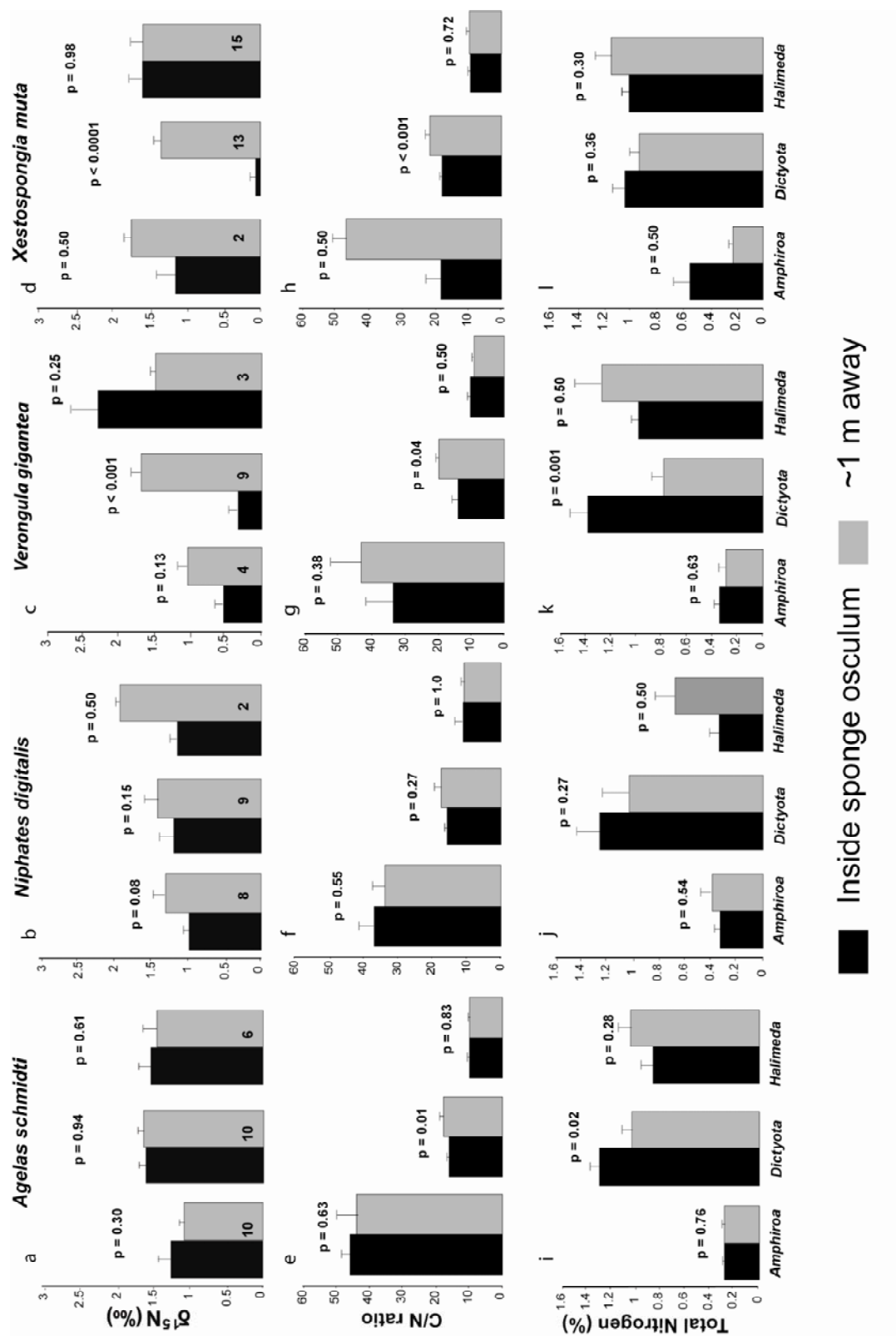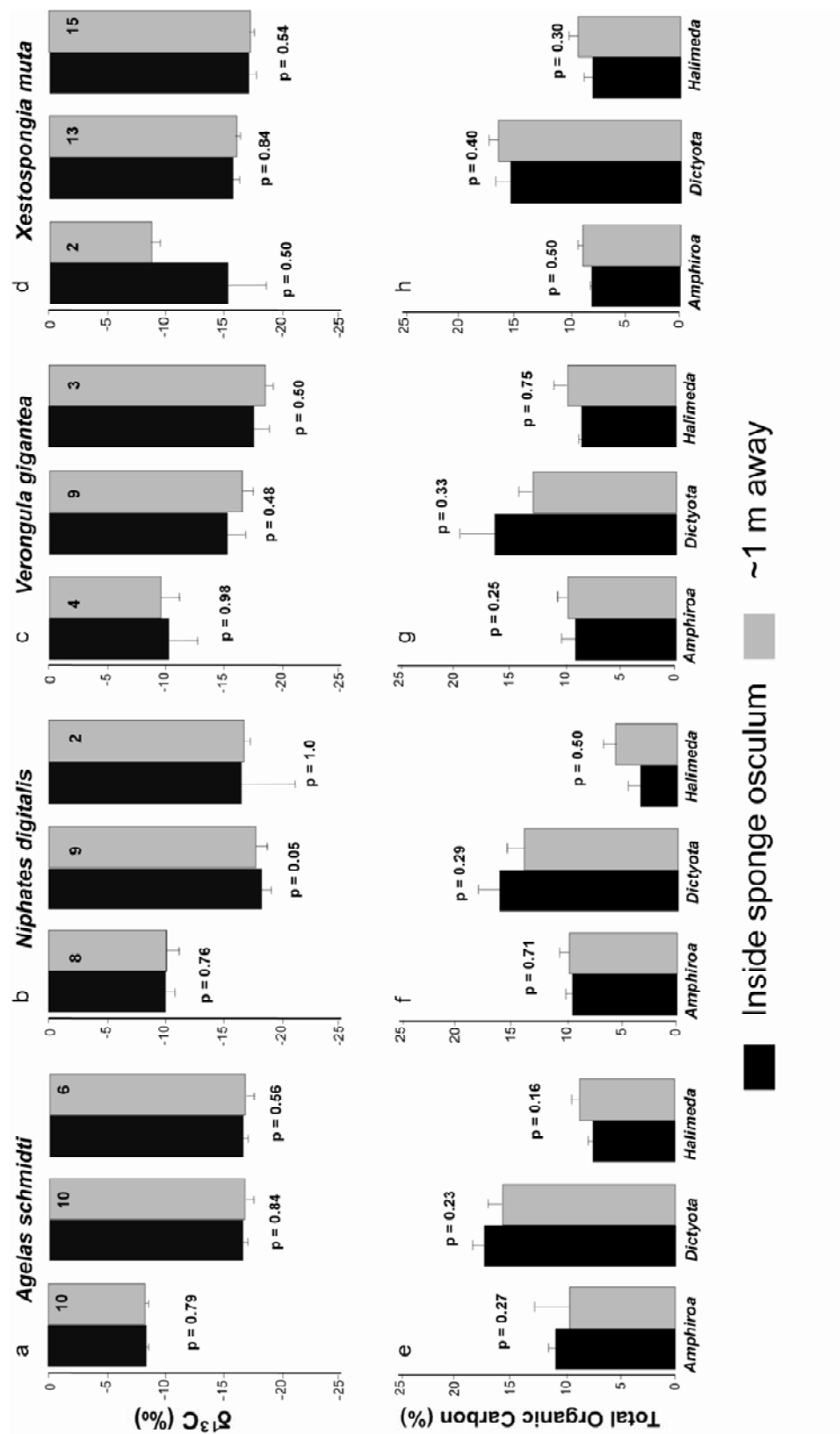
Figure 1

Figure 2

Figure 3

Figure 4

Control Seaweeds:
Away from Sponges

Treatment Seaweeds:
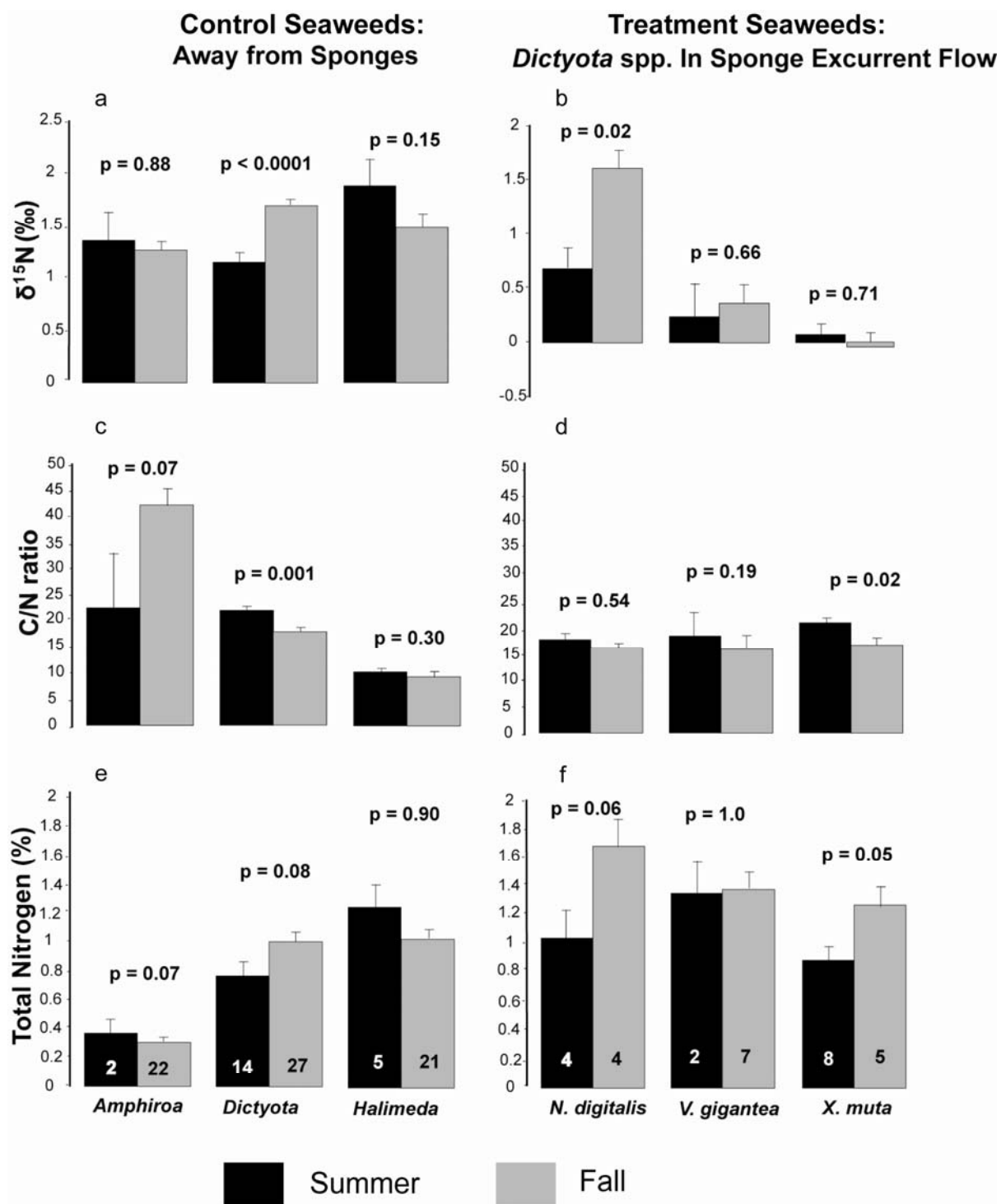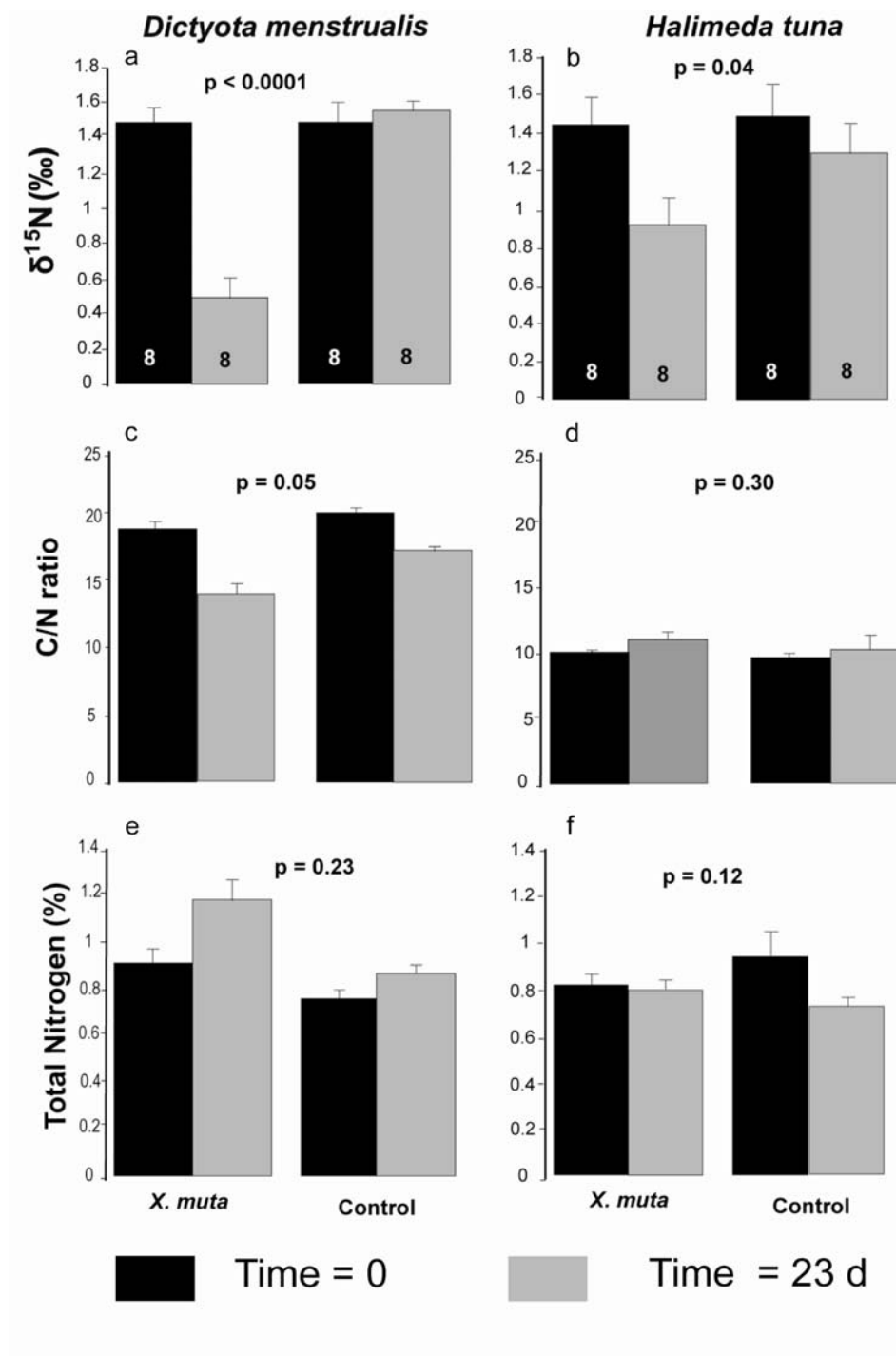*Dictyota* spp. In Sponge Excurrent Flow

Figure 5

Figure 6

38

REFERENCES

Alongi DM, Trott LA, Pfitzner J (2008) Biogeochemistry of inter-reef sediments on the northern and central Great Barrier Reef. Coral Reefs 27:407-420

Aronson RB, Precht WF, Toscano MA, Koltes KH (2002) The 1998 bleaching event and its aftermath on a coral reef in Belize. Marine Biology 141:435-447

Bayer K, Schmitt S, Hentschel U (2008) Physiology, phylogeny and *in situ* evidence for bacterial and archaeal nitrifiers in the marine sponge *Aplysina aerophoba*. Environmental Microbiology 10:2942-2955

Beach K, Walters L, Borgeas H, Smith C, Coyer J, Vroom P (2003) The impact of *Dictyota* spp. on *Halimeda* populations of Conch Reef, Florida Keys. Journal of Experimental Marine Biology and Ecology 297:141-159

Beach KS, Walters LJ, Borgeas HB (2006) Irradiance and nutrient limitation of *Dicytota* spp. populations on Conch Reef, Florida Keys, USA. Journal of Experimental Marine Biology and Ecology 329:101-112

Bracken MES, Stachowicz JJ (2006) Seaweed diversity enhances nitrogen uptake via complementary use of nitrate and ammonium. Ecology 87:2397-2403

Bracken MES, Stachowicz JJ (2007) Top-down modification of bottom-up processes: selective grazing reduces macroalgal nitrogen uptake. Marine Ecology-Progress Series 330:75-82

Burkepile DE, Hay ME (2008) Herbivore species richness and feeding complementarity affect community structure and function on a coral reef. Proceedings of the National Academy of Sciences of the United States of America 105:16201-16206

Carpenter RC (1985) Sea urchin mass-mortality: effecrs on reef algal abundance, species composition, and metabolism and other coral reef herbivores. Proceedings of the 5th International Coral Reef Congress 4:53 - 60

Chiappone M, Sullivan KM (1997) Rapid assessment of reefs in the Florida Keys: Results from a synoptic survey. Proceedings of the 8th International Coral Reef Symposium 2:1509-1514

Corredor JE, Capone GG (1985) Studies of nitrogen diagenesis in coral reef sands. Proceedings of the 5th International Coral Reef Congress 3:395-399

Corredor JE, Wilkinson CR, Vicente VP, Morell JM, Otero E (1988) Nitrate release by Caribbean reef sponges. Limnology and Oceanography 33:114-120

Diaz MC, Ward BB (1997) Sponge-mediated nitrification in tropical benthic communities. Marine Ecology-Progress Series 156:97-107

Done TJ (1992) Phase-shifts in coral reef communities and their ecological significance. Hydrobiologia 247:121-132

Fong P, Boyer KE, Kamer K, Boyle KA (2003) Influence of initial tissue nutrient status of tropical marine algae on response to nitrogen and phosphorus additions. Marine Ecology-Progress Series 262:111-123

Foster NL, Box SJ, Mumby PJ (2008) Competitive effects of macroalgae on the fecundity of the reef-building coral *Montastraea annularis*. Marine Ecology-Progress Series 367:143-152

Fourqurean JW, Escorcia SP, Anderson WT, Zieman JC (2005) Spatial and seasonal variability in elemental content, delta C-13, and delta N-15 of *Thalassia testudinum* from South Florida and its implications for ecosystem studies. Estuaries 28:447-461

France R, Holmquist J, Chandler M, Cattaneo A (1998) delta N-15 evidence for nitrogen fixation associated with macroalgae from a seagrass-mangrove coral reef system. Marine Ecology-Progress Series 167:297-299

Hay ME (1981) Spatial patterns of grazing intensity on a Caribbean barrier reef - herbivory and algal distribution. Aquatic Botany 11:97-109

Hay ME (1984) Predictable spatial escaped from herbivory - How do these affect the evolution of herbivore resistance in tropical marine communities. Oecologia 64:396-407

Hentschel U, Usher KM, Taylor MW (2006) Marine sponges as microbial fermenters. Fems Microbiology Ecology 55:167-177

Hughes TP (1994) Catastrophies, phase-shifts, and large-scale degradation of a Caribbean coral-reef. Science 265:1547-1551

Kuffner IB, Walters LJ, Becerro MA, Paul VJ, Ritson-Williams R, Beach KS (2006) Inhibition of coral recruitment by macroalgae and cyanobacteria. Marine Ecology-Progress Series 323:107-117

Lapointe BE (1997) Nutrient thresholds for bottom-up control of macroalgal blooms on coral reefs in Jamaica and southeast Florida. Limnology and Oceanography 42:1119-1131

Lapointe BE (1999) Bottom-up control of macroalgal blooms in south Florida and Jamaica. Journal of Phycology 35:18

Lapointe BE, Barile PJ, Matzie WR (2004) Anthropogenic nutrient enrichment of seagrass and coral reef communities in the Lower Florida Keys: discrimination of local versus regional nitrogen sources. Journal of Experimental Marine Biology and Ecology 308:23-58

Leichter JJ, Paytan A, Wankel S, Hanson K (2007) Nitrogen and oxygen isotopic signatures of subsurface nitrate seaward of the Florida Keys reef tract. Limnology and Oceanography 52:1258-1267

Leichter JJ, Stewart HL, Miller SL (2003) Episodic nutrient transport to Florida coral reefs. Limnology and Oceanography 48:1394-1407

Leichter JJ, Wing SR, Miller SL, Denny MW (1996) Pulsed delivery of subthermocline water to Conch Reef (Florida Keys) by internal tidal bores. Limnology and Oceanography 41:1490-1501

Levitan DR (1988) Algal urchin biomass responses following mass mortality of *Diadema antillarum* Philippi at Saint John, United States Virgin Islands. Journal of Experimental Marine Biology and Ecology 119:167-178

Lewis SM, Wainwright PC (1985) Herbivore abundance and grazing intensity on a Caribbean coral reef. Journal of Experimental Marine Biology and Ecology 87:215-228

Lirman D (2001) Competition between macroalgae and corals: effects of herbivore exclusion and increased algal biomass on coral survivorship and growth. Coral Reefs 19:392-399

Lirman D, Biber P (2000) Seasonal dynamics of macroalgal communities of the northern Florida reef tract. Botanica Marina 43:305-314

Littler MM, Littler DS (1980) The evolution of thallus form and survival strategies in benthic marine macroalgae- field and laboratory tests of a functional form model. American Naturalist 116:25-44

Littler MM, Taylor PR, Littler DS (1987) Dominant macrophyte standing stocks, productivity and community structure on a Belizean barrier reef. Atoll Research Bulletin 302:1-24

Lopez-Victoria M, Zea S (2004) Storm-mediated coral colonization by an excavating Caribbean sponge. Climate Research 26:251-256

McClanahan TR, Muthiga NA (1998) An ecological shift in a remote coral atoll of Belize over 25 years. Environmental Conservation 25:122-130

McCook L, Jompa J, Diaz-Pulido G (2001) Competition between corals and algae on coral reefs: a review of evidence and mechanisms. Coral Reefs 19:400-417

McManus JW, Polsenberg JF (2004) Coral-algal phase shifts on coral reefs: ecological and environmental aspects. Progress in Oceanography 60:263-279

McMurray SE, Blum JE, Pawlik JR (2008) Redwood of the reef: growth and age of the giant barrel sponge. Marine Biology

Morrison D (1988) Comparing fish and urchin grazing in shallow and deeper coral reef algal communities. Ecology 69:1367-1382

Norris JN, Fenical W (1982) Chemical defense in tropical marine algae, Vol Smithsonian Contributions to the Marine Sciences 12. Smithsonian Institution Press, Washington D.C.

Norstrom AV, Nystrom M, Lokrantz J, Folke C (2009) Alternative states on coral reefs: beyond coral-macroalgal phase shifts. Marine Ecology-Progress Series 376:295-306

Nugues MM, Smith GW, Hooidonk RJ, Seabra MI, Bak RPM (2004) Algal contact as a trigger for coral disease. Ecology Letters 7:919-923

Paul VJ, Hay ME (1986) Seaweed susceptibility to herbivory - Chemical and morphological correlates. Marine Ecology-Progress Series 33:255-264

Reiswig HM (1971) In-situ pumping activities of tropical Demospongiae. Marine Biology 9:38-50

Reiswig HM (1974) Water transport respiration and energetics of 3 tropical marine sponges. Journal of Experimental Marine Biology and Ecology 14:231-249

Reiswig HM (1981) Partial Carbon and Energy Budgets of the Bacteriosponge *Verongia fistularis* (Porifera: Demospongiae) in Barbados. Marine Ecology 2:273 - 293

Seitzinger SP, D'Elia CF (1985) Preliminary studies of denitrification on a coral reef. The Ecology of Coral Reefs Symposia Series for Undersea Research NOAA's Undersea Research Program 1

Shulman MJ, Robertson DR (1996) Changes in the coral reefs of San Blas, Caribbean Panama: 1983 to 1990. Coral Reefs 15:231-236

Southwell MW, Popp BN, Martens CS (2008a) Nitrification controls on fluxes and isotopic composition of nitrate from Florida Keys sponges. Marine Chemistry 108:96-108

Southwell MW, Weisz JB, Martens CS, Lindquist N (2008b) In situ fluxes of dissolved inorganic nitrogen from the sponge community on Conch Reef, Key Largo, Florida. Limnology and Oceanography 53:986-996

Steneck RS (1983) Quantifying herbivory on coral reefs: just scratching the surface and still biting off more than we can chew. Symposia Series for Undersea Research- NOAA's Undersea Research Program 1:103- 111

Steneck RS, Dethier MN (1994) A functional-group approach to the structure of algal-dominated communities. Oikos 69:476-498

Szmant AM, Forrester A (1996) Water column and sediment nitrogen and phosphorus distribution patterns in the Florida Keys, USA. Coral Reefs 15:21-41

Titlyanov EA, Yakovleva IM, Titlyanova TV (2007) Interaction between benthic algae (*Lyngbya bouillonii, Dictyota dichotoma*) and scleractinian coral *Porites lutea* in direct contact. Journal of Experimental Marine Biology and Ecology 342:282-291

Todd BR (2008) Nutrient distribution across the insular shelf of La Parguera Puerto Rico: assessment by algal tissue nitrogen. University of Puerto Rico

Umezawa Y, Miyajima T, Tanaka Y, Koike I (2007) Variation in internal delta N-15 and delta C-13 distributions and their bulk values in the brown macroalga *Padina australis* growing in subtropical oligotrophic waters. Journal of Phycology 43:437-448

Umezawa Y, Miyajima T, Yamamuro M, Kayanne H, Koike I (2002) Fine-scale mapping of land-derived nitrogen in coral reefs by delta N-15 in macroalgae. Limnology and Oceanography 47:1405-1416

Walters LJ, Beach KS (2000) Algal bloom in the Florida Keys. Underwater Naturalist 25:27-19

Ward-Paige CA, Risk MJ, Sherwood OA, Jaap WC (2005) Clionid sponge surveys on the Florida Reef Tract suggest land-based nutrient inputs. Marine Pollution Bulletin 51:570-579

Weisz JB, Hentschel U, Lindquist N, Martens CS (2007) Linking abundance and diversity of sponge-associated microbial communities to metabolic differences in host sponges. Marine Biology 152:475-483

Wulff JL (1984) Sponge-mediated coral reef growth and rejuvenation. Coral Reefs 3:157-163

Wulff JL (2008) Life-history differences among coral reef sponges promote mutualism or exploitation of mutualism by influencing partner fidelity feedback. American Naturalist 171:597-609