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

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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.
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TABLE OF CONTENTS

LIST OF TABLES...........................................................................................................viii
LIST OF FIGURES........................................................................................................ix
LIST OF SYMBOLS AND ABBREVIATIONS.................................................................x

CHAPTER 1..................................................................................................................1
INTRODUCTION...........................................................................................................1
MATERIALS AND METHODS.......................................................................................5
  Study Site..................................................................................................................5
  Study Organisms.....................................................................................................5
  Natural Experiment.................................................................................................6
  Seaweed Transplant Experiment............................................................................6
  Mass Spectral Analysis............................................................................................8
  Statistical Analyses.................................................................................................9
RESULTS....................................................................................................................11
  Natural Experiment.................................................................................................11
  Seasonal Effects......................................................................................................12
    Control Seaweeds: Seaweeds away from sponges............................................12
    Treatment Seaweeds: *Dictyota* spp. in Sponge Excurrent Flow....................13
  Seaweed Transplant Experiment..........................................................................14
DISCUSSION...............................................................................................................16
  Implications for Sponge DIN on Conch Reef.......................................................16
LIST OF TABLES

Table

1: Number of seaweed samples collected per sponge species.................................28

2: $\delta^{15}$N values for Dictyota spp. and Halimeda spp. ...............................................29
LIST OF FIGURES

Figure

1: Map of field site…………………………………………………………………………………..33
2: Seaweed Transplant Experiment Set-up………………………………………………………….34
3: Natural Experiment: $\delta^{15}$N, C/N ratio, and total nitrogen (%) values…………………35
4: Natural Experiment: $\delta^{13}$C and total organic carbon (%) values……………………..36
5: Seasonal Effects: $\delta^{15}$N, C/N ratio, and total nitrogen (%) values ……………………..37
6: Seaweed Transplant Experiment: $\delta^{15}$N, C/N ratio, and total nitrogen (%) values…….38
LIST OF SYMBOLS AND ABBREVIATIONS

δ^{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:

\[ \delta^{N X} = \left( \frac{R_{\text{sample}} - R_{\text{std}}}{R_{\text{std}}} - 1 \right) \times 1000 \] 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).

δ^{15}N: Expression of the isotopic composition of nitrogen, analogous to the above equation, but where \( R = \left( \frac{^{15}\text{N}}{^{14}\text{N}} \right) \) and the standard is atmospheric \( \text{N}_2 \).

‰: Units in which δ values are expressed.

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

DOM: Dissolved organic matter.

LMA: Low microbial abundance (\textit{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.

\( \text{NH}_4^+ \): Ammonium.

\( \text{NO}_3^- \): 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 nucula* 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 μmol m$^{-2}$ h$^{-1}$ and 57 ± 73 μmol 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 ≥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 ≤ 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 δ¹⁵N NO₃⁻ 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 NO₃⁻ versus NO₃⁻ in subthermocline 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 ¹⁵N-depleted NO₃⁻ released by HMA sponges, upwelled NO₃⁻ has a high δ¹⁵N 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 δ¹⁵N 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 subthermocline 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 NO₃⁻, whereas the LMA species releases only NH₄⁺. The re-mineralized NO₃⁻ excreted by all of these HMA sponges is known to have unusually low δ¹⁵N 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 cm² 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: $\delta^{N}X = [(R_{sample} – R_{std})/R_{std} – 1] \times 1000$ in units of parts per thousand (‰). Where $^{N}X$ is the coefficient of the heavy isotope (i.e. $^{15}N$
or \textsuperscript{13}C), and \( R \) is the ratio of the heavy to light isotope in both the standard and sample (i.e. \textsuperscript{15}N/\textsuperscript{14}N or \textsuperscript{13}C/\textsuperscript{12}C). The standard for nitrogen is atmospheric N\textsubscript{2} (0.0‰) and the standard for carbon is Pee Dee Belemnite (0.0‰). Samples were compared to a pure acetonilide 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 \( \delta^{15} \)N and \( \delta^{13} \)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 (\( \delta^{15} \)N, \( \delta^{13} \)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,
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 $t_{\text{final}}$ and $t_0$ data and comparing the between treatment differences of $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, 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 $\delta^{15}N$ 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 $\delta^{15}N$ values between summer and fall (Fig. 5a). There were no seasonal differences in the $\delta^{13}C$ 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 ≥ 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 δ$^{15}$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. in 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:**
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 $\delta^{15}N$ 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 $\delta^{15}N$ 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 $\delta^{15}N$ values at $t_0$, and by $t_{final}$ the $\delta^{15}N$ 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 $\delta^{15}N$ value than *H. tuna* (0.42 vs. 0.87‰: $t_{14} = -2.61$, $p = 0.02$). Neither the $\delta^{13}C$ 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 $\delta^{15}$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 $\delta^{15}$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 $\delta^{15}$N value of NO$_3^-$ in *A. schmidti* effluent have not been measured, it is possible that this sponge’s effluent has a $\delta^{15}$N value
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 $\sim$2.5‰. While this $\delta^{15}N$ value is $\sim$2‰ lower than that of the ambient NO$_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 ($\sim$1.8 $\mu$mol L$^{-1}$ vs. $\sim$0.84 $\mu$mol 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 δ¹⁵N 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 NO₃⁻ 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 δ¹⁵N values for Dictyota spp. vary dramatically (Table 2). For example, the δ¹⁵N 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 δ¹⁵N 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 NO₃⁻ and that sponges are an important source of NO₃⁻ on Conch Reef. However, the low δ¹⁵N 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 NH₄⁺ 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 oligotrophic 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). Leichter 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
higher (by ~2‰) during the summer upwelling season than at other times of the year. The fact that the δ¹⁵N 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 NO₃⁻ 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 δ¹⁵N 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 δ¹⁵N 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 NO₃⁻ to NH₄⁺ 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 δ¹⁵N value of the excreted DIN. Although, because some HMA sponges, including Xestospongia muta, nitrify virtually all the NH₄⁺ before it exits the sponge, no net change in the δ¹⁵N 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 δ¹⁵N 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
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. Fourquarean 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 $\delta^{15}$N value. Because a change in the concentration of the DIN pool seemed to affect the $\delta^{15}$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 $\delta^{15}$N values typically increase with increasing growth rate due to high N needs and less discrimination against $^{15}$NH$_4^+$ and $^{15}$NO$_3^-$ (Fourquarean 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.
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Table 2: $\delta^{15}$N values for *Dictyota* spp. and *Halimeda* spp.

<table>
<thead>
<tr>
<th>Seaweeds</th>
<th>$\delta^{15}$N value</th>
<th>Location</th>
<th>Region</th>
<th>Citation</th>
</tr>
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<tbody>
<tr>
<td><em>Dictyota</em></td>
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<tr>
<td><em>bartayresiana</em></td>
<td>0 ± 0.08</td>
<td>Puerto Rico mid-shelf</td>
<td>Caribbean</td>
<td>Todd 2008</td>
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<tr>
<td></td>
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<td>Puerto Rico inshore</td>
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<tr>
<td><em>D. bartayresiana</em></td>
<td>3.48 ± 0.09</td>
<td>Looe Key Back Reef</td>
<td>Florida Keys</td>
<td>Todd 2008</td>
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<tr>
<td><em>D. cervicornus</em></td>
<td>~2</td>
<td>Looe Key Fore Reef</td>
<td>Florida Keys</td>
<td>Lapointe et al. 2004</td>
</tr>
<tr>
<td><em>D. menstrualis</em></td>
<td>~5.5</td>
<td>Looe Key Back Reef</td>
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<td>Lapointe et al. 2004</td>
</tr>
<tr>
<td><em>D. pulchella</em></td>
<td>~6</td>
<td>Looe Key Fore Reef</td>
<td>Florida Keys</td>
<td>Lapointe et al. 2004</td>
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<tr>
<td><em>D. pulchella</em></td>
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<td>Lapointe et al. 2004</td>
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<tr>
<td><em>Dictyota</em> sp.</td>
<td>1.9 ± 0.11</td>
<td>Belize</td>
<td>Caribbean</td>
<td>Abed-Navandi &amp; Dworshak 2005</td>
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<td><em>Dictyota</em> sp.</td>
<td>~1.6</td>
<td>Curaco reef</td>
<td>Caribbean</td>
<td>de la Moriniere et al. 2003</td>
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<td><em>Dictyota</em> sp.</td>
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<td>Mediterranean</td>
<td>Lepeoint et al. 2000</td>
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<td><em>Dictyota</em> sp.</td>
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<td>Todoroki river reef</td>
<td>Indo-Pacific</td>
<td>Umezawa et al. 2002</td>
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<td><em>Dictyota</em> sp.</td>
<td>4.7 ± 0.1</td>
<td>Maezato reef</td>
<td>Indo-Pacific</td>
<td>Umezawa et al. 2002</td>
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<td><em>Dictyota</em> sp.</td>
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<td>Shiraho reef</td>
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<td>Umezawa et al. 2002</td>
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<td><em>Dictyota</em> sp.</td>
<td>2.8 ± 0.4</td>
<td>Nagura Bay</td>
<td>Indo-Pacific</td>
<td>Umezawa et al. 2002</td>
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<td><em>Dictyota</em> sp.</td>
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<td>Kabira Reef</td>
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<td>Umezawa et al. 2002</td>
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<td>Conch Reef</td>
<td>Oceanside</td>
<td>This study</td>
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<td><em>Halimeda</em></td>
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<td><em>goreau</em></td>
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<td>Oceanside</td>
<td>Lapointe et al. 2004</td>
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<td><em>H. incrassata</em></td>
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<td>Lapointe et al. 2004</td>
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<td><em>H. monile</em></td>
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<td>Lapointe et al. 2004</td>
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<td><em>H. opuntia</em></td>
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<td>Florida Keys</td>
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<tr>
<td>Seaweeds</td>
<td>δ¹⁵N value</td>
<td>Location</td>
<td>Region</td>
<td>Citation</td>
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<td>Lapointe et al. 2004</td>
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<td>Florida Bay</td>
<td>Lapointe et al. 2004</td>
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<tr>
<td><em>H. opuntia</em></td>
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<td>Lapointe et al. 2004</td>
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<td><em>H. tuna</em></td>
<td>1.3 ± 0.3</td>
<td>Corsica</td>
<td>Mediterranean</td>
<td>Lepointe et al. 2000</td>
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<tr>
<td><em>H. tuna</em></td>
<td>5.0 ± 0.7</td>
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<td><em>H. tuna</em></td>
<td>1.56 ± 0.11</td>
<td>Conch Reef</td>
<td>Oceanside</td>
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<td><em>Halimeda</em> sp.</td>
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<td>Curaco reef</td>
<td>Caribbean</td>
<td>de la Moriniere et al. 2003</td>
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<td><em>Halimeda</em> sp.</td>
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<td><em>Halimeda</em> sp.</td>
<td>2</td>
<td>Palau</td>
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<td>Yamamuro et al. 1995</td>
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</table>
FIGURE CAPTIONS

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 cm²) 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: δ¹⁵N, 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 δ¹⁵N (‰), 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: δ¹³C 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 δ¹³C (‰) 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 δ¹⁵N (‰), 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 δ¹⁵N (‰), 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: δ¹⁵N (‰), C/N ratio and total nitrogen (%) values for *Dictyota menstrualis* (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 $t_{\text{final}} - t_0$ datum for replicate seaweeds in the *X. muta oscula* versus next to the sponge. The within treatment differences between $t_{\text{final}}$ and $t_0$ were compared using Student’s t-tests.
Figure 3

A. *Agelas schmidtli*
- $p = 0.39$
- $p = 0.54$
- $p = 3.91$

B. *Niphates digitalis*
- $p = 0.60$
- $p = 0.15$
- $p = 0.50$

C. *Verongula gigantea*
- $p < 0.05$
- $p = 0.001$
- $p = 0.26$

D. *Xestospongia muta*
- $p = 0.60$
- $p < 0.0001$
- $p = 0.88$

E. C/N ratio
- $p = 0.63$
- $p = 0.01$
- $p = 0.83$

F. C/N ratio
- $p = 0.56$
- $p = 0.27$
- $p = 1.0$

G. C/N ratio
- $p = 0.33$
- $p = 3.04$
- $p = 0.5$

H. C/N ratio
- $p = 3.50$
- $p < 0.001$
- $p = 0.72$

I. Total Nitrogen (%)
- $p = 0.76$
- $p = 0.64$
- $p = 0.26$

J. Total Nitrogen (%)
- $p = 0.27$
- $p = 0.50$
- $p = 0.63$

K. Total Nitrogen (%)
- $p = 0.01$
- $p = 0.50$
- $p = 0.53$

L. Total Nitrogen (%)
- $p = 0.26$
- $p = 0.33$

- Inside sponge osculum
- ~1 m away
Figure 4

(a) *Agelas schmidtii*  
(b) *Niphates digitalis*  
(c) *Verongula gigantea*  
(d) *Xestospongia muta*

- **$\delta^{13}C$ (%)**
  - *Agelas schmidtii*: 0, 10, 3, p = 0.79, p = 0.44, p = 0.353
  - *Niphates digitalis*: 0, 3, 2, p = 0.76, p = 3.65, p = 1.6
  - *Verongula gigantea*: 4, 9, 3, p = 0.98, p = 0.48, p = 0.56
  - *Xestospongia muta*: 2, 13, 15, p = 0.60, p = 0.54, p = 0.64

- **Total Organic Carbon (%)**
  - *Amphiroa*: 5, 6, 8, p = 3.27, p = 0.23, p = 0.16
  - *Dictyota*: 12, 13, 14, p = 0.71, p = 0.26, p = 0.50
  - *Haliclona*: 11, 12, 13, p = 0.33, p = 0.33, p = 0.36

- **Legend**
  - Black bar: Inside sponge osculum
  - Gray bar: ~1 m away
Figure 5
Figure 6
REFERENCES


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


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


