Ecosystem Impacts of Carbon and Nitrogen Cycling by Coral Reef Sponges

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ABSTRACT

PATRICK J. GIBSON: Ecosystem Impacts of Carbon and Nitrogen Cycling by Coral Reef Sponges
(Under the direction of Christopher S. Martens)

Sponges and their associated microbial communities are capable of dramatically altering the water chemistry of their surrounding environment by rapidly pumping water through their tissues where nutrients are absorbed and waste products released. This study focuses on the impact of sponge populations on reef ecosystem carbon and nitrogen cycles and describes four principle findings: 1) Sponges with large associated microbial communities obtain the bulk of their carbon from dissolved organic matter (DOM), while sponges without large associated microbial communities feed only on particulate organic matter (POM). 2) Respiration by large sponge populations results in localized acidification of the coral reef ecosystem through the release of CO₂. 3) Some coral reef sponges host coupled nitrification-denitrification and impact the ecosystem N cycle in complex and significant ways. 4) There exists an autocatalytic feedback loop between sponge and nuisance macroalgal populations in which sponges consume algal-derived DOM and algae utilize sponge derived dissolved inorganic nitrogen (DIN). This interaction benefits both the sponge and algal populations in the competition for the limiting resource of space on the reef and may be shifting the reef ecosystem into a new, stable community structure. These findings were only made possible through the use of
novel, highly precise *in situ* underwater instrumentation including mass spectroscopy and spectrophotometric elemental analysis used to identify, quantify, and observe in time-series the various sponge mediated biogeochemical processes.
For Dewey
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CHAPTER 1:
IN SITU OBSERVATION AND EXPERIMENTAL INVESTIGATION OF SPONGE MEDIATED ECOSYSTEM PROCESSES ON A FLORIDA KEYS CORAL REEF: AN INTRODUCTION

Marine environments are inherently dynamic and complex ecosystems. Coastal ecosystems where the land and sea interact are particularly dynamic, where the confluence of changing ocean currents and tides, land based inputs, seafloor communities and anthropogenic activities creates the potential for dramatic variability across wide range of spatial and temporal scales. Marine sciences have traditionally relied on occasional expeditionary sampling and experiments which cannot sufficiently capture the conditions defining ecosystem structure and function. To understand the marine environment would require investigating processes as they occur and in their natural context. The application of in situ instrumentation and experimental manipulation has the ability to observe ecosystem dynamics in real time, and to observe the effects of modifications on these processes.

Herein I present the results of in situ coral reef observatory deployments and lab and field based experiments investigating the role of sponges on coral reef ecosystem biogeochemistry. There are two chapters with a focus on carbon dynamics. The first deals with sponge respiration of dissolved organic carbon (Chapter 2), while the next studies the products of this respiration in the reduction of pH in sponge excurrent waters (Chapter 3). The following two chapters deal with the reef nitrogen cycle and how sponges may influence it. Nitrification has long been associated with sponges and here we quantify nitrate flux and oxygen demand as a function of sponge pumping rate in a
high-resolution, *in situ* time-series analysis (Chapter 4). Consequent to the factors resulting in rapid nitrification are conditions favoring a N sink process of anammox or denitrification. I describe a collection of *in situ* enclosure experiments where we track sponge mediated processing of N compounds within a controlled volume of reef water outfitted with an array of chemical sensors. We observe and quantify coupled processes of nitrification and N$_2$ production, and present a case that the likely mechanism of N$_2$ production is indeed canonical denitrification instead of anammox. While the sponge population on the reef does host a N removal process, the rate of nitrification in the most dominant sponges far exceeds the rate of denitrification. This imbalance will influence the ecological significance of these processes at different temporal scales. The research findings directed a lab-based aquarium experiment that synthesized some of the processes described above with a model sponge-algal system. This final research chapter (Chapter 6) pursues a putative autocatalytic feedback loop manifest through an exchange of carbon and nitrogen compounds that benefits the sponge and algal populations of a reef ecosystem while consequently creating conditions non-conducive to the growth and survival of reef-building hard corals.

**CHAPTER 2: RESPIRATION OF DISSOLVED ORGANIC MATTER BY CORAL REEF SPONGES**

Sponges are widely recognized as effective filter feeding organisms capable of removing from the surrounding water column particles ranging from 0.1 to roughly 5 μm diameter. The energetics of tropical sponges was the subject of a number of seminal papers by H.M. Reiswig (1971, 1974, 1981) who observed that certain sponges could not meet their metabolic demands with the amount of particulate organic carbon (POC) they
were consuming. Reiswig astutely associated this carbon deficit with the presence of large microbial communities living inside some of the sponges he studied and postulated that these “bacteriosponges” would meet their carbon demands through the respiration of dissolved organic carbon (DOC). The microbial associations of sponges have been actively studied over the last few decades and we now recognize the “bacteriosponges” of Reiswig’s research as High Microbial Abundance (HMA) sponges, whose endosymbiotic bacterial and archaeal population numbers are several orders of magnitude over that of the ambient water or sponges of lesser associations (Low Microbial Abundance, or LMA). Subsequent studies on sponge metabolism, namely those of research groups led by G. Yahel (2003) and J.M. de Goeij (2007, 2008), directly measured DOC uptake by a number of sponge species. We build upon this body of work by directly measuring and comparing DOC uptake among a variety of HMA and LMA sponges and conclude that only HMA sponges respire DOC. We find significant uptake of DOC by three of four HMA sponges studied but no uptake by the three LMA species researched. Indeed, we discover that the giant barrel sponge, *Xestospongia muta*, the dominant animal on our Florida Keys fore-reef ecosystem, met nearly 90% of its C demand with DOC. Most of the carbon in the oceans is in dissolved form, and DOC is generally elevated in reef environments. The ability or respire DOC may give HMA sponges an evolutionary advantage to succeed in environments with low or inconsistent particle loads. This process and other activities of abundant sponge populations will have implications for biogeochemical cycling on the greater reef ecosystem.

**CHAPTER 3: SPONGE RESPIRATION DRIVES LOCAL REEF ACIDIFICATION**
Sponge populations of coral reef ecosystems will rapidly respire both particulate and dissolved organic carbon. The process of this respiration yield diminished dissolved oxygen concentrations and large injections of CO$_2$ into the benthic water column. This autochthonous CO$_2$ input will alter the seawater carbonate equilibria and directly reduce pH of the receiving water column. We directly measured reductions of seawater pH in sponge excurrent water relative to the ambient reef water column. Measurements of seawater pH were conducted in situ with high temporal and analytical resolution using a submersible SEASII-pH spectrophotometric analysis system. The SEASII-pH was deployed on the reef and collected alternating ambient and sponge excurrent samples for over 10 consecutive days each in 2009 and 2010. By adding the instrument to our cabled observing system we provided consistent power and had real-time access to instrument data and control capabilities. Significant differences in reef pH were observed between ambient and excurrent waters from two common sponge species, *Xestospongia muta* and *Ircinia strobilina*. Mean depletions in pH of 0.020 and 0.055, respectively, were measured; figures sufficient to reduce reef aragonite saturation state and potentially inhibit calcification rates of reef building hard corals. Within the context of global-scale ocean acidification and the measured expansion of fore-reef sponge populations, the effect of this localized acidification process should be closely monitored.

**CHAPTER 4: DIEL VARIABILITY IN NITRATE FLUX BY THE DOMINANT CARIBBEAN REEF SPONGE XESTOSPONGIA MUTA**

Sponge animals are ubiquitous in marine hard-bottom habitats. They prosper by obtaining food resources by actively pumping water from their surrounding environments
through a complex network of aquiferous channels and chambers which retain nutrients and expel wastes. The rate of this pumping is typically many thousand times the sponge’s own volume, and each parcel processed by the sponge bears the signature of the chemical transformations effected upon it. As the resultant compounds of sponge activity can have an impact on the surrounding environment, understanding to rate and extent of these activities is paramount to the ecology of their ecosystem. There is strong evidence that populations of the giant barrel sponge *Xestospongia muta*, and perhaps other HMA sponges, are expanding on upper Florida Keys fore-reef communities. The diverse associations of microbial communities characteristic of HMA sponges facilitate a wide array of biogeochemical transformations. Principle among these processes is the aerobic oxidation of ammonium \(\text{NH}_4^+\) and subsequent nitrification to nitrate \(\text{NO}_3^-\). The source of amine compounds supporting this reaction are derived from the remineralization or ammonification of particulate and dissolved organic matter (POM and DOM) respired by the sponges. We conducted in situ, high-resolution time series measurements of pumping rate, \(\text{O}_2\) demand, and \(\text{NO}_3^-\) flux for 40 hours from a *X. muta* individual at Conch Reef in July 2010. Sponge excurrent velocity was measured with a vertically mounted acoustic Doppler velocimeter in the excurrent plume, while dissolved oxygen (DO) was sampled from both ambient benthic and sponge excurrent water masses by paired DO optodes. Measurements of \(\text{NOx} (\text{NO}_2^- + \text{NO}_3^-)\) were collected by an automated submersible spectrophotometric analyzer (SEASII-NOx) deployed on the reef. All instruments were components of our cabled reef observatory system and could be monitored and controlled from land or surface based scientists. All parameters were sampled at 1 min or smaller intervals and data were coordinated to quantify synchronous
changes in pumping velocity and chemical fluxes. Pumping rate was variable in time and displayed diel trends. Occasional cessations in pumping were observed, exclusively during nighttime hours, along with corresponding spikes in O₂ demand and NOx concentration. The fluxes of these compounds were flow controlled, in that the pumping rate term of the flux calculation (not the concentration term) accounted for most of the variability in NOx flux. Daytime patterns in biogeochemical transformations indicate that the sponge may receive an O₂ subsidy from photosynthetic endosymbiotic cyanobacteria as no significant O₂ demand was measured yet the products of oxidative reactions were observed in NOx production. After conducting a survey for *X. muta* abundance on the fore-reef habitat, we estimate that the normalized NOx flux from *X. muta* at Conch Reef is approximately 5.1 (±2.0) mmol N m⁻² day⁻¹. This value is higher than other NOx sources on the reef (such as reef sediments) and is a major source of bioavailable inorganic nitrogen. Considering that elevated nutrients can exacerbate coral disease and bioerosion, detailed analysis of sponge mediated N cycling and transport processes may elucidate driving forces of reef ecosystem stability.

**CHAPTER 5: COUPLED NITRIFICATION-DENITRIFICATION IN CARIBBEAN REEF SPONGES**

The complex chemical gradients and diverse microbial associations within sponges are favorable conditions for a number of biogeochemical transformations. The documented process of nitrification results in conditions of low O₂ and high NOx, an environment suitable to the two known N removal processes, denitrification and anammox. We used enclosure experiments to monitor *in situ* sponge activity within a controlled volume of reef water where chemical sensors and amendments of ¹⁵N
isotopically labeled tracers are used to discern the variety of N transformation mechanisms carried out by the sponge. Monitoring of reactants, intermediaries, and products of putative cycling mechanisms along with the source and fate of $^{15}$N tracer elucidated the mechanisms of N cycling along with the conditions in which they operate. These experiments provided evidence, rates, and mechanisms across a range of induced conditions of reactant concentrations for two prominent N cycling pathways, nitrification and N$_2$ production. Distinguishing between the two mechanisms leading to N$_2$ production is difficult but there is strong evidence in support of denitrification over anammox as the primary pathway. There was variability in process rates between the sponges studied but for X. muta the dominant sponge on the reef, the rate of nitrification was far greater than that of denitrification. Results from other sponge species and a single observation from boring and encrusting sponge aggregations reveal that denitrification may be significantly higher than in X. muta. These processes need to be further elucidated and their controls constrained before the impacts to reef scale N budgets are known.

CHAPTER 6: SPONGE-ALGAL INTERACTIONS ON A DEGRADED CORAL REEF ECOSYSTEM: A FEEDBACK TOWARDS STABILITY?

The Florida Keys fore-reef was once characterized by thickets of branching Acropora corals and a number of mounding species forming a structurally complex tropical habitat. Like many coral ecosystems around the globe, the Keys reef tract has suffered major declines in hard coral cover over the last few decades. The Florida system was particularly damaged by disease outbreaks and extreme weather events that killed the
reef building hard corals. Little or no recovery has been observed in the offshore fore-reef habitats of the keys reef, and present day benthic communities are dominates by macroalgae, sponges, and a number of soft coral species. This ecosystem phase-shift seems to have become somewhat stable, with marked increases in sponge populations, no reduction of fleshy macroalgae, and continued decline of the few remaining hard corals. I hypothesize that the sponge and macroalgal populations are succeeding with the help of an autocatalytic feedback loop that benefits their growth and survival while consequently further inhibiting recovery of hard corals. The putative feedback mechanism contends that algal derived organic matter is providing a C food source for the HMA sponge populations who in turn remineralize this organic matter into bioavailable nutrients that support high algal productivity. This feedback mechanism is tested in a lab-based aquarium experiment by following the path of isotopically labeled C and N compounds as they cycle through a model sponge-algal system. We find that while algal organic matter is indeed respired by our system, we cannot conclusively attribute this respiration to the sponge specimens. We do find conclusive evidence that remineralization of fixed N compounds from algal organic matter is being carried out by the sponge and that this bioavailable N is subsequently reincorporated into biomass by the algal population. The apparent mutualistic benefits to these dominant reef inhabitants may increase the ecological stability of the sponge-algal system, and decrease the likelihood of a phase-reversal back to coral dominance.

The research presented in this dissertation comprises a significant contribution to our understanding of the role of sponge populations in reef ecosystem ecology.
number of the discoveries presented here have management implications. By identifying the rates, mechanisms, and controls of key biogeochemical processes, we may identify trends in reef ecosystem dynamics and clues to system trajectory. Due to their abundance and capacity to drive ecosystem change, careful consideration of sponge populations and their interactions with algal and coral communities is advised. Further application of in situ observing systems may be a valuable tool in understanding the complex dynamics of marine ecosystems. The research described here was able to discover critical mechanisms of ecosystem change that would not have been possible without the resolution of the novel technologies employed. The greatest benefit from these technologies will be found in directed research into the local scale processes governing ecosystem development. For instance, on reef ecosystems such as the Keys fore-reef community studied here, organisms responsible for ecosystem structure and function are tied to the benthic substrate and must contend with the environment of the benthic boundary layer. Observations of upper water column processes are a poor indicator of conditions present on the reef benthic surface where ecosystem development is taking place. Further development and deployment of in situ monitoring equipment of sufficient resolution and scale will help identify the key players and processes in marine ecosystem dynamics. We may be able to improve management and conservation efforts of our valuable marine ecosystems by identifying these key ecosystem components and focusing on their crucial role in shaping ecosystem structure and function. This dissertation is an early example of how these new technologies can be employed and it is the hope of the author that this research will guide and inspire the future of in situ marine investigations.
CHAPTER 2:
RESPIRATION OF DISSOLVED ORGANIC MATTER BY CORAL REEF SPONGES

Introduction

Sponges (phylum Porifera) are ubiquitous in hard-bottom marine ecosystems. One of the earliest evolved animals, sponges are essentially a body of cells organized around aquiferous channels through which water is actively pumped, nutrients are absorbed, and wastes are expelled. This generic construction belies the structural and chemical complexity evolved by sponges to survive in nearly all marine habitats. Highly diverse, over 9,000 extant species of poriferans have been documented (Brusca & Brusca 1990). Although often cryptic and overlooked in ecological studies, sponge species have evolved to perform a variety of key ecosystem functions including the cycling of nutrients (Wilkinson & Fay 1979, Corredor et al. 1988, Diaz & Ward 1997, Southwell et al. 1998, Gibson 2011, this volume), stabilization of substrate (Wulff 1984), bioerosion of carbonate structures (Neumann 1966, Rutzler 1975, 2004), provision of habitat (Duffy 2002, Henkel & Pawlick 2004), and as forcers of benthic-pelagic coupling (Lesser 2006; see also review by Bell, 2008). In some degraded Caribbean coral reef ecosystems, sponges are emerging as the heir to space made available by the declining coverage of hard corals (Aronson et al. 2002, Maliao et al. 2008, McMurray et al. 2010).

Marine sponges are regarded for their capacity as efficient filter feeders and many articles have reported on the ability of all sponge species to remove particulate matter (POM) from the water column as a food source (e.g. Reiswig 1974, Pile et al. 1997,
Yahel et al. 2005, Lesser 2006, Hadas et al. 2009). Early studies on sponge respiration failed to balance the carbon demand of some sponges and an alternative feeding strategy of utilizing dissolved organic matter (DOM) was postulated (Putter 1908, 1914 as per Reiswig 1974, Stephens & Schinske 1961, Reiswig 1971, 1974, 1981, Wilkinson and Garrone 1980). Although these early investigations provided no direct evidence of DOM uptake, recent studies have directly implicated sponges in the respiration of the DOC pool as a food source (Yahel et al. 2003, de Goeij & van Duyl 2007, de Goeij et al. 2008). Yet, both the historical and modern studies have found variability among species in the apparent DOM demand. Reiswig (1974) insightfully attributed this disparity to the presence of microbial communities living within “bacteriosponge” specimens.

Many sponge species have developed associations with large populations of microbes hosted in their tissues (Dosse 1939 as per Wilkinson 1978, Hentschel et al. 2002, 2006, Lafi et al. 2009). Sponges hosting these large microbial communities, generally with population sizes >10^3 higher than the surrounding water column, are classified as High Microbial Abundance (HMA) (Hentschel et al. 2006). Sponges without populations of microbes significantly higher than the surrounding water column are classified as Low Microbial Abundance (LMA) (Hentschel et al. 2006, Weisz et al. 2007). While not yet well described (Hoffmann et al. 2005, Siegl et al. 2011) these microbial populations contribute to the overall metabolism of the host sponge animal and this sponge-microbial consortium is thought to alter the biogeochemical processes carried out by the holobiont (van Duyl et al. 2008, Hentschel et al. 2006, Hoffman et al. 2005, 2009, Reiswig 1974, Southwell et al. 2008).
In this study we show evidence of the uptake and respiration of DOM by three HMA sponge species but not by three LMA species abundant on the Florida Keys reef ecosystem. This is the first direct quantitative study comparing these two classifications. This finding implicates DOM uptake as a function of the sponge-microbe holobiont, rather than the sponge animal itself. This newly identified metabolic pathway may influence the ecosystem state and trajectory of the Keys coral reef, a system which has already undergone dramatic ecological changes.

The Florida Keys reef ecosystem has been well described in its benthic habitats and communities over the past few decades (Miller at al. 2000, Somerfield et al. 2008). These benthic communities have dramatically changed since the 1970’s (or before), experiencing a rapid decline in hard coral populations. Particularly large reductions of the once prominent branching *Acropora palmata* and *Acropora cervicornis*, as well as the mound forming *Montastrea spp.* have characterized this decline. Many factors have contributed to the decline of coral cover in the Florida Keys, but central among them are temperature stress (both warm and cold), disease outbreaks (infecting corals as well as other keystone species such as the urchin *Diadema antillarum*), and the impact of occasional hurricanes (see Precht & Miller (2007) for a thorough review of factors influencing the Florida Keys reef tract). As aspects of the reef ecosystem structure have changed, so have aspects of ecosystem function. Here we identify a prominent pathway of C cycling emerging on the reef and discuss its significance for this ecosystem in transition.

**Methods**
STUDY AREA

The Florida coral reef tract extends in an arc along the Atlantic side of the Keys archipelago from the Dry Tortugas in the south and west northward beyond West Palm Beach. Much of this area is encompassed by the Florida Keys National Marine Sanctuary (FKNMS) which provides varying degrees of protection and resource management. This study was conducted at Conch Reef in the Upper Florida Keys (Fig. 2.1). Conch Reef is designated as a Special Protected Area within the FKNMS and is a no-take zone closed to all activities other than permitted research. Conch Reef is also the location of Aquarius Reef Base, and underwater research habitat that has supported extensive research in the area since 1994 including both surface diving and saturation diving missions led by our group at UNC-Chapel Hill.

SAMPLE COLLECTION

Seven species of sponges were sampled in this study: Callyspongia vaginalis, Ircinia strobilina, Mycale laxisima, Niphates digitalis, Spheciospongia vesparium, Xestospongia muta, and Verongula gigantea (Fig. 2.2). Sponge species selected are all of erect morphology, feature distinct and directed oscula, and are abundant in the Florida Keys reef ecosystem. X. muta and V. gigantea feature a massive barrel morphology with a large central cavity. M. laxisima is commonly a smaller round barrel shape. C. vaginalis and N. digitalis are generally elongate tube or vase shaped, though N. digitalis is singular while C. vaginalis generally occurs with multiple erect tubes. I. strobilina and S. vesparium are round and squat with multiple distinct oscula collected into a central oscula group. Specimens were chosen by random (haphazard) selection by divers among
healthy looking, actively pumping sponges were sampled. These species cover a range of natural abundance, pumping rates, tissue density and microbial abundance (Table 2.1) (Southwell et al. 2008, Weisz et al. 2008).

Examining the impact of sponge metabolism on reef water quality involves measuring differences in water quality parameters before and after sponge processing. Sampling of ambient reef water before it enters the sponge and comparing it with samples from the water exhausted by the sponge in its excurrent plume is the basis of this study. Sponges can pump at impressive rates and the residence time of a water mass within sponge tissue is a function of sponge morphology, tissue density, and pumping rate. Water residence time is extremely short for rapidly pumping, thin walled LMA sponges like *C. vaginalis* and *N. digitalis*. These sponges will process a water mass in a period of a few seconds. Conversely, a squat, dense, HMA sponge such as *I. strobilina* will have a much longer time in which to impact a given water mass as it passes through its tissues. Water residence times in an actively pumping *I. strobilina* can be as fast as 20-30 sec, but dye tracer has shown that some water can stay within an active sponge for as long as 3 mins. The barrel sponges, such as *X. muta* and *V. gigantea*, fall in the middle of this range and actively pumping individuals exhibit water residence times on the order of <10 to 30 sec, although some water may remain in the sponge for up to a minute (C. Martens unpublished data). All of these intervals are less than the time required to collect samples of ambient and excurrent waters and we therefore assume our paired ambient/excurrent collections to be representative of their respective water masses.

Uptake of DOM was measured by analyzing the difference in DOC concentration between paired samples of ambient and sponge excurrent water. Ambient samples were
collected by syringe (60 mL) adjacent to the outer sponge wall (within 10-20 mm) where water intake occurs. Sponge excurrent samples were collected with a syringe and 15 cm uptake tube inserted in the sponge oscula with minimal disturbance to the excurrent water plume and avoiding contact with the sponge. Excurrent samples were slowly drawn at an estimated rate of 2 ml s\(^{-1}\). Observations using fluorescein dye to trace sponge excurrent indicate that this sampling strategy does not disturb the sponge or the excurrent plume in such a way as to compromise the sample with stagnant or entrained ambient water. Syringe sampling of massive sponges has proven effective in other studies of sponge metabolism (Yahel et al. 2005, Southwell et al. 2008). Samples were collected in triplicate syringes by a SCUBA diver at depths ranging from 12 m to 30 m across a range of dates in July and October 2008, May, July, and September 2009, and July and October 2010. Samples were filtered \textit{in situ} upon uptake through an inline 25 mm pre-combusted glass fiber filter (Whatman GF/F) with a 0.7 µm nominal pore size. GF/F filters were selected to correspond with the previous study of sponge Caribbean sponge DOC uptake by Yahel et al. (2003). All filters and other glassware used in the experiment were pre-combusted at 450°C for >4 hours prior to use. All plastic used for sample collection or storage, including syringes, fittings, filter holders, and pipette tips were either HDPE or polycarbonate and were extensively washed prior to use (Tupas et al. 1994). All new plastics were sonicated with lab detergent for 30 mins, rinsed and soaked overnight in ultrapure water, acid washed in 0.1 M HCl for >2 hours then thoroughly rinsed in ultrapure water. Previously used plastics were acid washed for >2 hours and rinsed with ultrapure water between uses. Each syringe was fully rinsed 3x with sample water prior to collection. Upon return to the surface, sample syringes were placed in an ice bath.
before preservation in the lab (usually within 6 hr). Samples were placed in replicate pre-combusted, 3x sample rinsed 20 ml scintillation vials with washed HDPE screw caps. Samples were preserved with 100 µL of 50% H$_3$PO$_4$ and maintained at 4°C until analysis.

For a comparison of relative metabolic demand of different carbon sources, *X. muta* was also sampled for uptake of POM (N. Lindquist, unpublished). Particulate samples were collected with an *in situ* vacuum filtration apparatus utilizing atmospheric and at depth pressure differential to draw a water mass through a 0.7 µM filter (pre-combusted 47 mm Whatman GF/F). Sampling rate was regulated with a flow-control valve. Typical flow rates of approximately 2 L hr$^{-1}$ were used and each filter integrated 8 to 12 L of total water sample volume. POM sample water was simultaneously drawn from both ambient water near the outer sponge wall and excurrent water from the sponge oscula. These samples were collected over several days in July, September, and October 2007.

**SAMPLE ANALYSIS**

Dissolved organic carbon concentrations were measured via high temperature catalytic oxidation (HTCO) and non-dispersive infrared (NDIR) detection on a Shimazdu TOC-5000 instrument. Samples were acidified to a pH <2 and sparged with CO$_2$ free “zero air” for 10 mins to remove inorganic C. Concentrations are reported as non-purgable organic carbon (NPOC), but referred herein simply as DOC. Standards were generated from dilution of commercially prepared potassium pthallate (La-Mar-Ka Inc., Baton Rouge, LA) with 18.2 MΩ ultrapure water. All standard curves achieved $R^2$ of 0.999 or better and blanks and standards were interspersed for quality control. Of the
three replicate samples collected for each ambient or excurrent water mass, generally two were analyzed unless the sample failed to pass quality control as described below. Each replicate sample vial was dispensed into two analysis vials. Each analysis vial was sampled for three to five injections of 30 µL for a CV <2%. Thus the mean DOC concentration reported for each water mass represents the average of N=24 to 60 distinct measurements. Outlier removal occurred with measurements >1.5 the interquartile range of a sample’s concentration distribution and any sample with a difference greater than the mean ambient DOC concentration of 88.75 µM between replicates was rejected. With few exceptions, these rejected samples all occurred on days of strong current or high swell and the triplicate syringe collections are likely not representative of a distinct water mass. After QA/QC mean difference between replicate sample vials was 3.46 µM.

Particulate sample filters were folded after collection with the sample side inward, and were frozen in aluminum foil until return to the lab. Filters were lyophilized to remove all water prior to analysis preparation. Filters were pulverized and placed in pre-combusted silver cups that were exposed to concentrated HCl vapors overnight to remove inorganic C. Vapor fluxed samples were dried overnight in an 80°C oven to remove excess acid before being sealed for analysis. Samples were analyzed for total C and total N. Elemental results were normalized to volume filtered.

**Dissolved Oxygen Measurements**

An Aanderaa Data Instruments (AADI) Seaguard system equipped with a 10 sensor digital oxygen optode string (Martens et al. unpublished) was deployed on Conch Reef from June 9-17 and July 9-18, 2010. Oxygen optode sensors were placed to sample
ambient and excurrent waters of four *X. muta* sponges in order to determine O$_2$
drawdown during sponge respiration. Sponge stations were located at 65, 51, 52, and 73 ft. of depth. The Aanderaa string collected O$_2$ concentration data at 30 sec intervals from all sensors. The optode sensors have a reported long-term precision of 1 μM, however this was not tested during this deployment. Data were compared and the difference observed between ambient and excurrent waters represents sponge holobiont (sponge plus associated microbial communities) O$_2$ demand. Although O$_2$ demand and C uptake measurements were not concurrent, both datasets comprise healthy, medium sized, randomly selected representatives of the species on the same reef and can be considered a representative population sample.

**Results**

**DOC UPTAKE BY HMA Sponges**

The mean DOC concentration of ambient reef water from all samples was 88.75 μM (SD=30.82). Three of the four HMA sponges sampled in this study were found to significantly reduce the concentration of DOC in the waters they processed (matched pairs, Student’s t-test vs. 0) (Fig. 2.3). *I. strobilina* had the greatest removal rate with a mean DOC uptake of 26.82 μM (SEM=9.54, N=9, p=0.0228). The two common HMA barrel sponges also showed an uptake significantly different from zero: *V. gigantea* had a mean uptake of 22.73 μM (SEM=8.35, N=6, p=0.0417) while *X. muta* retained 9.81 μM (SEM=1.63, N=65, p<0.0001). The remaining HMA sponge tested, *S. vesparium* (commonly called Loggerhead Sponge) did not show a significant DOC uptake (-1.27 μM, SEM=7.23, N=6). Possible explanations for these findings are discussed below. Of
the 3 LMA sponges studied, *C. vaginalis*, *M. laxissima*, and *N. digitalis*, no significant change in DOC concentration was observed (Fig. 2.3, Table 2.1).

**POM Uptake by *Xestospongia muta***

A total of 12 filter pairs were collected from *X. muta* ambient and excurrent waters. Carbon content of the particles collected from excurrent water was lower in 11 of the 12 samples. Mean ambient POC content was 3.9 (SD=1.3) μM and mean excurrent POC content was 2.9 (SD=0.8) μM (Fig. 2.4). Ambient-excurrent sample pairs were significantly different in POC, with sponges retaining an average of 0.96 (SD=0.28, paired t-test p<0.0001) μM from the water they processed (Fig. 2.4). This value can be directly compared to the DOC results above, indicating that nearly 90% of average *X. muta* C uptake is met with dissolved compounds (Table 2.2). Particulate results also demonstrate particulate organic nitrogen (PON) uptake by *X. muta*. Mean ambient and excurrent PON content was 0.36 (SD=0.04) and 0.17 (SD=0.02) μM, respectively. There was a significant difference between paired ambient-excurrent samples, with a mean PON uptake of 0.20 (SD=0.03, paired t-test p<0.0001) μM (Fig. 4). Analysis of the C:N ratio of POM in ambient and excurrent waters indicates preferential retention of N rich particles by *X. muta*. Mean POM C:N content increased from 11.3 (SD=3.3) in ambient waters to 18.6 (SD=4.0) in excurrent waters. This trend was observed in all of the 12 samples collected (N. Lindquist unpublished). This may be the result of active selection of N rich fraction of the POM from the water column or a consequence of variable lability and C:N content of components of the POM pool. Comparing the relative uptake of POC and PON, we estimate the C:N ratio of retained particles is 4.8:1 (Table 2.3).
However, it is unrealistic to assume this C:N ratio for all sponge catabolism considering the large majority of its energy demands are fulfilled through DOM which typically has a much higher C:N (Hopkinson & Vallino 2005).

**DISSOLVED OXYGEN DYNAMICS**

Four *X. muta* individuals were monitored for dissolved oxygen (DO) demand for over 16 days in the summer of 2010. DO concentration in both ambient and excurrent waters varied widely, although some patterns were observed. When considering data from all four sponge stations, mean ambient O$_2$ was 178 (SD=11) μM while mean excurrent O$_2$ was 166 (SD=16) μM, and these results were significantly different (t-test p<0.0000). The difference between paired ambient and excurrent concentrations is an estimate of sponge O$_2$ demand. Pooled DO data from the four sponge stations results in an average O$_2$ demand of 12 (SEM<1) μM. Observations of DO dynamics in time revealed diel patterns to DO concentration and sponge O$_2$ demand. Oxygen measurements were grouped by Day (07:00 to 19:00) or Night (17:00 to 07:00) readings for descriptive statistics. Samples categorized as Day measurements exhibited mean pooled ambient DO concentration, excurrent DO concentration, and O$_2$ demand of 182 (SD=11), 171 (SD=13), and 11 (SEM<1) μM, respectively. Sample statistics of these same parameters from the night category were 176 (SD=11), 161 (SD=17), and 14 (SEM<1) μM. Night estimates of all parameters were significantly lower than Day estimates (t-test p<0.000). Each individual sponge station was also significantly different from all other stations in all parameters (each pair Student’s t-test p<0.0001 for all tests).

Detailed results of these DO monitoring efforts will be presented elsewhere (Martens et
al. in prep). For the purposes of this study the pooled data from all sponge stations will be used.

**Discussion**

The DOC uptake data support the hypothesis that the microbial associations present in some HMA sponges use or facilitate DOM as a carbon source. LMA sponges that appear to lack this association do not take up DOC. A number of previous studies have implicated DOC uptake by coral reef sponges through investigations of their energetics. Seminal work by Reiswig (1971, 1974, 1981) examined the uptake of POM as a C source relative to growth and respiration of three Caribbean sponge species. His studies on sponge C uptake and respiration indicated that a greater imbalance occurred between particulate C uptake and O₂ demand in “bacteriosponges” (now HMA, including *V. gigantea* studied here) than in LMA species. The symbiont-free LMA sponge *Tethya crypta* was able to balance its C demand through ingestion of POM alone while *V. gigantea* showed a deficit; a property that Reiswig postulated as being met with the uptake of DOC. Further work by Ribes et al. (1999) indicated a disparity among some Caribbean sponges’ abilities to satisfy their metabolic demands exclusively with POM. Recent studies by Yahel et al. (2003, Yahel 2003) examined the C uptake by the cryptic HMA reef sponge *Theonella swinhoei* and found far greater utilization of DOC than POC. Work by van Duyl et al. (2008) shows DOC fixation by a known HMA cold-water sponge and ascribes this capacity to the “sponge-microbial consortia” (cf. holobiont). A body of work by de Goeij et al. (2007, 2008a, 2008b, 2009) using, among other strategies, bulk DOC concentrations and ¹³C tracer experiments found major utilization of
DOC by cavity dwelling coral reef sponges (Table 2.2). Additionally, these studies presented data showing far greater DOC than POC removal by sponges in coral cavities. The $^{13}$C tracer studies and fatty-acid biomarker analyses of the sponge *Haliscarca caerulea* by de Goeij et al. (2008a) implicate both the sponge animal directly and its associated microbial community in the uptake of labeled DOM. They specifically imply that LMA sponge species may also be capable of utilizing DOM, although no direct evidence is presented.

A study on the feeding behavior of the Red Sea LMA sponge *Negombata magnifica* (Hadas et al. 2009) indicated uptake of a range of naturally occurring particles, including planktonic prokaryotes and eukaryotes as well as detritus. Removal efficiencies reached as high as 100% for some particulate fractions. However, the C and N flux measurements in this study also failed to meet the sponges metabolic demands and Hadas et al. (2009) postulate that the deficiency is made up by the uptake of DOM, although no direct measurements of DOM were made. Schläppy et al. (2010), based on laboratory experiments with small (<0.5 g) sponge tissue sections, also identify LMA sponges as hosting microbially mediated nitrification and denitrification activity, even at rates higher than an HMA sponges, but do not address C cycling in LMA versus HMA sponges. Thus, there are several studies throughout the literature that implicate both HMA and LMA sponge species in the uptake of DOM through either direct evidence or through carbon balance. Consequently, there is some evidence to suggest that although LMA sponges may have relatively lower concentrations of microbes, these microbes may yet be capable of diverse biogeochemical processes. The present study is the first to
directly measure DOM uptake by several species of HMA and LMA sponges in situ and examine the impact of microbial associations on this significant ecological function.

One objective for estimating C and N demand by *X. muta* is to look for stoichiometric balance. Considering that all DOC samples were collected during daylight hours, the initial analysis will use the pooled daylight statistics. The mean O$_2$ demand and C uptake (both particulate and dissolved) for *X. muta* failed to achieve balanced respiration stoichiometry. Using Redfield (1963) stoichiometry as a guide (138 O$_2$:106 C:16 N:1 P), we would expect to see an O$_2$:C uptake ratio of 1.3:1. The Redfield ratio is intended to apply only to oceanic POM and does not necessarily apply to coastal or benthic ecosystems. Regardless, Redfield stoichiometry serves as a useful guide and benchmark for comparing production and respiration processes among various ecosystems. Using our average values of O$_2$ uptake (11 μM) and C uptake from DOC+POC (9.81+0.96 = 10.77 μM), we achieve a O$_2$:C ratio very near to 1. Apparently our estimated C demand is too great or our O$_2$ demand is too small. Many factors confound attempts at this calculation, not the least of which is the use of different measurements on different sponges at different times. All reported rates are averaged for randomly selected, apparently healthy *X. muta* of moderate size. One possible explanation of the observed imbalance considers that daytime O$_2$ demands may be partially satisfied by photosynthetic O$_2$ generation by symbionts within the sponge. This amendment would go unnoticed by our paired optode approach for measuring bulk O$_2$ uptake and would bring our estimates closer to those predicted by Redfield. To estimate the impact of endosymbiotic oxygenic photosynthesis on apparent sponge stoichiometry, we analyze the nighttime O$_2$ uptake and assume that DOC remains constant between day
and night periods. This approach yields uptake values of 14 μM O₂ and 10.8 μM C, or a O₂:C ratio of 1.3:1, exactly that predicted using Redfield stoichiometry (Table 2.3).

Estimates of sponge N uptake, however, fail to balance according to Redfield (Gibson 2011, see Chapters 4, 5). This is in part explained by the large proportion of DOM comprising the sponge diet (with generally much higher C:N ratios (Hopkinson & Vallino 2005)). Considering the results from POM uptake by X. muta, we find that the sponge selects for N rich POM with an average C:N of 4.8:1, a value that is more N rich than theoretical Redfield organic matter (C:N of 6.6:1; Table 2.3). However, the finding that this sponge obtains >90% of its C from the DOC pool requires that the C:N ratio of DOM be measured or estimated if attempting to find a balanced stoichiometry. Data for generating an estimated C:N can be found in the results of a separate study by the author (Gibson 2011, see Chapter 4) in which X. muta O₂ demand and NO₃⁻ flux were simultaneously measured. The study found an average O₂ demand of 7 μM and a mean NO₃⁻ production of 0.6 μM. If we assume the X. muta O₂:C ratio calculated above (1.3:1) holds true for the specimen of this other experiment, then we can calculate an estimated C uptake of 5.4 μM. The resulting estimate for the C:N ratio of sponge respired POM+DOM is 9:1. Correcting for the uptake of N-rich POM as ~10% of the sponge’s diet, we calculated a DOM C:N ratio of 9.5:1, a value that is, as expected, somewhat higher than Redfield (Table 2.3). This estimate is in good agreement with the C:N estimated for labile oceanic DOM of 9.95:1 (C:N:P of 199:20:1) by Hopkinson & Vallino (2005). Our calculated C:N ratio is likely to be a slight underestimate as it may be affected by denitrification occurring within the sponge (Gibson 2011, Chapter 5) and the ensuing unaccounted for loss of N. Additional studies are needed to coordinate
measurements of sponge O$_2$ demand, POM and DOM uptake along with photosynthesis and N flux in an attempt to balance the stoichiometry of sponge metabolism.

The ecological significance of rapid DOM uptake by sponges on coral reefs has not yet been elucidated. Sponge populations are known to release large amounts of inorganic N, generally as NH$_4^+$ by LMA sponges and as NO$_3^-$ by HMA sponges (Southwell et al. 2008a). The evidence of rapid respiration rates and release of excess N could indicate C rather than N limitation of sponge holobiont productivity. The composition of DOC found in reef ecosystems is also not well understood. The fraction of DOC consumed by the sponge community is not presently known, and no assertions can be made about the source of the DOM. That said, most of the DOM on the Upper Keys fore-reef is likely autochthonous in origin (Yamashita et al. 2008, personal communication). Additionally, there is a high abundance of macroalgae on Conch Reef, particularly the chemically defended phaeophyte Dictyota spp. Dictyota spp. are known to be leaky with respect to DOC (Brylinsky 1977) and they may be contributing recently fixed, relatively labile DOM to the reef benthic boundary layer (BBL). Indeed, Khalilov & Burlakova (1969) found that phaeophytes in the Black Sea release up to 69% of their gross production as DOM over the course of their existence. Diel and seasonal variability in the supply and bioavailability of DOM to the reef BBL may drive alterations in other water quality parameters such as DIN and O$_2$ concentration at these scales.

Sponge respiration of DOM may have beneficial effect on coral reef ecosystems. Elevated reef DOC concentration has been directly associated with increased coral mortality (Kuntz et al. 2005, Kline et al. 2006), although these lab-based studies were
performed with very high concentrations of carbohydrates and not natural DOM. Smith et al. (2006) evinced in a lab-based experiment indirect coral mortality by macroalgae by stimulation of deleterious microbial communities via algal DOM. Conversely, a field-based study by Vu et al. (2009) found that macroalgae in close proximity to corals did increase DOC concentrations but did not increase mortality of diseased or healthy corals. Sponge respiration of DOM may influence the community structure of microorganisms found in the reef environment. Actively pumping water may allow the sponge community greater access to the DOM pool compared to free-living or surface associated microbes, potentially limiting their growth. Coincidentally, both HMA and LMA sponge populations are still functioning as filter feeders, with removal efficiencies of heterotrophic bacteria as high as 90% (Hadas et al. 2009). Inconclusive evidence regarding the role of DOM on coral reefs prevents assessment of the ecological impact of sponge respiration of DOM.

The uptake of DOM may provide the sponges with an energetic advantage in oligotrophic reef ecosystems where water column productivity and particle load is relatively low. We found the concentration of the DOC pool exceeded that of the POC pool by over 20 fold, although the relative lability of these pools is unknown. Some of the species studied, notably the dominant barrel sponge *X. muta*, host photosynthetic cyanobacterial symbionts which may help offset sponge metabolic demands through resource sharing of fixed C and N, as well as a subsidy of O₂ (Wilkinson & Fay 1979, Wilkinson 1983). Analyses of sponge community composition on reef ecosystems may provide insight into reef water quality (Wilkinson 1987). A large HMA to LMA ratio may indicate DOM as a significant C cycling pathway. Clearly, much more study is
required to understand the various roles of sponges on reef ecosystems. Their interactions with corals, algae, microbes, and other reef ecosystem components will influence ecosystem structure and function and should be well understood if management strategies are to be successful.
Fig 2.1. Map of South Florida depicting the study site location, Conch Reef on the upper Florida Keys reef tract.
Fig 2.2. Photos of sponge species investigated in this research. From left to right, top to bottom: *Xestospongia muta; Ircinia strobilina* (left, dark grey) and *Niphates digitalis* (right, blue-green); *Ircinia strobilina* exhausting fluorescein dye in its excurrent plume; *Callyspongia vaginalis; Verongula gigantea; Spheciospongia vesparium* being sampled by divers and a vertically mounted acoustic Doppler velocimeter. All photographs courtesy of Christopher S. Martens.
Table 2.1. Characteristics, abundance, and DOC uptake of sponges from Conch Reef, Florida Keys. HMA/LMA designates High Microbial Abundance or Low Microbial Abundance classification. DOC Uptake represents the mean concentration difference between paired samples from ambient water column and the sponge excurrent plume. Negative DOC Uptake values represent a release of DOC relative to ambient water column.

<table>
<thead>
<tr>
<th>Species</th>
<th>HMA/LMA</th>
<th>Pumping Rate (l s⁻¹ l⁻¹)</th>
<th>Abundance c (L m⁻²)</th>
<th>DOC Uptake µM(±S.E.)</th>
<th>DOC Uptake (% Ambient)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. vaginalis</em></td>
<td>LMA</td>
<td>0.374 a</td>
<td>0.13</td>
<td>2.44(±2.85)</td>
<td>3.0</td>
</tr>
<tr>
<td><em>I. strobilina</em></td>
<td>HMA</td>
<td>0.093 a</td>
<td>0.08</td>
<td>26.83(±9.54)</td>
<td>20.7</td>
</tr>
<tr>
<td><em>M. laxissima</em></td>
<td>LMA</td>
<td>0.21-0.27 b</td>
<td>&lt;0.01</td>
<td>1.35(±4.14)</td>
<td>1.3</td>
</tr>
<tr>
<td><em>N. digitalis</em></td>
<td>LMA</td>
<td>0.365 a</td>
<td>0.05</td>
<td>-1.80(±1.57)</td>
<td>-2.8</td>
</tr>
<tr>
<td><em>S. vesparium</em></td>
<td>HMA</td>
<td>0.176 a</td>
<td>-0.03</td>
<td>22.73(±8.35)</td>
<td>23.6</td>
</tr>
<tr>
<td><em>V. gigantea</em></td>
<td>HMA</td>
<td>0.05-0.10 b</td>
<td>0.03</td>
<td>9.81(±1.63)</td>
<td>15.1</td>
</tr>
</tbody>
</table>

a Weisz et al. (2008)
b Reiswig (1974)
c Southwell et al. (2008) (Excluding *X. muta*, this study)
Table 2.2. Published DOC and POM uptake results. Sampling techniques using Ambient/Excurrent methods cannot be directly compared to those using an incubation (Incub.) method. LvPOC denotes living particulate matter from Yahel et al. 2003.

<table>
<thead>
<tr>
<th>Species</th>
<th>Habitat</th>
<th>DOC Uptake (µmol)</th>
<th>POM Uptake (µmol)</th>
<th>Method</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. vaginalis</td>
<td>Coral reef</td>
<td>2.44(±2.85)</td>
<td></td>
<td>Amb/Exc</td>
<td>This study</td>
</tr>
<tr>
<td>I. strobilina</td>
<td>Coral reef</td>
<td>26.83(±9.54)</td>
<td></td>
<td>Amb/Exc</td>
<td>This study</td>
</tr>
<tr>
<td>M. laxissima</td>
<td>Coral reef</td>
<td>1.35(±4.14)</td>
<td></td>
<td>Amb/Exc</td>
<td>This study</td>
</tr>
<tr>
<td>N. digitalis</td>
<td>Coral reef</td>
<td>-1.80(±1.57)</td>
<td></td>
<td>Amb/Exc</td>
<td>This study</td>
</tr>
<tr>
<td>S. vesparium</td>
<td>Coral reef</td>
<td>-1.27(±7.23)</td>
<td></td>
<td>Amb/Exc</td>
<td>This study</td>
</tr>
<tr>
<td>V. gigantea</td>
<td>Coral reef</td>
<td>22.73(±8.35)</td>
<td></td>
<td>Amb/Exc</td>
<td>This study</td>
</tr>
<tr>
<td>X. muta</td>
<td>Coral reef</td>
<td>9.81(±1.63)</td>
<td>0.96(±0.28)</td>
<td>Amb/Exc</td>
<td>This study</td>
</tr>
<tr>
<td>Theonella swinhoei</td>
<td>Coral reef</td>
<td>10(±8)</td>
<td>2.4(±1.1)</td>
<td>Amb/Exc</td>
<td>Yahel et al. 2003</td>
</tr>
<tr>
<td>Haliscara caerulea</td>
<td>Coral reef (cryptic)</td>
<td>17.1(±2.4)</td>
<td>0.7(±0.4)</td>
<td>Incub.</td>
<td>de Goeij et al. 2008</td>
</tr>
<tr>
<td>Haliscara caerulea</td>
<td>Coral reef (cryptic)</td>
<td>13.1(±2.5)</td>
<td>0.6(±0.1)</td>
<td>Incub.</td>
<td>de Goeij et al. 2008</td>
</tr>
<tr>
<td>Merlia normani</td>
<td>Coral reef (cryptic)</td>
<td>13.6(±3.1)</td>
<td>0.4(±0.1)</td>
<td>Incub.</td>
<td>de Goeij et al. 2008</td>
</tr>
<tr>
<td>Mycale microsigmatosa</td>
<td>Coral reef (cryptic)</td>
<td>15.2(±0.9)</td>
<td>0.6(±0.1)</td>
<td>Incub.</td>
<td>de Goeij et al. 2008</td>
</tr>
<tr>
<td>Higginsia thielei</td>
<td>Cold-water reef</td>
<td>3.83(±1.2)</td>
<td></td>
<td>Incub.</td>
<td>van Duyl et al. 2008</td>
</tr>
<tr>
<td>Rosella nodastrellae</td>
<td>Cold-water reef</td>
<td>2.02-3.66(±1.2)</td>
<td></td>
<td>Incub.</td>
<td>van Duyl et al. 2008</td>
</tr>
</tbody>
</table>
Table 2.3. Stoichiometric ratio for O, C, and N estimated for *Xestospongia muta* relative to the Redfield ratio. Oceanic DOM after Hopkinson & Vallino (2005).

<table>
<thead>
<tr>
<th>Stoichiometric Ratio</th>
<th>O₂</th>
<th>C</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Redfield</td>
<td>138</td>
<td>106</td>
<td>16</td>
</tr>
<tr>
<td>Redfield (normalized to N)</td>
<td>8.6</td>
<td>6.6</td>
<td>1</td>
</tr>
<tr>
<td><em>X. muta</em></td>
<td>11.7</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td><em>X. muta</em> POM</td>
<td>6.2</td>
<td>4.8</td>
<td>1</td>
</tr>
<tr>
<td><em>X. muta</em> DOM</td>
<td>12.4</td>
<td>9.5</td>
<td>1</td>
</tr>
<tr>
<td>Oceanic DOM</td>
<td>9.95</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 2.3. Mean DOC uptake rates as the difference between paired ambient and excurrent water samples for sponges from Conch Reef in the Florida Keys. Sponge species are grouped by high or low microbial abundance classification. Numbers in or near the bar represent sample size, error bars are the standard error of the mean, and * denotes significant differences.
Fig. 2.4. Difference in POC and PON concentration and the POM C:N ratio between ambient and excurrent water from *Xestospongia muta*. Error bars are standard deviation of each sample group.
CHAPTER 3:  
SPONGE RESPIRATION DRIVES LOCAL REEF ACIDIFICATION

Introduction

Global ocean uptake of anthropogenic CO\textsubscript{2} from the burning of fossil fuels has resulted in a significant reduction in pH and carbonate saturation state of surface waters (e.g. Doney et al. 2009). The exchange of CO\textsubscript{2} between the atmosphere and the ocean has shifted the inorganic C equilibrium, reducing the activity of carbonate ion ([CO\textsubscript{3}\textsuperscript{2-}]). This shift in water chemistry creates environmental conditions that are less favorable for a large diversity of marine calcifying organisms, particularly aragonite producers such as reef building hard corals (Hoegh-Goldberg et al. 2007, Kleypas & Yates 2009, Reis et al. 2009, 2010). Acidification processes follow the reactions:

\[
\text{CO}_2(aq) + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^-
\]

and

\[
\text{CaCO}_3(s) + \text{H}^+ \rightleftharpoons \text{Ca}^{2+} + \text{HCO}_3^-
\]

Global ocean acidification is one of the most threatening issues for the coming century (Orr et al. 2005, Fabry et al. 2008, Guinotte & Fabry 2008) and requires understanding of impacts at all scales of influence. Just as fossil fuel combustion processes oxidize organic C compounds into inorganic CO\textsubscript{2}, water and other end products, aerobic respiration by living organisms meets their metabolic energy demands through essentially the same pathway. Ecosystems populated by organisms capable of rapid respiration of organic matter may experience local buildup of CO\textsubscript{2}, particularly if
their ecosystem is in a state of transition or imbalance (Odum 1969). The result of this rapid respiration in marine ecosystems could be a localized ocean acidification process. This study identifies a coral reef ecosystem where local scale organismal respiration may be contributing significantly to ecosystem acidification and driving changes in reef ecosystem structure. Novel, *in situ* instrumentation is employed to observe large reductions in reef-water pH resulting from the metabolic activities of abundant sponges in a Florida Keys fore-reef community.

Coral reef sponge populations have long been recognized as important players in reef ecosystem structure and function (see reviews by Diaz & Rutzler 2001, Wulff 2001, Bell 2008). Evolutionarily ancient and highly diverse, sponges have adapted successful strategies that permit their habitation throughout marine hardbottom communities (Brusca & Brusca 1990). The ability of sponges to respire large quantities of dissolved and particulate organic matter while actively pumping vast amount of water results in a normalized impact to ecosystem water chemistry of far greater magnitude than more passive benthic populations. Capable of processing seawater in excess of 50,000 times their own volume each day (Weisz et al. 2008), even a small sponge population can imprint upon their marine environment the signature of their internal biogeochemistry (Corredor et al. 1988). Sponges often host large communities of microbial organisms (Wilkinson 1978a-c, Hentschel et al 2002, 2006), and the complexity of aquiferous channels and cell structures within the sponge body facilitates a wide range of chemical gradients and biogeochemical processes (Wilkinson 1978a-c, Wilkinson & Fay 1979, Corredor et al. 1988, Diaz & Ward 1997, Hentschel et al. 2006, Southwell et al. 2008, Hoffman et al. 2009, Gibson 2011, see Chapters 2, 5, this volume).
Of primary importance to ecosystem scale processes are the cycling of C and N by sponge populations. Sponges actively pump water from their environment to bring in essential nutrients in both particulate and dissolved forms. As heterotrophic animals, sponges require a supply of fixed C compounds to satisfy their energetic requirements, which in some circumstances may be supplemented by symbiotic photosynthetic microbes (Wilkinson 1978a). Sponges have long been recognized as efficient filter feeders and the uptake of some particle size fractions has been recorded up to 100% (Pile 1997). A number of studies on reef sponges have examined their capacity to take up DOM in addition to POM (Van Duyl et al. 2001, Yahel et al. 2003, de Goeij et al. 2008, Gibson 2011, see Chapter 2, this volume). DOC has been found to fulfill the majority of C demand by a number of common HMA Caribbean reef sponges, although the rate of uptake and contribution of DOC to the animal’s diet have been variable for the handful of species studied. The capacity to efficiently respire both POM and DOM pools ensures a continual supply of energy and nutrients to the sponge population. Yet, as the benthic water column is repeatedly processed by the sponge populations, the waste products of their rapid metabolism are injected back to the reef environment.

One of these waste products, CO$_2$, is the result of respiration of organic matter. Injection of CO$_2$ into seawater will directly affect and can be indicated by changes in pH (Robert-Baldo et al. 1985). Here we employ in situ instrumentation to measure the pH and other parameters of sponge effluent, and to monitor the effect of sponge metabolism on acidification in the benthic boundary layer (BBL, described below) of the reef environment. We observe measureable and significant differences between ambient seawater and the sponge effluent and propose that this process amplifies within the reef.
BBL with ecosystem level impacts. The goal of this chapter is to convey a theoretical and methodological approach to investigating local scale impacts of benthic respiration on BBL carbonate system parameters including pH. This study is part of several NOAA-NURC supported projects investigating chemical, physical, and biological processes that impact carbonate equilibria in the BBL of Florida Keys coral reef ecosystems. Novel pH data collected in the BBL at Conch Reef, Florida Keys, will be described in this chapter, but the results are ultimately intended to be incorporated into future papers that include a broader range of research efforts and data.

**Coral Reef Benthic Boundary Layer**

Coral reefs are typified by a rough, geometrically complex, biogenic bottom cover. This structure influences the fluid mechanics of the overlying seawater at a range of scales extending from that of a single coral polyp (mm’s) to the regional coastal zone (km’s; see review by Monismith (2007)). This study focuses on the impact of reef complexity on seawater fluid dynamics at the reef scale (generally 1 to 100 m) where the roughness of the reef may affect a significant drag of the flow of water over the reef. Turbulent boundary layer processes over smooth bottoms adhere to the “law of the wall” in that velocity will vary logarithmically and the dissipation of turbulent kinetic energy will decrease with distance away from the bottom (Reidenbach et al. 2006). This BBL is limited in its exchange with the overlying water-mass relative to the mixing taking place within the near-bottom region. Factors controlling the exchange between the BBL and the overlying water column depend in part on physical processes operating a larger scales which may not function at rates sufficient to mix these two watermasses (Genin et al.)
As such, biological and geochemical processes deriving from benthic communities and substrates can affect gradients in chemical constituents of the BBL. Studies have shown that this theory also applied to complex bottom structures, such as reefs (Reidenbach et al. 2006), and that grazing by reef organisms can result in severely depleted abundances of phytoplankton in the BBL (Yahel et al. 2005, Genin et al. 2009, Monismith et al. 2010). It is logical to contend that the chemical compounds required to support this active grazing (ie. O$_2$), and the subsequent waste products (ie. CO$_2$) would exhibit similar patterns of relative depletion (or concentration) within the BBL. The strength and depth of the BBL with vary with a number of physical and environmental conditions; the theory of these processes are beyond the scope of this paper and will not be discussed here. Previous studies of BBL dynamics on reef ecosystems, including one conducted on Conch Reef, the site of the present study, have identified a BBL depth on the order of 1-3 m (Yahel et al. 1998, Reidenbach et al. 2006, Monismith et al. 2010). Here we examine the impact of sponge respiration within the BBL on reef-water pH, a key parameter of coral reef ecology.

**Methods and Experimental Approaches**

**INSTRUMENTATION FOR IN SITU pH MEASUREMENTS**

Seawater pH was measured *in situ* using a Spectrophotometric Elemental Analysis System (SEASII) instrument, developed by a team led by Dr. Bob Byrne from the University of South Florida. Unique characteristics of this system include high precision (<0.002 pH units), rapid sampling (>1 Hz), small sampling volume, and 2 sample intake sources. Cabled benthic deployment of this autonomous sensor facilitated
a number of novel deployment scenarios for investigating reef carbonate dynamics including near-simultaneous sampling of pH of ambient reef water and sponge excurrent water, or benthic boundary layer pH and upper water column pH. These real-time, calibration-free analyses (Byrne & Brelan 1989, DeGrandpre et al. 1999) are based on spectrophotometric pH theory as presented by Robert-Baldo et al. (1985), Byrne (1987), Byrne & Brelan (1989), Clayton & Byrne (1993), and Zhang & Byrne (1996).

**PRINCIPLES OF ANALYSIS:**

Spectrophotometric pH depends on the protonation of a sulfonephthaline indicator dye with dissimilar absorbance spectra among ion states. For pH values within the range of seawater, all such indicators can exist as only 2 forms, HI$^-$ or I$^2^-$, with (I) denoting the indicator. These forms adhere to H$^+$ ion (and thus pH) dependent equilibrium as:

$$\text{HI}^- \leftrightarrow \text{H}^+ + \text{I}^2^-$$

Solution pH can be determined if the indicator dissociation constant ($K_1$) and concentrations of its associated states [HI$^-$] and [I$^2^-$] are known from:

$$\text{pH} = \text{p}K_1 + \log \frac{[\text{I}^2^-]}{[\text{HI}^-]}$$

For each indicator, $K_1$ is known for a given temperature (T), salinity (S), and pressure (P). As different indicators will have different properties, selection is based on the expected range of environmental conditions where the sample will be taken. The indicator dye Thymol Blue has been shown to be most effective for pH of shallow warm seawater solutions (Zhang & Byrne 1996). Relative concentrations of HI$^-$ or I$^2^-$ can be determined by measuring the ratio ($R$) of absorbance ($A$) at their respective distinct absorbance maxima ($\lambda$), which for thymol blue is:
\[ R = \frac{\lambda_{596}A}{\lambda_{435}A} \]

which also will be impacted by T, S, and P. De-convoluting the final spectra requires solving for the absorbance of each indicator state on both measurement wavelengths following the equation:

\[
pH = pK_1 + \log \left( \frac{R - (\frac{\epsilon_{596}}{\epsilon_{435}})}{\frac{\epsilon_{596}1}{\epsilon_{435}1} - R(\frac{\epsilon_{435}}{\epsilon_{435}1})} \right)
\]

with \( \epsilon_{596} \) and \( \epsilon_{435} \) representing the molar absorptivities at each wavelength for the corresponding indicator state. For a more detailed methodological explanation of the approach used in this experiment see Liu et al. (2006).

**SEASII Instrument & Deployment:**

The SEASII instrument is essentially a submersible, peristaltic pump-based, flow-through spectrophotometer employing a liquid-core optical waveguide or cell through which absorbance is measured. The instrument is versatile and can be used for a wide array of seawater analyses (Byrne et al. 2000, Adornato et al. 2007). The SEASII-pH used in this study was configured with two sample intake pumps, a single indicator dye pump, and a 10cm PEEK cell connecting an incandescent light source to a fiber optically coupled field spectrophotometer (OceanOptics USB2000+).

The instrument was deployed on the seafloor of the fore-reef habitat at Conch Reef in the upper Florida Keys at a depth of 15 to 24 m (Fig. 3.1). Conch Reef is home to the *Aquarius Reef Base* underwater research habitat and the site of many contemporary reef studies (for more info, see http://aquarius.uncw.edu/). The SEASII instrument was
cabled to the *Aquarius Life Support Buoy (LSB)* with power and communications infrastructure to maintain real-time data and instrument control from any internet connected computer. Sampling programs could be modified or analytical parameters adjusted based on feedback from real-time data, ensuring good data quality for much of the deployment. Deployments were made for near-continuous time-series of pH observations under a variety of experimental conditions from 9-10-2009 to 9-21-2009 and 7-08-2010 to 7-20-2010.

Deployment configuration consisted of the SEASII instrument mounted in a PVC cradle together with a coupled SBE CTD for environmental parameters of temperature (T), salinity (S), and pressure (P). These data are recorded along with spectrophotometer readings and are used to correct the measurements for the influence of T and S on the pH indicator reaction (Zhang & Byrne 1996). Sample seawater was collected with one of 2 peristaltic pumps from equal lengths of 1/8 in. ID PTFE tubing snuggly connected to the peristaltic pump tubing (Pharmed). A third pump was used for indicator (thymol blue) injection. Intake tubing was connected to a common line for indicator mixing and absorbance detection at a Y-connector fitted with one-way check valves. Indicator was injected through a check valve to a Y-connector directly after the sample lines join the common line. Care should be taken in selection of plastic fittings and check valves to ensure minimal dead volume and cracking pressure. Intake pumps were programmed to turn on and off at alternate intervals of typically 10 minutes. Sample water was filtered inline by 5µm pore-size syringe filters to prevent blockage of the flow cell. Filters were changed and pump tubing was inspected daily by SCUBA divers. Sample pump speed was set to maximum in order to minimize instrumental delay between collection and
analysis. Typical flow rates were approximately 8 ml min\(^{-1}\). Sample flow was continuous but absorbance spectra were collected at intervals of 5 to 10 s depending on deployment objectives. A time interval was incorporated after intake pump switching to allow for flushing of previous sample water with the new source. For analysis of pH time series, these data points were typically bin-averaged to 1 min intervals. A background reference spectrum was collected typically every 2-3 hours by combining sample sources with both intake pumps on and turning off the indicator pump in order to measure background seawater absorbance only. Intake lines and flow-cell were occasionally flushed \textit{in situ} with 1M HCl to prohibit biofouling.

\textbf{EXPERIMENTAL APPROACH:}

The research project consisted of three pH monitoring efforts. The first was to measure the pH of sponge excurrent waters of common reef sponges, namely \textit{Xestospongia muta} and \textit{Ircinia strobilina}, and was the focus of the 2009 deployment. The results of this approach are detailed below. The second approach was to measure the difference in pH and other water quality parameters between the reef benthos at 5 cm off the bottom and the overlying water column 5 m above bottom. This approach was the focus of the 2010 deployment. Both 2009 and 2010 deployments utilized the SEASII-pH for the third experimental approach: to measure the change of pH inside sponge enclosure experiments designed to elucidate C and N transformation processes carried out by various sponge species and reef substrates (see Chapter 5, this volume). Results from the latter two approaches are mentioned briefly here, however, broader analyses are reserved for future publications.
Results

Sponge Excurrent vs. Ambient pH:

The pH values for each pair of ambient-excurrent sampling periods were evaluated for both \textit{X. muta} and \textit{I. strobilina} from September 2009. A total of 122 paired sampling periods were collected, each covering roughly 24 mins of combined ambient-excurrent observations. All pH measurements were corrected for T and S according to Zhang & Byrne (1996). Mean ambient pH for all ambient water column samples collected near \textit{X. muta} was 8.027 (SD=0.010), while mean excurrent pH was 8.007 (SD=0.011) (Fig 3.2). Samples from the sponge \textit{I. strobilina} had a mean ambient pH of 8.048 (SD=0.17) and a mean excurrent pH of 7.990 (SD=0.021) (Fig. 3.2). Differences between paired ambient and excurrent collections resulted in a mean reduction of pH from both sponges. \textit{X. muta} respiration changed pH by an average of -0.020 (SD=0.003) units. \textit{I. strobilina} respiration had a greater effect, with a mean pH change of -0.055 (SD=0.022). These results are significant for both sponge species (ANOVA, $p<0.001$ in both tests). These results are consistent with our findings of \textit{I. strobilina} having a higher water residence time and higher O$_2$ consumption rate than \textit{X. muta} (Martens et al. 2006, see Chapter 2). The maximum pH change was observed in \textit{I. strobilina} excurrent with a drawdown of -0.103 pH units from ambient water.

Interestingly, comparisons of source water between sponges also resulted in significant differences. If reef water was truly well mixed throughout the interval of the study, it would be expected that ambient samples for both \textit{X. muta} and \textit{I. strobilina} would be similar. However, when considering all associated ambient data, the mean pH
ambient samples collected next to *X. muta* (pH = 8.032, N=1792) are significantly lower than those collected near *I. strobilina* (pH = 8.049, N=851; ANOVA, p<0.001) (Fig. 3.2). Differences in excurrent pH values between sponges would be expected, and this is indeed what we observe. Mean pH values of all excurrent samples collected (60s bin-averaged) for *I. strobilina* and *X. muta* were 7.992 (N=805) and 8.014 (N=1710), respectively (ANOVA, p<0.001). Differences between ambient values for each sponge could be representative of variations in the reef microhabitat surrounding each specimen or could demonstrate temporal dynamics of the reef BBL as samples were collected on different days.

**Benthic Substrate on and Enclosure Experiments:**

Observations of pH in the reef BBL and overlying water column were conducted over different substrate types including erect sponges, boring sponges, reef sand, and macroalgae. Both water mass and substrate type had an impact in reef pH. The results were highly dynamic and influenced by chemical, physical, and biological forces impacting the system. These forces have not yet been elucidated and no conclusive statements will be made here regarding pH dynamics in these experiments. These data will be forthcoming in a future paper.

Enclosure experiments involving sponge specimens always resulted in a dramatic pH decline corresponding with a drawdown of enclosure O$_2$ concentration and associated increases in pCO$_2$. Declines in pH were extreme; a reduction of nearly 0.4 units was observed in one experiment (Fig. 3.3). Although this closed system manipulation is not representative of natural processes, it does provide insight into the capability of sponge
populations to impact a given water mass. The results of such sponge mediated acidification under strong BBL conditions may stress carbonate structured organisms. Analysis of these data will appear in a forthcoming synthesis of sponge enclosure experiments.

Discussion

CO$_2$, pH, and Calcification

This study reports on large reductions in pH of sponge excurrent plumes. Sponges now populate at least 10% of the benthic area of upper Florida Keys fore-reef communities >10 m and represent the largest biomass of any invertebrate reef ecosystem component (McMurray et al. 2008, 2010). Regardless of the areal or volumetric extent of reef sponge populations, their active filtration of the water column results in a disproportionate biogeochemical effect on reef waters relative to more passive functional groups such as corals and algae. Rapid respiration of POM and, in the case of HMA sponges, DOM, results in O$_2$ consumption and CO$_2$ enrichment of reef benthic waters. Increases in aqueous CO$_2$ will reduce the relative concentration of CO$_3^{2-}$ ions in seawater, a factor significant to the rate of biological calcification (Gattuso et al. 1998, Marubini & Atkinson 1999, Langdon & Atkinson 2005, Schneider & Erez 2006, Marubini et al. 2008, Reis et al 2009). Marine carbonate organisms respond differently to acidification (Reis et al 2009). Many coral species have been shown in laboratory aquaria experiments to continue calcification at lowered aragonite saturation states, albeit at reduced rates (Langdon & Atkinson 2005, Schneider & Erez 2006, Reis et al. 2010). Coral calcification has even been observed in environments of aragonite undersaturation.
(Gattuso et al. 1998, Reis et al. 2010). The energetic expense of this process and its impact of coral survival and viability remain to be determined. No coral species has been shown to increase calcification with increasing \( p\text{CO}_2 \) (Doney et al. 2009).

Sponges are cited as forcing bentho-pelagic coupling on the ecosystems they inhabit (Lesser 2006, Bell 2008). However, the benthic and pelagic environments are dynamic and rarely defined in ecological studies. Physical dynamics of reef ecosystems will dictate the size and intensity of BBLs (Reidenbach et al. 2006, 2007, Monosmith et al. 2010). Systems with relatively high energy and structural complexity will exhibit BBL’s that persist only mm’s away from benthic surfaces, while on the opposite end of the spectrum stratification can setup many meters above the reef into the water column. The chemical, physical, and biological characteristics of the BBL and overlying water column can exhibit dramatic differences (Yahel et al. 1998, Genin et al. 2009, Monismith et al. 2010). Samples collected for water quality monitoring on reef ecosystems from the water column or even 1m off the bottom are unlikely to represent the conditions with which their benthic organisms actually contend. These organisms will be exposed to a range of benthic processes taking place on the reef that are rarely expressed at distances more than centimeters above the benthic substrate. Does the activity of sponge pumping truly couple the parameters characteristic to these two water masses? While this is certainly possible for the massive barrel sponge morphologies with powerful and upwardly directed excurrent plumes, many sponge species grow as encrusting, boring, and other morphologies with little vertical extent that do not necessarily direct their excurrent plumes away from the benthic substrate. The BBL of ecosystems with large,
actively pumping sponge populations is likely to bear the signature and ecological consequences of their metabolism.

The effect of sponge-derived CO\(_2\) injection to the benthic boundary layer is complex, non-linear, and a species specific process that impacts not only inorganic carbon system balance and aragonite saturation state but also proton-dependent transport mechanisms across diffusive boundary layers and tissue surfaces (see McConnaughey & Whelan 1997, McConnaughey et al. 2000, and references therein). These mechanisms are inherent to the success of carbonate organisms hosting photosynthesis. Indeed, work by McConnaughey and colleagues (1997, 2000) has argued that proton generation and export from calcification facilitates improved photosynthesis and nutrient assimilation and explains the prevalence of massive carbonate structures in highly oligotrophic habitats. However, a direct study by Marubini et al. (2008) found no effect of pH on photosynthesis, a process more analogous to the mechanism of sponge respiration. Regardless, altering [H\(^+\)] in the environment of these mechanisms is likely to affect their rate or the energetic costs to the organism.

Localized ecosystem scale acidification would not be expected in a mature, healthy ecosystem (Odum 1969), particularly on a coral reef where tightly coupled nutrient cycling and productivity-respiration rates are defining characteristics (Odum & Odum 1955). Autochthonous acidification processes may have come about on the Florida Keys fore-reef with an increase in HMA sponge populations beyond a putative threshold that represents the capacity of ecosystem to buffer products of sponge respiration. The faculty of DOC respiration by HMA sponges (Gibson 2011, see Chapter 2) potentially allows biological access to a previously refractory C-pool, or accelerates
the oxidation rate of the labile pool. If this DOC pool is derived from prolific algal
population on the reef, oxidation of photosynthetic products by heterotrophic sponges
will result in remineralization and cycling of nutrients that may stimulate further growth
of algal population as a positive feedback loop (Gibson 2011, see Chapter 6). Perhaps
the reef is indeed living up to its expectation as a tightly coupled system, but in a state
different from and antagonistic to historical scleractinian dominance.

**FUTURE DIRECTIONS**

The roles of respiration, CO$_2$, and pH on coral reef structure and function are not
fully understood. Results of this research indicate that high-resolution, *in situ* techniques
can provide a new perspective on local scale ecosystem processes. Our research has
made several important discoveries of reef biogeochemical processes that would have
impossible or likely been missed though traditional sampling techniques. Investigations
into whole-ecosystem metabolism and calcification would benefit from this finer scale
resolution of the impact of specific functional groups such as HMA sponges (Bates et al.
2010, Gledhill et al. 2010). The SEASII instruments are capable of sampling at very fine
spatial and temporal scales for long durations, often with minimal maintenance. One
study on a Puerto Rican reef employed an autonomous SAMI pH instrument (Sunburst
Sensors, Missoula, MT) and found dramatic seasonal variability of reef carbon system
parameters (Cullison 2010). Their study revealed important information about ecosystem
carbonate parameters, but did not focus on specific processes or reef localities of critical
importance. It would be valuable to investigate, for instance, pH dynamics at the surface
of a coral head or within the interstices of reef crevices where “hot-spots” of calcification
or dissolution may be found. Long-term investigations of carbon and nutrient dynamics could help elucidate the impact of regional scale phenomena on a specific reef system or process. For instance, the common occurrence of tidal bore upwelling of cold, nutrient rich waters is well documented in the Upper Florida Keys (Leichter et al. 1996, 2003) and has been identified as a major source of nutrients to the reef (Gibson et al. 2008). However, deployment of a SEASII-NOx for the fore-reef community for a period of nearly two weeks revealed no such nutrient pulses, although physical measurements confirmed a number of events indicative of tidal bores (Gibson, Hench et al. unpublished data). Long-term, process oriented deployments would help reveal patterns and dynamics in such synoptic events and change the way we think about ecosystem scale processes. Technological advances in the fields of chemical engineering and ocean observing systems are starting to make such approaches a reality.
Fig. 3.1. Photo of two SEASII instruments deployed in a sand patch on Conch Reef in the Florida Keys. Photo courtesy of Chris Martens.
Fig 3.2. Distribution of pH measurements from paired consecutive ambient/excurrent sampling periods from the coral reef sponges I. strobilina and X. muta. The box represents the distribution of the middle 50% of data points with the bisecting line denoting the median. Whiskers extent to the 5 and 95 percentile and jittered points represent suspected outliers.
Fig. 3.3. Time-series of SEASII-pH measurements collected on Conch Reef in July 2009. Blue data represent ambient seawater pH and orange data are sponge excurrent pH. Data from enclosure experiments have been excluded (see Fig. 3.3).
Fig. 3.4. Selection from time-series of SEASII-pH measurements collected on Conch Reef in July 2009. Including enclosure experiments. Blue data represent ambient seawater pH and orange data are sponge excurrent pH. Green data are from enclosure where the sponge excurrent influenced the pH of a confined watermass.
Fig. 3.5. Time-series of SEASII-pH measurements collected on Conch Reef in July 2010. Blue data represent ambient seawater pH and orange data are sponge excurrent pH. Data from enclosure experiments have been excluded (see Fig. 3.3).
CHAPTER 4:  
DIEL VARIABILITY IN NITRATE FLUX BY THE DOMINANT CARIBBEAN 
REEF SPONGE XESTOSPONGIA MUTA 

Introduction

Sponges are evolutionarily ancient animals of the phylum *Porifera*. Organized as a matrix of cells surrounding aquiferous channels, sponges possess the ability to pump water directionally through their bodies, supplying the animal with nutrients and expelling waste products. Sponges often host large communities of microbial organisms within their cell matrix (Wilkinson 1978a-c, Hentschel et al. 2006). Continual pumping and the potential regulation of internal ventilation levels by the sponge generate a wide range of chemical gradients within the sponge body (Hoffman et al. 2005, 2008). These gradients facilitate a dynamic range of biogeochemical reactions that affect the water quality of the resultant sponge effluent. Evidence of sponge hosted nitrification has been presented by several studies (Corredor et al. 1985, Southwell et al. 2008b, Hoffman et al. 2009, Diaz & Ward 1997, Jimenez & Ribes 2007). On reefs and hard bottom communities where sponges are abundant, this nitrification can be the dominant source of dissolved inorganic nitrogen (DIN) to the system and represent a critical pathway in the ecosystem nutrient budget (Corredor et al. 1988, Southwell et al. 2008a). Variability in this process has the potential to regulate other reef processes dependent upon the availability of DIN, possibly including photosynthesis and overall system production, coral disease mortality, and coral fecundity. This study examines the variability of NO$_3^-$ and NO$_2^-$ (hereby NOx) flux from *Xestospongia muta*, a giant barrel sponge common on
Caribbean reef ecosystems, by high-resolution *in situ* sampling of NOx concentration, O₂ consumption, and sponge pumping rate. The resulting time series are the first evidence of dynamic biogeochemical variability by these important reef ecosystem components.

*Xestospongia muta* is the dominant animal on Florida Keys reef ecosystems at depths below 10m and has a greater biomass than any other benthic invertebrate (McMurray et al. 2008). Growing up to 2 m in both height and diameter (Hentschel et al. 2006), *X. muta* is a conspicuous reef component and serves as habitat structure for a number of other organisms (Henkel & Pawlik 2005). Occupying the exterior surface layer of *X. muta* and contributing to its distinctive brown-orange color are symbiotic cyanobacteria of the genus *Synechococcus* capable of carbon fixation through oxygenic photosynthesis (Wilkinson 1983). These microbes, in addition to large communities of both bacteria and archaea found deeper within the sponge cell matrix, occur with abundances on the order of 8x10⁹ cells per g sponge (Hentschel et al. 2006), roughly 3 orders of magnitude over the ambient water column and place this sponge in the category of High Microbial Abundance (HMA) species. Generally robust, *X. muta* individuals are thought to persist for 100’s to 1000’s of years and feature complex population dynamics in both genetic connectivity and local demographics (Lopez-Legentil & Pawlik 2008, McMurray et al. 2008, 2010). On Conch Reef, Florida, the research location of this study, *X. muta* populations have increased by as much as 46% from 2000 to 2006 (McMurray et al. 2010). *X. muta* can typically be found at a density of 0.2 individual sponges m⁻², populating the fore-reef habitat generally between 10 and 30 m (McMurray et al. 2010). Areal density has been measured up to 4.5% of benthic cover and individuals as large as 133 L have been recorded on Florida Keys reefs (Bertin &
Callahan 2008). Although rarely preyed upon by reef fishes or turtles (Dunlap & Pawlik 1998), *X. muta* does experience limited top-down population reduction through the actions of disease (Cowart et al. 2006, Angermeier et al. 2011), fatal bleaching events (Vicente 1990, Lopez-Legentil et al. 2010), and physical damage by anchors, tackle, and marine debris (Chiappone et al. 2005). In the light of continued decline of hard coral populations in this ecosystem (Schutte et al. 2010), *X. muta* is becoming a critical player in reef ecology in terms of both structure and function. Understanding the ecological role of these animals is paramount to understanding the future of these vital marine ecosystems.

The role of nutrients on Florida coral reefs has been heavily debated (Lapointe 1997, 1999, Hughes et al. 1999, Miller et al. 1999, Szmant 2002, Precht & Miller 2007). The debate centers upon the role of nutrients in providing fleshy macroalgae a competitive advantage on the reef, resulting in the overgrowth and mortality of corals. There is general consensus that macroalgal expansion on the reef is a consequence of coral mortality rather than a cause McCook et al. 2001, Aronson & Precht 2006). Nutrients alone are unlikely to instigate macroalgal blooms on a reef, but they may have this effect in the absence of grazing pressure from herbivores. Large populations of macroalgae have persisted for decades on the outer Florida reef tract (Aronson et al. 2004) and there is some indication the grazer population is presently unable to reduce standing algal biomass (Paddock et al. 2006). Nutrients are detrimental to coral reefs through mechanisms unrelated to algal growth, such as the exacerbation of coral pathogens (Bruno et al. 2003, Voss & Richardson 2006) and stimulating rate of bioerosion (Rose & Risk 1985, Holmes 2000, Chazottes et al. 2002). Thus, we should
continue to investigate the sources and sinks of nutrients on reef ecosystems. Here we use novel, *in situ* instrumentation to present evidence of a dynamic, autochthonous DIN source from coral reef sponges and begin to constrain the rates and mechanisms of this important process.

**Methods**

**STUDY AREA**

The study was conducted at Conch Reef in on the Florida Keys Reef Tract in the upper Keys. Our investigation was conducted on the fore-reef community which has in recent decades become dominated by sponge and macroalgal population and features <5% hard coral cover (Aronson et al. 1994). A single *Xestospongia muta* (identified as Station 1) was selected for focused observation of pumping rate, O$_2$ consumption, and NOx concentrations (Fig. 4.1). These measurements were conducted over July 10-12, 2010, and were a component of a larger investigation of sponge processes that will be presented elsewhere.

**PUMPING RATE**

Sponge excurrent Velocities were measured using 6 MHz acoustic Doppler velocimeter (ADV; Nortek Vector, Rud, Norway). The ADV was programmed for burst sampling at 8 Hz for 30 sec every 1 minute. The sampling volume was 14 x 14 mm, located 15.7 cm from the acoustic transmitter. The ADV was positioned vertically above the sponge so that the sampling volume of the instrument was located centrally within the sponge cavity, below the upper rim of the sponge. The placement of sampling volume
within the sponge cavity reduced interference of ambient flows across the reef. The ADV was held in place by a weighted tripod system and its position was secured by tensioning cables (Fig. 4.1). Individual ADV pings with beam-to-beam correlations less than 50 were rejected. The remaining pings were bin averaged into 1-min time series.

**SEASII Nitrate + Nitrite Analysis**

NOx concentration of sponge effluent was measured *in situ* using a Spectrophotometric Elemental Analysis System (SEASII-NOx) underwater autoanalyzer (Byrne et al. 2000, Adornato et al. 2005, 2007). The SEASII instruments are submersible, peristaltic pump-based, flow-through spectrophotometric analyzers utilizing a flexible liquid-core waveguide (LCW) as the optical cell (Fig. 3.1). The instruments are highly versatile and can be configured to analyze a wide range of seawater chemistries. Changes to reagents, light source, LCW, and spectrophotometer parameters are easily conducted and many alterations can be made *in situ* while submerged. The pathlength of the LCW (Teflon AF-2400) cell can be adjusted to match expected analyte concentrations range. LCW length will influence instrument detection levels according to the Beer-Lambert equation:

\[ A = \varepsilon \lambda \times l \times c \]

where absorbance \( A \) is a function of indicator molar absorptivity at a given wavelength \( \varepsilon \lambda \) \((M^{-1} cm^{-1})\), \( l \) is cell pathlength (cm), and \( c \) as analyte concentration (M).

Increasing the length of the LCW effectively lowers analyte concentration required to reach a detectable absorbance. Lengths exceeding 1 m have been applied for sub-nanomolar detection of some analytes.
Nitrate detection method was based on the Griess Reaction, via Cd reduction of NO$_3^-$ to NO$_2^-$ and subsequent reaction with sulfanilamide and N-1-napthylethylenediamine dihydrochloride (NED) for azo dye formation, followed by broad range spectrophotometric detection of molecular absorptivity. Concentration of NO$_2^-$ alone can be measured by manually bypassing the Cd reduction column with a valve manifold, although this is not the optimal method. A buffer solution of NH$_4$Cl and EDTA at pH 8.5 was used to ensure reduction over the Cd column. A 15 cm pathlength LCW was used. The instrument was calibrated using a linear standard curve prior to deployment with a range of 50 nM to 20 µM NO$_3^-$ and a resulting R$^2$ of >0.99. Detection was made at 543 nm, although at concentrations >10 µM an off-peak absorption wavelength of 590 nm, with its own calibration factors, would have been applied (Adornato et al. 2007). Calibration standards were diluted with ultrapure 18.2 MΩ DI H$_2$O from commercially prepared standard stock. Reduction efficiency of the Cd column was testing by running consecutive standards of NO$_2^-$ and NO$_3^-$ of the same concentration. Reduction efficiency always exceeded 98% in calibrations and was tested frequently throughout the deployment. Confidence levels for the instrument were established through measurements of an analytical blank of 18.2 MΩ DI H$_2$O. The SEASII-NOx minimum detection level (MDL) at 543 nm was calculated as the mean blank measurement (10.4 nM, N=6) plus 3xSD (1.2 nM) to be 14 nM. Measured concentration values falling below 14 nM were replaced with a value of 7 nM, half the calculated MDL, assuming normal distribution of NO$_3^-$ concentration below our detection limit. Injections of NO$_3^-$ standards were conducted in situ by a SCUBA diver for a daily calibration check. Calibration was conducted by filling pre-rinsed polyvinyl IV bags
with standard solutions and connecting them in sequence to a valved luer fitting inlet. Real-time data access from the surface vessel revealed instrumental performance during the \textit{in situ} calibration period.

The SEASII-NOx was deployed directly on the fore-reef community at a depth range of 15 to 24 m. A pair of divers could readily handle the instrument for deployment and retrieval. A cable connected to the \textit{Aquarius Reef Base LSB} surface buoy was used for power and communications with the instrument (battery powered deployments are possible) and facilitated real-time data access and instrument control from an internet connected computer. Samples were collected through a 1/8 in ID PTFE tube connected to the instrument’s peristaltic pump tubing. The sampling rate (~3 ml min$^{-1}$) and inlet size (<2 mm) are sufficiently small to place in a number of microenvironments. For this study we positioned the SEASII-NOx intake inside the oscula of the \textit{X. muta} (Station 1), approximately halfway down near the inner wall of the sponge. The inlet was fitted with a mesh filter to prevent suspended particles from entering the instrument flowpath. Samples were collected at approximately 10 sec intervals for the duration of the experiment. Data points were binned to 60s periods and averaged to generate a time series of mean NOx concentrations. Outlier detection was applied and points exceeding 1.5 times the interquartile range for each binned minute distribution were removed.

**Oxygen Consumption**

In order to measure sponge oxygen consumption, a pair of O$_2$ optode sensors (Aanderaa Data Instruments) were deployed at each sponge. Each station consisted of an ambient optode sensor outside the sponge and an excurrent optode inside the sponge.
cavity. These sensors have a reported precision of 1 μM O₂, but this term was not tested for this study. Oxygen concentration was sampled from each optode pair every 30 sec and the difference represents instantaneous sponge O₂ demand. Observations were 60 sec bin-averaged for coordination with corresponding NOx and pumping velocity time series.

**SAMPLING PERIOD**

Observations of sponge pumping velocity, excurrent NOx concentration, and sponge O₂ demand were conducted from 17:00 July 10 2010 to 09:00 July 12 2010 EDT, representing 40 hours of coordinated analytical data points at 1 min resolution. All instruments were time synchronized and data was aligned by time of detection. This required an empirically derived offset for the SEASII-NOx analyzer resulting from time required for the sample to travel through the instrument’s sample and pump tubing. This time-offset was tested *in situ* using a small injection of fluorescein dye and timing its transition from inlet to waste. This 40 hr record is the longest consecutive deployment of the three instruments from the study period.

**CALCULATING FLUX**

The average flux (\( \bar{F} \)) (mass over time) of a substance in a fluid is the product of the volumetric flow (cm³ s⁻¹) through an area of discharge and the concentration (c) (mol cm⁻³) of the item in consideration over the time period of interest (T):

\[
\bar{F} = a \frac{1}{T} \int_0^T u(t)c(t) \, dt
\]

where volumetric flow is the product of fluid velocity (u)(cm s⁻¹) and boundary area (a) (cm²), in this case the planar area of the sponge osculum. This simple formula is
complicated in the case of sponge excurrent plumes in that those plumes do not discharge uniformly over the area of the oscula. The vector field of velocity across the plane of the oscula is therefore not equal at all points. The injection of sponge effluent from its oscula into the overlying water column alters both the dimensions of the plume and, through mixing with the receiving water mass, alteration of analyte concentration. Hench et al. (2010) have characterized these interactions for X. muta by mapping spatial and temporal variations in the physical and chemical dynamics of excurrent plumes in the field (Fig. 4.3). Further investigations may develop a relationship or correction factor between the centerline velocity measurement used in this study and a more accurate description of the variability of flow across the sponge oscular plane. However, this study will consider the calculation of flux to be an over-estimate or maximum potential value until such a correction can be applied.

The X. muta at Station 1 had a circular osculum with a diameter of 23 cm and a planar area of 415 cm$^2$. Sponge volume is estimated through measurements of circumference around the base, middle, and top of the sponge, sponge height, oscula depth and shape. The volume of the oscula void is removed from the total sponge volume to estimate the sponge tissue volume (Fig. 4.2). The total biovolume of the Station 1 sponge is estimated at 78 L. Normalizing by sponge biovolume gives representative rates for NOx flux from an X. muta of given volume and may be used to estimate ecosystem scale NOx flux from a known sponge abundance. A survey of the Conch Reef X. muta population was conducted in order to scale our findings from individual to fore-reef ecosystem. A total of 15 belt transects of 50 m length and 2 m width were conducted along with two additional transects of 20 and 25 m, for a total
survey area of 1590 m$^2$. Each *X. muta* individual encountered by the belt transect was estimated for volume. Sponge volume was approximated using diameter measurements from the base, midpoint, and top of the sponge, sponge height, and oscula depth (Fig. 4.2).

**Results**

**ABUNDANCE OF *XESTOSPONGIA MUTA***

A survey of the *X. muta* population at Conch Reef was conducted in July 2010 (Martens et al. unpublished). The survey found a total of 169 individuals, with a mean *X. muta* density at Conch Reef of 0.15 individuals m$^{-2}$. This result is very similar to previous studies which estimate *X. muta* density 0.20 and 0.17 individuals m$^{-2}$ (Southwell et al. 2008a, McMurray et al. 2010). The size of individuals varied widely, with a range of 0.03 to 218.32 L. The size distribution was skewed towards smaller individuals, a finding consistent with McMurray et al. (2010), as the mean sponge volume was 23.56 L but the median volume was only 6.16 L. At an estimated 78 L, the specimen studied here was a large representative of the population at Conch Reef. The mean areal volume of *X. muta* in the survey was estimated at 2.77 L m$^{-2}$ (SD=1.38), excluding a single outlier exceeding 14 L m$^{-2}$. This result is higher than previous estimates of 2.33 L m$^{-2}$ from Southwell et al. (2008a) and 1.3 L m$^{-2}$ by McMurray et al. (2010). We will used our current data to extrapolate sponge flux results to reef scale estimates based on sponge abundance.

**PUMPING RATE**
The mean excurrent velocity created by the *X. muta* sampled during the July 2010 experiments was 7.78 (SD=2.95) cm s\(^{-1}\) over the 40 hour time series. The mean excurrent pumping rate calculated from this data was 3.23 (SD= 1.23 SD) L sec\(^{-1}\), or approximately 3577 L L\(_{sponge}\)\(^{-1}\) day\(^{-1}\). Notably, the range of excurrent velocities spread from -1.07 to 11.40 cm s\(^{-1}\), with four distinct occurrences of near zero excurrent velocity and a number of smaller reductions in pumping velocity. These dramatic changes in flow occurred only during dark nighttime conditions and persisted for approximately 30 to 90 minutes.

These low flow events correspond loosely to variable ambient O\(_2\) concentrations in the benthic water column (Martens et al. unpublished). Interestingly, this correlation is stronger in the dark than in daylight conditions. This phenomenon may be explained by the presence of oxygenic photosynthetic algae present in the sponge exterior layers. The oxygen produced by photosynthesis from these associated microorganisms may supply a sufficient oxygen subsidy to diminish the effect of low ambient O\(_2\) on sponge pumping rate.

Because of the diel patterns present in pumping rate, estimates of sponge flow rates at scale of 1 day or greater should be normalized to a standard 24 hr period rather than the 40 hr period sampled here. The period of 00:30 July 11 to 00:30 July 12 was chosen to represent a typical 24 hr period (Fig. 4.4). Midnight to midnight was avoided due to several minutes of NOx data missing during routine instrument maintenance around midnight on July 11. Using only the data from this 24 hour period, the flow estimates increase to 8.85 (SD=2.49) cm s\(^{-1}\) and 3.68 (SD=1.04) L sec\(^{-1}\) (Table 4.1). The sponge normalized volumetric flow rate is then revised to 4076 L L\(_{sponge}\)\(^{-1}\) day\(^{-1}\). A forthcoming analysis of this dataset will frequency average all 24 hr periods within the 40
hr dataset at 1 min resolution for a more robust estimation of sponge volumetric flow, although significant changes are not expected. The effect of this pumping rate on the reef ecosystem can be conceptualized by extrapolating our results to the population of sponges on Conch Reef. Using our measured mean abundance (2.77 L m\(^{-2}\)) and calculated mean diel pumping rate (4076 L L\(_{sponge}\) \(^{-1}\) day\(^{-1}\)), we find the \textit{X. muta} population Conch Reef could filter a 11 m water column in less than 1 day.

NOx Flux

NOx concentration data were collected for 1308 of the 1440 minutes within the 24 hour period described above, with missing data resulting from maintenance or internal optical reference calibration periods. Sponge excurrent NOx concentration had a mean value of 0.656 \(\mu\)M but the concentration distribution was skewed towards lower values. The median NOx concentration was 0.501 \(\mu\)M with a range of 0.183 to 9.119 \(\mu\)M. Ambient benthic NOx concentration sampled overnight on July 12-13 in a nearby sand patch often fell below the 14 nM MDL with a mean of 29 nM and a maximum value of 163 nM (N=846). This low ambient NOx persisted even during establishment of a dynamic overnight benthic boundary layer (BBL; see Chapter 3) as well as a significant cold water intrusion that lowered the benthic water temperature by >1.5\(^\circ\)C in less than 10 minutes at 01:45 on July 13. This intrusion likely resulted from a cold water tidal bore, similar to those previously observed at Conch Reef and that have been reported to have a \(\text{NO}_3^-\) concentration of over 4 \(\mu\)M (Leichter et al. 1996, 2003). However, a significant increase in NOx was not detected by our \textit{in situ} SEASII-NOx instrument. Because the concentration of ambient NOx was consistently low and varied little relative to sponge
excurrent NOx, the mean ambient concentration was removed from all excurrent concentrations to calculate the NOx fluxes described here.

Sponge pumping rates and NOx concentrations were used to calculate NOx fluxes. Mean NOx flux from the Station 1 *X. muta* was 1.66 (SD=0.81) µmol N s⁻¹, with a range of 0.03 to 9.62 µmol N s⁻¹. This calculation excludes 22 values where negative flow rates resulted in negative flux. These points resulted from extremely low sponge pumping activity that likely allowed some vertical mixing to occur at the sponge osculum. Each calculated flux is multiplied by 60 sec to convert to minute-based fluxes. The sum of all calculated fluxes with the substitution of the mean value for all missing observations (N=112) results in a total 24 hour NOx flux of 143.1 (SD=69.3) mmol N from this single sponge. Using our estimated volume of the sponge of 78 L we calculate a sponge normalized NOx flux of 1.83(±0.89) mmol N L⁻¹ sponge⁻¹ day⁻¹. With an average *X. muta* density of 2.77 L m⁻² at Conch Reef, this results in a daily NOx flux of 5.08 mmol N m⁻² day⁻¹ from *X. muta* alone (Table 4.1). To compare our NOx flux measurements to a previous study at Conch Reef (Southwell et al. 2008a), we will use our normalized NOx flux rate with their estimated sponge abundance of 2.33 L m⁻². This adjusted estimate comes to 4.26 (SD=2.06) mmol N m⁻² day⁻¹. This value is lower than the previous estimate of 11.8 (SD=1.92) mmol N m⁻² day⁻¹ by Southwell et al. (2008a), a study that did not use *in situ* instrumentation but employed discrete syringe collections for NOx concentration and a dye-front video technique for estimating sponge pumping rate. Our daily NOx flux value calculated under natural conditions is in agreement with rates calculated from closed-system sponge enclosure experiments (see Chapter 5). Enclosure experimental flux calculations resulted in 3.9 mmol N m⁻² day⁻¹, lower than the
estimated mean for natural conditions. The lower value from enclosure experiments is unsurprising as that nitrification would become limited as O$_2$ was consumed during the enclosure period. Additionally, the process of denitrification could also have been occurring, resulting in a lower net nitrification rate (Gibson 2011, see Chapter 5).

**OXYGEN DEMAND**

Oxygen samples were collected from ambient and *X. muta* excurrent waters every 30 sec for the 40 hr deployment. These measurements were bin-averaged to 60s intervals. There was significant difference between mean ambient and excurrent O$_2$ concentration, with estimates of 160 (SD=13) and 151 (SD=31) µM respectively. Mean O$_2$ demand (excurrent – ambient) by the sponge was 9 (SEM<1) µM, and paired sample analysis showed significant uptake (matched pairs t-test vs. 0, p<0.0001). Diel patterns were observed in the O$_2$ time series with a number of large reductions in excurrent O$_2$ during nighttime hours (Fig. 4.4). As with pumping rate and NOx flux, O$_2$ will be analyzed for a single 24 hr period (see above) so that all hours will be sampled evenly. As such, the descriptive statistics for O$_2$ concentration distributions become 163 (SD=13) µM for mean ambient, 156 (SD=33) µM for mean excurrent, and a mean O$_2$ demand of 7 (SEM<1) µM. Compared with the 40 hr time series which over-weighted nighttime concentrations in its distributions, these values represent consistently higher O$_2$ concentration. Oxygen demand was still significant between paired samples from the 24 hr time series (matched pairs t-test p<0.0001). Examining O$_2$ concentration patterns among Day and Night periods, all O$_2$ distributions were significantly lower at night (ANOVA p<0.0001, all tests) (Table 4.1). Notably, O$_2$ demand varied significantly
across day and night groups. Daytime samples of paired ambient-excurrent O₂ showed no significant difference (matched pairs t-test p=3.447). Thus, all diel O₂ uptake must be taking place at night, indeed these paired samples show a difference of 15 (SEM=1) µM O₂ (matched pairs t-test p<0.0001). This result may be isolated to the specific 24 hr period in question; the same analysis on a longer O₂ time series comprised of four X. muta individuals sampled simultaneously for several days yielded a significant mean daytime difference between ambient and excurrent O₂ of 11 (SE<1) µM (matched pairs t-test p<0.0001) (Gibson 2011, see Chapter 2). This result questions the validity of our assumption that our 24 hr time series is representative of diel patterns.

Discussion

Nitrification by HMA sponges has been shown to be a significant N cycling mechanism and DIN source on coral reef ecosystems (Corredor et al 1988, Diaz & Ward 1977, Jimenez & Ribes 2007, Southwell et al. 2008a). Observations of bulk sponge metabolism and pumping rates in this study clearly identify the NOx flux as a process controlled by sponge pumping. Variability in sponge pumping has been previously reported (Reiswig 1974) with large excursions occurring commonly within diel periods and with the majority of pumping variability occurring at night. These pumping reductions appear to be in response to changes in ambient water column O₂ concentration (Martens et al. unpublished). These changes are likely driven by regional scale physical processes bringing different water masses across the reef and may have significant seasonal patterns not identified here. Reiswig (1974), for example, identified diel and
seasonal scale patterns in the pumping rate of the sponge *Tethya crypta* at Discovery Bay, Jamaica.

Changes in *X. muta* pumping velocity directly impacted the sponge oxygen demand and NOx flux. There was an inverse relationship between pumping velocity and excurrent plume NOx concentration and apparent sponge O₂ consumption. The normalizing effect of pumping rate on excurrent NOx concentrations results in a generally consistent NOx flux relative to the dramatic changes in NOx concentration in sponge excurrent water. Although excurrent NOx concentration reached over 9 µM during our observation, an exceedingly high value for coral reef waters, the net increase in NOx flux was minimal due to the accompanying cessation of sponge pumping (Fig. 4.5). When the sponge was pumping rapidly, delivering nearly 5 L s⁻¹ of effluent to the benthic water column, NOx concentrations were generally very low. High flow conditions were characterized by NOx concentrations typically less than 200 nM above typical ambient benthic concentrations. After distinct cessations in sponge pumping, brief periods of very high NOx flux were observed, persisting for only a few minutes, due to the expulsion of highly concentrated NOx when pumping is resumed. These events quickly returned to more typical flux values when pumping rate returned to normal. This process can be expressed quantitatively by calculating the coefficient of variation (CV) for NOx concentration and NOx flux across the time series. Sponge excurrent NOx concentration had a CV of 127% while NOx flux for the same periods had a CV of only 53%. While still somewhat variable, the flux term is mediated by the inverse relationship between concentration and pumping rate (Fig. 4.4).
COMPARISON TO OTHER STUDIES

Quantification of NOx flux from *X. muta* was previously measured by Southwell et al. (2008a) using discrete paired syringe collections for ambient versus excurrent NOx concentrations and a mean pumping velocity estimated using dye-front video averaged from a number of individuals. Their results were somewhat higher than the values presented here, and the disparity is likely even greater considering our probable over-estimation of flux due to variability in velocity across the oscular plane. Other differences between the two studies may arise from the concentrations of NOx measured by the previous study that were consistently higher than those measured here utilizing a SEASII-NOx instrument, a fully *in situ* method we believe produces more accurate results. Interestingly, as NOx flux is flow controlled, an argument could be made for reduced sampling intensity for long term quantification of sponge NOx inputs. Recent knowledge of pumping velocity’s principal role on NOx concentration and, consequently, NOx flux, brings into question the need for expensive *in situ* instrumentation for chemical analyses. The value of deploying advanced *in situ* instrumentation, like the SEAS-NOx, must be weighed against that of extending ADV deployments supplemented with discrete nutrient sampling. Further work is required to determine the efficacy of this approach.
Fig. 4.1. Photo of the Station 1 *X. muta* instrumented with an ADV and two oxygen optodes. The SEASII-NOx is not pictured here but the sample intake tubes were deployed along-side the O₂ optode sensors. Photo courtesy Chris Martens.
Fig. 4.2. Diagram of sponge volume estimation parameters. Figure courtesy Dr. Johanna Rosman.
Fig. 4.3. Plot of O$_2$ concentration (% saturation) and current vectors (cm s$^{-1}$) of the excurrent plume of *Xestospongia muta*. Note the variability of velocity and DO concentration across the oscular plane of the sponge. Figure courtesy of Dr. Jim Hench.
Fig. 4.4. Time series plot of *X. muta* excurrent velocity (cm s$^{-1}$), NOx concentration (µM) and O$_2$ concentration (µM). NOx concentration can been seen to rise in concert with reduction in pumping velocity and drawdowns of excurrent O$_2$.
Fig. 4.5. Time series plot of *X. muta* excurrent velocity (cm s\(^{-1}\)), NOx flux (µM s\(^{-1}\)) and sponge O\(_2\) demand (µM). NOx flux remains relatively constant throughout the time series due to inverse response of flow and concentration.
Table 4.1. Comparison of 24 hour diel and separate day and night periods for *X. muta* NOx flux, O$_2$ demand, and pumping rate.

<table>
<thead>
<tr>
<th>Period</th>
<th>NOx (µM)</th>
<th>O$_2$ Demand (µM)</th>
<th>Pumping Velocity (cm s$^{-1}$)</th>
<th>NOx Flux (µmol l$_{sponge}$ h$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hr.</td>
<td>0.656(±1.054)</td>
<td>7(±1)</td>
<td>8.85(±2.49)</td>
<td>76.25(±37.06)</td>
</tr>
<tr>
<td>Day</td>
<td>0.367(±0.154)</td>
<td>0(±4)</td>
<td>10.27(±0.71)</td>
<td>70.00(±25.83)</td>
</tr>
<tr>
<td>Night</td>
<td>0.945(±1.425)</td>
<td>15(±1)</td>
<td>7.45(±2.79)</td>
<td>82.91(±45.02)</td>
</tr>
</tbody>
</table>
CHAPTER 5:  
COUPLED NITRIFICATION-DENITRIFICATION IN CARIBBEAN REEF SPONGES

Introduction

Coral reef ecosystems worldwide are in an era of change. Significant declines in hard coral cover have been observed over the last 40 years (Pandolfi et al. 2003, Gardner et al. 2003, Bruno & Selig 2007). The coral reef ecosystem of the Florida Keys has exhibited particularly rapid decline, resisting the stabilization of hard coral benthic cover observed in other Caribbean regions (Schutte et al 2010). Global and local factors have combined to shift these systems away from reef building scleractinian corals towards ecosystem components lacking massive carbonate frameworks such as soft corals, fleshy and turf macroalgae, and towards a diversity of sponges (Hughes 1994, Precht & Miller 2007, Norstrom et al. 2009). These shifts away from hermatypic organisms have previously been documented in the geological record of reef ecosystems (Hallock & Schlager 1986).

Although many factors have contributed to these shifts and affected reefs have been impacted by a different suite of forces, many have experienced increasing nutrient concentrations. It is important to note that eutrophication alone has been deemed unlikely to degrade a reef ecosystem (Hughes et al. 1999, Szmant 2002). However, increased nutrient concentrations on reef ecosystems have been shown to exacerbate outbreaks of coral disease (Bruno et al. 2003, Voss & Richardson 2006), increase rates of bioerosion of the reef carbonate framework (Hallock 1988, Holmes et al. 2000, Chazottes
et al. 2002) and may encourage the growth of previously nutrient-limited organisms such as fleshy macroalgae (Hatcher & Larkum 1983, Lapointe 1997; but see comment and reply by Hughes et al. 1999 and Lapointe 1999), particularly in the absence of top-down controls (Aronson & Precht 2000, Jompa & McCook 2002). Essential nutrients are characteristically cycled efficiently on reef ecosystems. Understanding rates and mechanisms impacting nutrient dynamics on reef ecosystems is thus a critical step in understanding mechanisms and trajectory of system change.

Sponges, animals of the phylum Porifera, are ubiquitous among marine hardbottom communities, particularly reef ecosystems. Essentially composed of a matrix of cells surrounding aquiferous channels, sponges actively pump water from their surrounding environment through their bodies to sequester nutrients and expel wastes. The flow of water through the sponge generates a variety of geochemical gradients that facilitate a diversity of nutrient element transformation reactions by the microbial communities that they host. These microbial populations are large, often with abundances 4 to 6 orders of magnitude above the ambient water column (Wilkinson 1978, Hentschel et al. 2006), diverse in species composition (Hoffmann et al. 2005, Taylor et al. 2007, Siegl et al. 2011), and are capable of a large array of biogeochemical transformations. Microbial communities occur throughout the diverse environments within the sponge body and are known to conduct nitrification (Corredor et al. 1985, Diaz & Ward 1997, Jimenez & Ribes 2007, Bayer et al. 2008, Southwell et al. 2008x, Hoffmann et al. 2009) and possibly N\textsubscript{2} fixation (Wilkinson & Fay 1979, Mohamed et al. 2008a) and N\textsubscript{2} production (Hoffmann et al. 2009, Schläppy et al. 2010). Sponges with
these large microbial communities are classified as High Microbial Abundance (HMA), those without as Low Microbial Abundance (LMA).

Here we investigate the role of sponge-hosted microbial communities in nitrogen dynamics of a degraded reef ecosystem, specifically the coupling between microbial nitrification and $N_2$ production occurring in common sponge species. This study investigates N cycling by two common HMA Caribbean reef sponges, *Xestospongia muta* and *Ircinia strobilina*. Results provide evidence of coupled nitrification-$N_2$ production, likely via denitrification, by the microbial communities associated with these sponges. Benthic substrates hosting other dominant organisms were also investigated and results from those investigations will be addressed in brief.

*Xestospongia muta*, the giant barrel sponge, is commonly found throughout Caribbean reef ecosystems. As a large erect sponge, *X. muta* is a conspicuous component of fore- and patch reef communities and has come to dominate the seascape of many degraded reef ecosystems following the recent losses of branching and other corals (Precht & Miller 2007, McMurray et al. 2008). Nicknamed the “Redwood of the Reef” (McMurray et al. 2008), *X. muta* individuals have been suggested to persist on reefs for hundreds to thousands of years. *X. muta* grows up to 2 m in both height and diameter, accounts for more biomass than any other invertebrate on Conch Reef (McMurray et al. 2008), and has become a major provider of habitat structure for a variety of organisms (Henckel & Pawlik 2005). *X. muta* is classified as a HMA sponge and hosts photosynthetic cyanobacteria (*Synechococcus spp.*) in its exterior cell layers and well as complex bacterial and archaeal communities throughout its body (Steindler et al. 2005, Lopez-Legentil et al. 2010).
*Ircinia strobilina*, commonly known as the stinker sponge, is a squat and generally round sponge that is gray-brown to black in coloration and features a cluster of multiple oscula in a shallow depression on the top of its body. Although smaller and less common than *X. muta*, *I. strobilina* is frequently found on fore-, mid-, and occasionally patch reef communities in the Florida Keys. Unlike *X. muta*, *I. strobilina* does not host photosynthetic microorganisms, but it is an HMA sponge with large densities of bacteria and archaea living within its tissues (Mohamed et al. 2008, 2010). It has been the focus of a number of ecological studies, principally involving investigations of its secondary metabolites and microbial communities (Hoppe 1988a, 1988b, Pawlik et al. 2002, Mohamed et al. 2008) as well as biogeochemical process studies including N transformations (Weisz et al. 2007, Southwell et al. 2008a,b).

Some internal regions of both *X. muta* and *I. strobilina* are hypoxic, as shown by precipitation of tetrazolium salt indicator (Weisz 2006) and through measurements with Clark-type oxygen microelectrodes (Hoffmann et al. 2005, Martens et al. 2006, Weisz et al. 2007). These hypoxic regions are presumably formed through rapid aerobic respiration by the sponge and its microbes (Hoffmann et al. 2005, 2008). Sponge respiration of particulate (POM) and dissolved organic matter (DOM) generates NH$_4^+$, among other waste products, that is in most but not all HMA species (Southwell et al. 2008), subsequently nitrified at a rapid rate by resident microbial communities.

Evidence of sponge nitrification has been reported for a number of HMA sponge species (Corredor et al. 1988, Southwell et al. 2008b, Diaz & Ward 1997, Jimenez & Ribes 2007, Hoffmann et al. 2009, Schläppy et al. 2010). Nitrogen is generally released directly in the form of NH$_4^+$ from Low Microbial Abundance (LMA) sponges. Thus, all evidence
suggests that the chemical speciation of sponge DIN release is species-specific and dependent on the composition and activities of the associated microbial communities. These fluxes of DIN from sponges are large relative to other inputs in reef ecosystems, sometimes to several orders of magnitude over other N sources (Corredor et al. 1988, Diaz & Ward 1997, Southwell et al 2008a).

There have been two previous, laboratory-based studies investigating sponge N\(_2\) production (Hoffmann et al. 2009, Schläppy et al. 2010). These lab-based studies were conducted on small (<0.5 g) explant sections of sponge cut from the body of a collected specimen and placed in lab glassware or aquaria. Evidence of sponge-hosted N\(_2\) production was observed in three sponge species from cold-water reef (Geodia barettii; Hoffmann et al. 2009) and Mediterranean environments (Dysidea avara and Chondrosi reniformis; Schläppy et al. 2010). Each of these three species also hosted coincident nitrification, presenting evidence of a coupled nitrification-N\(_2\) production process hosted within sponges. There are two known mechanisms for microbial production of N\(_2\) gas: denitrification and anaerobic ammonium oxidation (anammox). Bacterial (heterotrophic) denitrification is the anaerobic reduction of NO\(_3^-\) to N\(_2\) via intermediary compounds, as follows: \(NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2\). Alternatively, anammox proceeds without extracellular intermediary compounds through the oxidation of ammonium via nitrite: \(NH_3 + NO_2^- \rightarrow N_2\). Both reactions proceed only under hypoxic or anoxic conditions (Brandes et al. 2007). Sponge tissues, particularly those of HMA species, often contain regions featuring suboxic conditions (Hoffmann et al. 2005, Hentschel et al 2006, Martens et al. 2006, Weisz 2006). Coupled with a steady supply of organic matter and
autochthonous DIN production, these suboxic microenvironments create conditions suitable to both denitrification and anammox.

Here we hypothesize that active respiration and nitrification by HMA sponges creates conditions favorable for N₂ production. In marine sedimentary systems, coupled nitrification-N₂ production has been explained by vertical zonation of biogeochemical processes controlled by the availability of chemical reactants (Rysgaard et al. 1994, Risgaard-Petersen 2003). Analogously, in our sponge-microbial system the biogeochemical zonation is governed by unidirectional water flow and respiration within the sponge cell matrix and aquiferous channels. The present study is unique in its in situ investigations of complex coral reef biogeochemistry. This work utilized a novel suite of state of the art instrumentation including an underwater autoanalyzer for NOₓ, a submersible membrane inlet mass spectrometer (MIMS) for dissolved gas analyses, multiple oxygen optode sensors and a variety of complementary instrumentation for environmental observation. The results provide the first evidence for sponge-hosted N₂ production, argued here as likely via denitrification, in a tropical coral reef ecosystem. This process may be an important N sink for an ecosystem believed to thrive in low nutrient conditions.

Methods

Investigations of the role of sponge populations in coral reef C and N cycling were carried out on Conch Reef, a fore reef ecosystem part of the upper reef tract of the Florida Keys, USA. The Conch Reef field site is associated with NOAA-NURC’s Aquarius Reef Base facility in Key Largo, and the infrastructure and personnel associated
with the center were critical to the operations of this research. Nitrogen transformations and resulting cycling by *X. muta* and *I. strobilina* were studied using both direct *in situ* observational time series measurements of NOx flux and O2 concentrations, along with experimental enclosure studies (Fig. 5.1) that incorporated the $^{15}$N isotope pairing technique and mass spectrometry to trace the pathway of N as it was processed by the sponge holobiont.

**NO$_2^-$ + NO$_3^-$ Measurements**

Concentrations of NO$_2^-$ + NO$_3^-$ (hereby NOx) were measured *in situ* using a SEASII spectrophotometric auto-analyzer (developed under Dr. Bob Byrne at University of South Florida, St. Petersburg, FL) with a 15 cm waveguide (Fig. 3.1) (see Chapter 4 this volume, Adornato et al (2007) for a full description of the instrument). Analysis is via the Griess reaction conducted by Cd reduction of NO$_3^-$ to NO$_2^-$ and reaction with sulfanilamide reagent for azo dye formation (Morris & Riley 1963). Calibrated analytical range was linear from 20 nM to 20 µM NO$_3^-$, with peak absorption at 543nm being used up to 10 µM, after which off-peak wavelengths were used to determine analyte concentration (Adornato et al. 2007). Confidence levels for the instrument were established through measurements of an analytical blank of 18.2 MOhm DI H$_2$O completing the reaction pathway and mixing with all reagents. The SEASII minimum detection level (MDL) at 543 nM was calculated as the mean blank measurement (2009=12.8 nM, 2010=10.4 nM, N=6) plus 3SD (2009=0.4 nM, 2010=1.2 nM) to be 14 nM for both the Sept. 2009 and July 2010 deployments. Measured concentration values falling below 14 nM were replaced with a value of 7 nM or half the calculated MDL.
Cadmium reduction column efficiency was calculated using separate standard injections of $\text{NO}_2^-$ and $\text{NO}_3^-$ and was always >98%. Measurements were collected roughly every 10 sec, nearly continuously for several days of observations and experiments. The SEASII instrument remained underwater whenever possible and was serviced daily by SCUBA divers. Calibration curves and/or standard injections were conducted *in situ*, typically on a daily basis. Reagents were replaced every two days and the pump tubing, Cd reduction column, or waveguide was replaced *in situ* if the data indicated suboptimal performance. Optical parameters could be adjusted remotely to improve performance based on real-time data collections. Additionally, discrete syringe collections were conducted throughout the instrument deployments for analysis at the Southeast Environmental Research Center (SERC) at Florida International University for comparisons to traditional lab-based analytical techniques. Data were fit to the pre-deployment calibration curve and adjusted if necessary to align with *in situ* calibration standards. These excursions were derived from small variations in reagents, efficiency of the peristaltic pump tubing, and biological fouling of some optical components. As the instrument spectrophotometer records full intensity data at 10 wavelengths across the spectrum including dark (null) intensity and azo dye non-absorbing wavelength (700 nm), these corrections are derived from quantitative measurements.

**ENCLOSURE EXPERIMENTS**

An *in situ* experimental enclosure method was used to quantify the rates and mechanisms of nitrogen cycling by the sponge-microbial consortia (*cf.* holobiont). The enclosure chamber was placed over a specimen sponge and isolated from the surrounding
benthic substrate by plastic sheeting placed around the base of the sponge (Fig. 5.1). The chamber was secured to the plastic so that no water could be exchanged with either the surrounding water column or the benthic substrate. Two different enclosure chamber volumes were used depending on the size of the specimen sponge: a large enclosure of 147 L total volume and a smaller enclosure of 47 L. Water within the enclosures was continuously circulated by pumping water from the top of the enclosure back to the base with an external submersible pump. Valved fittings were incorporated into the rigid top-plate of the enclosure housing to facilitate the addition of N tracers and sampling of the enclosed water volume by instrument or syringe. All enclosure experiments were dark conditions. The enclosure was instrumented with an Aanderaa O₂ optode to monitor oxygen concentrations and provided sampling ports for continuous sampling by the underwater MIMS (Monitor Instruments, Cheswick, PA) and SEASII-NOₓ and pH instruments (for a description of SEASII-pH see Chapter 3 this volume, Liu et al. 2006). The volume of water used by the SEASII instruments was quite small, approximately 6 mL min⁻¹ removed from the chamber volume. Water sampled by the MIMS was recirculated back into the chamber as no reagents were involved in its analyses.

Experimental progress could be monitored in real-time by a computer on the diving vessel moored above the research site and in near real time at remote shore laboratory locations. Changes in enclosure O₂, pCO₂, N₂, pH, and NOₓ concentration could be observed with the information relayed to divers controlling the experimental procedures and was used to make decisions on when to collect discrete samples or end the enclosure experiments.
Nitrogen cycling mechanisms were elucidated by observing changes in NOx and O2 concentrations using the SEASII and optode sensors in combination with MIMS measurements of $^{15}$N-labeled N2 gas production following the addition of $^{15}$N labeled NH$_4^+$, NO$_3^-$, or NO$_2^-$. For instance, an increase in chamber NOx concentration after the addition of NH$_4^+$ would indicate nitrification, while the disappearance of NOx from the chamber following the addition of NO$_3^-$ would indicate N$_2$ production. N$_2$ production was measured directly as $^{29}$N$_2$ production by underwater MIMS (Martens et al., unpublished manuscript). The isotopically labeled substrates in the isotope pairing technique (Risgaard-Petersen et al. 2003, Trimmer et al. 2006) were utilized to indicate whether N$_2$ was produced via canonical heterotrophic denitrification or through anaerobic ammonium oxidation (anammox) pathways. Although removal of NOx from the closed enclosure system is likely indicative of denitrification, alternative mechanisms of anammox or even dissimilatory nitrate reduction to ammonium (DNRA) via NO$_2^-$ intermediary, followed by anammox (coupled DNRA-anammox) are possible (Kuypers et al. 2003, Kartal et al 2007, Francis et al. 2007). Discrete samples for enclosure NH$_4^+$ and $^{15}$N$_2$O concentration were collected in an effort to discern alternate N transformation pathways. Enclosure control experiments were conducted to estimate the impact of benthic water column processes without sponges present. Controls included zero additions or changes in the amounts of unlabeled DIN and $^{15}$N DIN tracer additions with and without sponges present in the enclosure. This chapter deals specifically with chamber NOx dynamics, while the analysis of isotopic tracing experiments will be discussed in a future manuscript (Martens et al. in prep).
Results

EVIDENCE OF SPONGE NITRIFICATION

Evidence of sponge hosted nitrification, here defined as the production of NO$_2^-$ and/or NO$_3^-$ from NH$_4^+$ or organic matter, was observed by the SEASII-NOx spectrophotometric autoanalyzer during all sponge enclosure experiments. Nitrification was observed during both *X. muta* and *I. strobilina* experiments under control (no treatment) and NH$_4^+$ addition experiments (Figs. 5.2, 5.3, Table 5.1). With the closed-system design of these benthic enclosure experiments the results do not represent real-world process rates, but are an indication of apparent N transformation mechanism. Depleted reactant concentration in the enclosure system will slow rates of concentration-dependent N transformations. Inherent variability of available nutrient supply in the open reef water column were not intended to be reproduced in these enclosures. Rather, this method was designed to bring to light specific hypothesized N transformation pathways. Evidence of sponge hosted nitrification under natural open conditions was observed in an associated study and is presented elsewhere (Gibson 2011, see Chapter 4).

Dramatic increases and decreases in NOx concentrations occurred during enclosure experiments with both *X. muta* and *I. strobilina*. The rates of NOx change were highly variable, however, distinct patterns were observed that were consistent with the hypothesis of a coupled nitrification-N$_2$ production pathway. Experiments involving the addition of NH$_4^+$ always exhibited an initial increase in NOx. Rates of nitrification were calculated as the change in enclosure NOx concentration from initial to peak over the duration of the rise, and are presented for NH$_4^+$ amended experiments only. The rates of nitrification (NOx increase) in the *X. muta* enclosure experiments ranged from 3330.6
to 13528.0 µmol N L\textsubscript{sponge}^{-1} d^{-1}, with a mean nitrification rate of 7272.0 (SD=5478.6, N=3) µmol N L\textsubscript{sponge}^{-1} d^{-1}. Two NH\textsubscript{4}\textsuperscript{+} amendment experiments were conducted on \textit{I. strobilina}, with resulting rates of 1143.8 and 4417.1 µmol N L\textsubscript{sponge}^{-1} d^{-1} and a mean of 2780.4(SD=2314.6, N=2) µmol N L\textsubscript{sponge}^{-1} d^{-1}. Control experiments without a DIN amendment were also conducted and always resulted in increased NOx, a result of the nitrification of ambient NH\textsubscript{4}\textsuperscript{+} and organic matter in the enclosure. The nitrification rates of control experiments were substantially lower for both species, indicating that the reaction rate is limited by the amount reduced nitrogen available for oxidation.

**EVIDENCE OF SPONGE N\textsubscript{2} PRODUCTION**

The addition of NO\textsubscript{3}\textsuperscript{-} (and one experiment with a treatment of NO\textsubscript{2}\textsuperscript{-}, 2010-4) was always followed by a rapid loss of NOx from the enclosure over time (Figs. 5.3-5.6, Table 5.1). In \textit{X. muta} this apparent N\textsubscript{2} production (loss of NOx) rate ranged from 248.9 to 502.0 µmol N L\textsubscript{sponge}^{-1} d^{-1}, with a mean value of 357.4 (SD=130.3, N=3) µmol N L\textsubscript{sponge}^{-1} d^{-1}. Three experiments on \textit{I. strobilina} resulted in apparent N\textsubscript{2} production rates between 235.9 and 2704.0 µmol N L\textsubscript{sponge}^{-1} d^{-1}, or a mean of 1310.5 (SD=1264.6, N=3) µmol N L\textsubscript{sponge}^{-1} d^{-1}. N\textsubscript{2} production appeared to begin in earnest after significant drawdown of enclosure O\textsubscript{2} and the onset of suboxic conditions. Some experiments treated with NH\textsubscript{4}\textsuperscript{+} exhibited a rise in NOx from nitrification followed by a decline of NOx at a slower rate from apparent N\textsubscript{2} production, a pattern suggestive of a coupled nitrification-N\textsubscript{2} production mechanism.

**UNDERWATER MIMS DETECTION OF N\textsubscript{2} GAS**
Dinitrogen gas concentration was observed to rise in the chamber experiments in coordination with reductions of NOx concentration and the onset of suboxic conditions (Martens et al. 2010, Martens unpublished data). Isotopic signatures of $^{15}$N label were observed in the N$_2$ gas. These results provide qualitative confirmation of N$_2$ production, however, inter-calibration of the underwater MIMS with standardized instrumentation are underway and will be presented in a forthcoming manuscript (Martens et al. in prep). These data should present revealing information on the mechanisms of N$_2$ production described below, but will not be further discussed here.

**ENCLOSURE EXPERIMENTS ON OTHER BENTHIC COMPONENTS**

Enclosure experiments conducted on the benthic water column isolated from the substrate (blank control), coral rubble occupied by boring sponges, and a coral head were also carried out for comparison. A small increase in NOx from 138 nM to 166 nM over a 92 min experimental duration was observed during the blank control experiment, and thus nitrification occurring in the reef water captured in the enclosure during all experiments is not considered a significant source of NOx to the enclosure experiments, but may be consequential to the reef ecosystem as a whole (roughly 9 µmol m$^{-3}$ day$^{-1}$). Some of this may be attributed to biofouling “bottle-effect” of the chamber or plastic sheeting. The enclosure experiment of a hard coral specimen (*Porites spp.*) resulted in no significant change in ambient NOx during an hour-long enclosure, with a decline in concentration of 3 nM from 128 nM to 125 nM. As all enclosure studies were conducted as dark experiments, uptake by photosynthetic symbionts should not have occurred.
Previous studies have shown that NO$_3^-$ uptake by corals occurs very slowly below concentrations of 200 nM (D’Elia & Webb 1977, Wafar et al. 1990).

The enclosure experiments conducted on coral rubble containing boring sponge populations resulted in measureable changes to the chamber NOx concentration. A control, non-treatment experiment over this sponge-rubble revealed a nitrification rate of 94 nmol N min$^{-1}$ while enclosure NOx concentration increased by only 282 nmol N min$^{-1}$ in an experiment amended with over 20 μM NH$_4^+$. Interestingly, N$_2$ production rates were high when sponge rubble chambers were amended with NO$_3^-$, yielding a NOx loss rate of 2726 nmol N min$^{-1}$. After the experiments, digestion of boring sponge organic matter in a sodium hypochlorite solution resulted in an estimated boring sponge biomass of 44 g of organic matter (N. Lindquist, unpublished data). The calculated rate of boring sponge-mediated N$_2$ production is thus $3.72 \mu$mol N g$_{sponge}^{-1}$ hr$^{-1}$ (89.21 μmol N g$_{sponge}^{-1}$ day$^{-1}$), an order of magnitude greater than the mean denitrification rate of $I$. strobilina. The results from this single experimental result must be viewed as preliminary and should be repeated, however, given the large abundance of coral rubble on Keys coral reefs, this flux of DIN out of the system could have a profound ecosystem level impact on the overall reef N budget.

**Discussion**

**CHANGES IN NO$_2^-+$NO$_3^-$**

Enclosure experiments indicate extremely rapid and efficient nitrification of NH$_4^+$ regardless of the source, including ambient NH$_4^+$, tracer $^{15}$NH$_4^+$ additions and/or organic matter present in the enclosure water volume. Rates of nitrification were calculated from
the rise of NOx concentration versus time in the enclosure experiments. Nitrification appeared to continue throughout the duration of the chamber experiments although rate of nitrate production did slow in time, presumably with consumption of available reactants. Thus, the nitrification process may be limited by the abundance of NH$_4^+$ and/or O$_2$ as these reactants are consumed to produce NO$_2^-$ and then NO$_3^-$. If production of nitrate is coincident with N$_2$ production within the sponge system, then these rates will be underestimated if calculated by the change in NOx concentration alone. Indeed, five of the *X. muta* enclosure experiments with NH$_4^+$ additions (2009-2, 7, 17, 18) and no treatment (2010-8) exhibited increases in NOx via nitrification followed by a subsequent loss of NOx via apparent N$_2$ production. In all five experiments, a rise in NOx concentration was followed by a fall as measured from initial and peak concentrations for nitrification followed by a systematic drop from peak to final concentrations for apparent N$_2$ production. Therefore, rate measurements obtained during enclosure experiments should be regarded as net rates and likely as underestimates of potential nitrification-N$_2$ production rates. Natural, open system nitrification rates have been calculated using long-term measurements of excurrent NOx flux (see Chapter 4).

**Sponge Nitrification**

The mean rate of nitrification by *X. muta*, 7272.0 (SD=5478.6, N=3) µmol N L$_{sponge}^{-1}$ d$^{-1}$, is substantially higher than that calculated by Southwell et al. (2008) using small incubation experiments, with an estimate of 2520 (SD=600, N=4) µmol N L$_{sponge}^{-1}$ d$^{-1}$. The *in situ* nitrification values reported here for *X. muta* and *I. strobilina* are also significantly higher than laboratory-determined rates found for other sponge species from
outside the Caribbean region. Hoffmann et al. (2009) performed lab based experiments on explants of three species of sponges: the cold water sponge *Geodia barretti*, and two species of Mediterranean sponges *Dysidea avara* and *Chondrisa reniformis*. Their study resulted in nitrification rates for these three sponges of 566, 218, and 242 µmol N L_{sponge}^{-1} d^{-1}, respectively. However, laboratory incubation measurements of nitrification rates by the Caribbean sponge *Chondrilla nucula* (Diaz & Ward 1997) yielded calculated rates (16-41 mmol N L_{sponge}^{-1} d^{-1}) that exceed our value for *X. muta* by 1000 fold.

Direct evidence of nitrification by HMA sponges has been corroborated in other microbiological and genomic investigations of associated microbial communities. The two steps of the aerobic nitrification process, oxidation of ammonia to nitrite and oxidation of nitrite to nitrate, are conducted separately by a variety of microorganisms. Both bacteria (Brock & Wagner 2006) and archaea (Hallam et al. 2006, Francis et al. 2007) have been found to conduct ammonia oxidation, although to date no archaea have been discovered that oxidize nitrite (Ward et al. 2007). Diverse populations of both ammonia-oxidizing (bacteria and archaea) and nitrite-oxidizing (bacteria) microorganisms have been identified through 16S rRNA gene sequencing in sponge tissues (Hentschel et al. 2006, Taylor et al. 2007, Bayer et al. 2008, Mohamed et al. 2010, Lopez-Legentil et al. 2010). These associations may represent an evolutionary adaptation to rid sponges of potentially toxic ammonia wastes through conversion to NO_{2}^{-} and NO_{3}^{-}. This process may be particularly advantageous during period of reduced sponge pumping, and indeed large concentrations of NO_{3}^{-} have been observed in sponges under these conditions (Gibson 2011, see Chapter 4).
**Sponge N₂ Production: The Case for Denitrification**

Hoffmann et al.’s (2009) study of *G. barretti* showed that N₂ production occurred through both denitrification and, at a far lower rate, anammox pathways. This was the first report of sponge N₂ production and the first evidence of any animal-hosted anammox. Schläppy et al.’s contemporaneous 2010 study reported denitrification, but not anammox occurring within the two Mediterranean sponges studied. Our apparent N₂ production rates for *X. muta* (357.4 µmol N L⁻¹ sponge⁻¹ d⁻¹) are higher than, but similar to those reported by Schläppy et al. (2010) for the sponges *D. avara* and *C. reniformis* of 218 µmol N L⁻¹ sponge⁻¹ d⁻¹ and 260 µmol N L⁻¹ sponge⁻¹ d⁻¹, respectively. However, these values are substantially higher than the measured denitrification rate of 92 µmol N L⁻¹ sponge⁻¹ d⁻¹ found for the cold water sponge *G. barretti* in the Hoffmann et al. (2009) study. Our apparent rate from *I. strobilina* (1310.5 µmol N L⁻¹ sponge⁻¹ d⁻¹) is currently the highest N₂ production rate reported for any sponge species (Table 5.2).

The presence of microbial genes related to these N₂ production pathways has also been studied. Hoffmann et al. (2009) found microbial 16s DNA sequences similar to both denitrifying and anammox bacteria in *G. barretti*. Work on a number of sponge species has revealed the presence of genes associated with denitrification, but to date none for anammox (Taylor et al. 2007). Indeed, genomic investigations into the candidate bacterial phylum *Poribacter*, a globally distributed obligate sponge symbiont (Fieseler et al. 2004, Lafi et al. 2009), revealed genes coding for two enzymes critical to the denitrification mechanism, nitrite reductase and nitric oxide reductase (Siegl et al 2011). The LMA tropical reef sponge *Mycale laxissima* was found to host bacteria associated with several N cycling processes including N₂ fixation, denitrification, and
both aerobic and anaerobic ammonia oxidation (Mohammed et al. 2008, 2010), an interesting discovery considering that LMA sponges feature low numbers of detectable microbes (Schmitt et al. 2007). Other reports of vertical transmission of associated bacteria in this species (Enticknap et al. 2006) give reason to question its classification as LMA. However, in the same study Mohammed et al. (2010) found no evidence of anammox bacteria in *Ircinia strobilina*, a sponge that features strong nitrification and large hypoxic zones (Weisz 2006, Southewll et al. 2008) and was one subject of the present study. Thus, there is evidence of sponge association with microbial populations capable of both denitrification and anammox, but these associations are variable among species and perhaps among individuals. Additionally, it is important to note that the lack of evidence of microbial populations does not necessarily mean they are absent, only that the techniques employed to look for them failed to produce results. Further investigations with different molecular techniques (or different individual specimens) may yield evidence of anammox bacteria or other important microbial populations.

Without considering the results of isotopic tracer in N₂ gas, a few additional tests have been conducted to help distinguish between denitrification and anammox as the NOx loss pathway. *Ircinia strobilina* has been tested for indicators of anammox bacteria in both genomic studies (Mohamed et al. 2010) and by attempted isolation of ladderene biomarkers (Santos & Fuentealba 2003; N. Lindquist unpublished). Neither of these studies yielded evidence of anammox bacteria. Furthermore, significant production of ¹⁵N₂O during our experiments (B. Popp, unpublished data), a product generated in abundance with denitrification and only in trace amounts with anammox (0.26% of NO₃⁻ pool, Kartal et al. 2007) suggests that canonical denitrification is the dominant N₂
production pathway in our sponges. Coupled DNRA-anammox could explain observations of NOx loss from the enclosure. Evolution of NH$_4^+$ was not observed to coincide with NOx loss from the enclosures, ruling out significant DNRA if anammox can be ruled out or deemed unlikely to proceed at a rate sufficient to account for NOx loss. Considering no observation of increasing DNRA intermediary compounds (other than potentially N$_2$O), we must implicate reduction of NO$_3^-$ to NO$_2^-$ as the rate limiting step in the removal process. However, Dalsgaard & Thamdrup (2002) found that this was not the case in marine sediments, for the reduction of nitrate to nitrite proceeded at a rate four times that of nitrite reduction and a buildup of NO$_2^-$ was indeed observed. We see no such buildup in our enclosure experiments, although the temporal resolution of our discreet sampling process may not capture all NO$_2^-$ dynamics. Thus, there is a body of evidence in support of denitrification as the primary N$_2$ production pathway and no observed support for anammox. This is particularly relevant with respect to *I. strobilina*, where the highest rates of N$_2$ production are measured and no indicators of anammox bacteria have been found. While it is impossible to rule out anammox altogether for the sponge species we studied, if the anammox process is present its contribution is expected to be minor relative to denitrification (Hoffmann et al. 2009). Future analysis of isotopically labeled N$_2$ generated in the experiments may help further elucidate the N$_2$ source mechanism.

Currently, the factors that regulate rates of N$_2$ production by various HMA sponges are not well understood. Work on marine sediments by Rysgaard et al. (1994) revealed complex interactions between overlying water column O$_2$ concentrations, nitrification rates, and porewater NO$_3^-$ concentrations as controls on denitrification rates.
Capone et al. (1992) found that high NO$_3^-$ porewater conditions stimulated denitrification in a concentration-dependent process. Our sponge enclosure experiments indicate that N$_2$ production, probably through denitrification, is stimulated by low oxygen, high nitrate conditions. *In situ* observations have revealed just such conditions occurring naturally in Conch Reef sponge populations (Gibson 2011, see Chapter 4).

**ECOSYSTEM IMPACTS OF SPONGE NITROGEN CYCLING**

The complex N cycling carried out by dominant coral reef HMA sponge populations fits well within the concept of reef ecosystems being efficient recyclers of essential nutrients (Odum & Odum 1955). The coupled, sponge-hosted microbial nitrification-denitrification reactions observed in this study and previous work can impact the reef ecosystem in a number of important ways. For instance, the efficient remineralization of POM and DOM by HMA sponges releases bioavailable DIN as large fluxes to the surrounding reef environment. This DIN source should contribute significantly to the increasing abundance of fleshy macroalgae on Caribbean reefs. While the growth of macroalgae on reefs is the outcome of a complex interaction of bottom-up and top-down processes, elevated concentrations of DIN will facilitate growth in the absence of grazing pressure (Littler & Littler 2007). Other studies have shown macroalgal communities to increase and decrease with coinciding increases or decreases in nutrients (Cuet et al. 1988, Smith et al. 1981). Although populations of herbivorous fish on Florida Keys reefs are considered to be relatively intact (Paddack et al. 2006), the growth of chemically defended *Dictyota spp.* continues largely unchecked in the absence of the disease-abated *Diadema antillarum* urchin populations (Lessios et al. 1984,
Carpenter 1990, Chiappone et al. 2002) which can consume it in large quantities. Interestingly, the interaction of sponge and macroalgal populations may be mutualistic where algae grows rapidly with elevated DIN and releases large amounts of DOM to serve as a C source for local HMA sponge populations (see Chapter 6).

Elevated concentrations of NOx on reefs have also been implicated in the spread of coral disease (Bruno et al. 2003, Voss & Richardson 2006). Coral pathogens have been a major factor in the decline of Florida Keys coral populations, with significant disease outbreaks occurring throughout the 1980’s-90’s on many Keys reefs (Precht & Miller 2007). Additionally, one recent study has correlated high DIN concentrations with increased prevalence of coral bleaching on Florida Keys reefs (Wagner et al. 2010). Studies have also shown increased boring sponge activity with increasing nutrients (Rose & Risk 1985, Ward-Paige et al. 2005, Holmes 2000). Any stimulation of boring sponge abundance or activity will reduce net ecosystem calcification, a property that has already been greatly diminished with the declining abundance of live hard corals.

Conversely, sponge-hosted N₂ production may be serving as an important and increasing long-term N sink on the reef. While our calculated rates of apparent denitrification are well below those of nitrification, the process is a system sink rather than a recycling process, as in the case of nitrification. In the absence of allochthonous inputs of N or local reef N fixation, sponge hosted N₂ production would in time remove excess N from the system. In addition, increased respiration derived from a general state of reef eutrophication may accelerate this N removal process because both denitrification and anammox pathways are stimulated by low oxygen conditions.
Our enclosure experiments found significantly higher apparent denitrification rates in *I. strobilina* than in *X. muta*. However, the abundance of *X. muta* on Conch Reef is much higher than that of *I. strobilina* (2.77 and 0.08 L\textsubscript{sponge} m\textsuperscript{-2}) (Gibson 2011, see Chapter 4; Southwell et al. 2008), and known to be increasing (McMurray et al. 2010). Thus, the impact of *X. muta* on the ecosystem N budget is much more substantial. The increased rates observed in *I. strobilina* are likely due to longer water residence time and a larger degree of hypoxia within the sponge tissue relative to *X. muta* (Weisz 2006, Martens et al. 2006). A relationship has been made between the abundance of sponge-hosted microbial communities and pumping rate (Weisz et al. 2008), where reduced flow rates in HMA sponges may facilitate hypoxic conditions that allow a greater degree of elemental cycling or energy extraction within the sponge (Hoffmann et al. 2005, Schläppy et al. 2007, Gibson 2011, see Chapter 4). Interestingly, at least one study has observed that the biogeochemical processes generally associated with HMA sponges also take place in LMA sponges (Schläppy et al. 2010). Microbial community composition within the sponge may thus have a role in sponge biogeochemical processing that would not be predicted from microbial abundance alone.

Compared with N fluxes from coral reef sediments, N inputs from sponge populations appear to have a greater impact on reef scale N budgets. Although few data exist for reef sediment nutrient fluxes, generally rates obtained from reef and coastal systems are less than those of sponge populations for DIN flux (Southwell et al. 2008, Diaz & Ward 1997, Capone et al. 1992, Corredor & Capone 1985). Potential rates for sponge-hosted denitrification are similar to rates calculated in studies on reef sediments (Capone et al. 1992, Corredor & Capone 1985, Seitzinger & D’Elia 1985, Alongi et al.
2007), although direct comparisons are difficult due to differences in sediment depth and characteristics and sponge verticle growth and biovolume (Table 5.2). As sponge abundances are increasing on Florida Keys fore-reef communities (McMurray et al. 2008), their role in reef N cycling becomes more critical. Indeed, considering their abundance, rates and mechanisms of N cycling, and capacity to process much of the reef water column in a single day (Gibson 2011, see Chapter 4), sponges likely control the N dynamics of this ecosystem. Management decisions should carefully consider the impacts of sponge populations on the future of the Florida Keys coral reef ecosystem.
Fig. 5.1. Instrument array and sponge enclosure experimental chamber deployed on Conch Reef, FL. Photo courtesy of Chris Martens.
Fig. 5.2. Time-series of parameters measured during *X. muta* enclosure experiment 2010-1. Discrete data are collected at distinct points during the enclosure while SEASII and Optode measurements are near continuous.
Fig. 5.3. Time-series of parameters measured during *X. muta* enclosure experiment 2010-8. Discrete data are collected at distinct points during the enclosure while SEASII and Optode measurements are near continuous.
Fig. 5.4. Time-series of parameters measured during an enclosure experiment of boring sponge blocks (2010-12). Discrete data are collected at distinct points during the enclosure while SEASII and Optode measurements are near continuous.
Fig. 5.5. Time-series of parameters measured during *I. strobilina* enclosure experiment 2010-4. Discrete data are collected at distinct points during the enclosure while SEASII and Optode measurements are near continuous.
Fig. 5.6. Time-series of parameters measured during *Ectoplasia ferox* enclosure experiment 2010-11. Discrete data are collected at distinct points during the enclosure while SEASII and Optode measurements are near continuous. Note in this plot the SEASII-pH is sampling both inside the enclosure and outside in the ambient reef water column for alternating periods.
Table 5.1. Table of enclosure experiments conducted on Conch Reef in the Florida Keys in 2009 and 2010 (continued on following page). Experiments with a and b designations denote coupled nitrification-denitrification.

<table>
<thead>
<tr>
<th>Year</th>
<th>Exp. #</th>
<th>Treatment</th>
<th>Species</th>
<th>Sponge Vol. (L)</th>
<th>Sponge Mass (g)</th>
<th>Chamber Vol. (L)</th>
<th>Duration (min)</th>
<th>ΔµM NOx</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>2a</td>
<td>Control</td>
<td>X. muta</td>
<td>29.54</td>
<td>4460</td>
<td>147</td>
<td>37</td>
<td>5.88</td>
</tr>
<tr>
<td>2009</td>
<td>2b</td>
<td>Control</td>
<td>X. muta</td>
<td>29.54</td>
<td>4460</td>
<td>147</td>
<td>52</td>
<td>-1.25</td>
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<tr>
<td>2009</td>
<td>3</td>
<td>14.7 µM NO$_3^-$</td>
<td>X. muta</td>
<td>29.54</td>
<td>4460</td>
<td>147</td>
<td>95</td>
<td>-4.26</td>
</tr>
<tr>
<td>2009</td>
<td>4</td>
<td>Control</td>
<td>I. strobilina</td>
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<td>47</td>
<td>96</td>
<td>1.44</td>
</tr>
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<td>5</td>
<td>15.7 µM NO$_3^-$</td>
<td>I. strobilina</td>
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<td>420</td>
<td>47</td>
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<td>8</td>
<td>Control</td>
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<td></td>
<td></td>
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<tr>
<td>2009</td>
<td>11</td>
<td>20.5 µM NH$_4^+$</td>
<td>I. strobilina</td>
<td>3.01</td>
<td>490</td>
<td>47</td>
<td>115</td>
<td>5.85</td>
</tr>
<tr>
<td>2009</td>
<td>12</td>
<td>15.5 µM NO$_3^-$</td>
<td>I. strobilina</td>
<td>3.01</td>
<td>490</td>
<td>47</td>
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<td>0.66</td>
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<tr>
<td>2009</td>
<td>14</td>
<td>Control</td>
<td>I. strobilina</td>
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<td>490</td>
<td>47</td>
<td>106</td>
<td>0.44</td>
</tr>
<tr>
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<td>15</td>
<td>15.8 µM NO$_3^-$</td>
<td>Sponge Rubble</td>
<td></td>
<td></td>
<td></td>
<td>103</td>
<td>-6.01</td>
</tr>
<tr>
<td>2009</td>
<td>16</td>
<td>15.3 µM NO$_3^-$</td>
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<td>37.02</td>
<td>5590</td>
<td>147</td>
<td>91</td>
<td>-7.99</td>
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<tr>
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<td>Control</td>
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<td>37.02</td>
<td>5590</td>
<td>147</td>
<td>38</td>
<td>4.24</td>
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<tr>
<td>2009</td>
<td>17b</td>
<td>Control</td>
<td>X. muta</td>
<td>37.02</td>
<td>5590</td>
<td>147</td>
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<td>Control</td>
<td>Porites spp. Coral Head</td>
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<td>0.00</td>
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<td>1</td>
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<td>135</td>
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<td>2010</td>
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<td>8 µM NH$_4^+$</td>
<td>X. muta</td>
<td>2.95</td>
<td>436</td>
<td>147</td>
<td>202</td>
<td>13.96</td>
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<td>2010</td>
<td>3</td>
<td>26.8 µM NH$_4^+$</td>
<td>I. strobilina</td>
<td>0.68</td>
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<td>47</td>
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<td>6.08</td>
</tr>
<tr>
<td>Year</td>
<td>Exp.</td>
<td>Treatment</td>
<td>Species</td>
<td>Sponge Vol. (L)</td>
<td>Sponge Mass (g)</td>
<td>Chamber Vol. (L)</td>
<td>Duration (min)</td>
<td>ΔµM NOx</td>
</tr>
<tr>
<td>------</td>
<td>------</td>
<td>-----------</td>
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<td>----------------</td>
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</tr>
<tr>
<td>2010</td>
<td>4</td>
<td>12.1 µM NO$_2^-$</td>
<td>I. strobilina</td>
<td>0.68</td>
<td>112</td>
<td>47</td>
<td>119</td>
<td>-3.23</td>
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<tr>
<td>2010</td>
<td>8a</td>
<td>11.4 µM NH$_4^+$</td>
<td>X. muta</td>
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<td>2010</td>
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<td>X. muta</td>
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<td>-1.93</td>
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<td>2010</td>
<td>10</td>
<td>13.4 µM NO$_3^-$</td>
<td>Sponge Rubble</td>
<td>47</td>
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<td></td>
<td>-7.20</td>
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<td>2010</td>
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<td>10.1 µM NO$_3^-$</td>
<td>E. ferox</td>
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<td>13.1 µM NO$_3^-$</td>
<td>Boring Sp. Blocks</td>
<td>8</td>
<td>47</td>
<td>110</td>
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<td>-16.24</td>
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</table>
Table 5.2. Potential denitrification rates from coral reef benthos. Data are not normalized and represent variable sediments depth or sponge abundance as noted.

<table>
<thead>
<tr>
<th>Denitrification Rate (µmol N m$^{-2}$ hr$^{-1}$)</th>
<th>Location</th>
<th>Integrated Sed. Depth or Sponge Vol.</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>Bahamas</td>
<td>25 cm</td>
<td>Seitzinger &amp; D'Elia (1985)</td>
</tr>
<tr>
<td></td>
<td>Back reef lagoon sediment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50-100</td>
<td>SW Puerto Rico &amp; Mona Island Fringing reef sediment</td>
<td>10 cm</td>
<td>Corredor &amp; Capone (1985)</td>
</tr>
<tr>
<td>0.12-13.3</td>
<td>Great Barrier Reef Fore, middle, &amp; back reef sediment</td>
<td>2 cm</td>
<td>Capone et al. (1992)</td>
</tr>
<tr>
<td>1.25-93.1</td>
<td>Great Barrier Reef Inter-reef sediments</td>
<td>8-10 cm</td>
<td>Alongi et al. (2007)</td>
</tr>
<tr>
<td>26.2-56.3</td>
<td>Conch Reef, Florida Keys Fore reef sponge X. muta</td>
<td>2.77 L$_{sponge}$</td>
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<td>0.2-8.6</td>
<td>Conch Reef, Florida Keys Fore reef sponge I. strobilina</td>
<td>0.08 L$_{sponge}$</td>
<td>This Study</td>
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</tbody>
</table>
CHAPTER 6:
SPONGE-ALGAL INTERACTIONS ON A DEGRADED CORAL REEF ECOSYSTEM: A FEEDBACK TOWARDS STABILITY?

Introduction

Reef building hard coral populations have declined globally over the last few decades (Pandolfi et al. 2003, Gardner et al. 2005, Bruno & Selig 2007). Drivers of coral loss vary greatly by location, yet often align with direct and indirect impacts of human populations (Hughes et al. 2003, Halpern et al. 2008). All coral reefs, and indeed all ecosystems, have been impacted by global scale environmental changes including climate change (Hoegh-Goldberg 1999) and ocean acidification (De’ath et al. 2009). Regional and local stressors including disease outbreaks (Green & Bruckner 2000), land-based pollution (Fabricius 2005), and over-fishing (Hughes 1994, Jackson et al. 2001) also negatively impact reef hard coral populations. As hard coral populations decline, space on the reef habitat is occupied by other benthic populations through opportunistic or competitive mechanisms. This transition to a new characteristic community of ecosystem structure is often termed a phase-shift. Classically, phase-shifts in tropical reef ecosystems result in dominance of fleshy macroalgae in place of hard corals (Done 1992), although other transitions have been documented (Norström et al. 2009).

Ecological literature, particularly in the field of coral reef ecology, is rich with debate of the definition, theory, and evidence regarding interconnected concepts of ecosystem phase-shifts, stable states, and resilience (see recent reviews by Ulanowicz (2002), Knowlton (2004), Petraitis & Dudgeon (2004), Bruno et al. (2009), Norström et
al. (2009), and Hughes et al. (2010) for an introduction to the concepts and controversies. Much of this debate centers on the concept of system stability, and the existence of alternate stable states of ecosystems. Although there is disagreement among some scientists as to what defines a “stable” system state, there is general acceptance that one mechanism through which stability is achieved is the formation of autocatalytic (self-reinforcing) feedback loops (Knowlton 1992, Ulanowicz 1995, Lee 2006, Norström et al. 2009, Hughes et al. 2010). These feedbacks serve to support the resilience of an alternative system state even after the disturbance(s) instigating the phase-shift have been removed (Knowlton 2004). If the alternative state is indeed a stable system, these autocatalytic feedback processes act to built system maturity and vigor through self-selection mechanisms that optimize ecosystem efficiency (Ulanowicz 1980, 1995, Costanza & Mageau 1999). This study tests for the presence of an autocatalytic feedback loop between emerging sponge and macroalgal populations on a degraded reef environment and discusses the possible implications for an alternate stable state on the reef ecosystem.

**Changes in Reef Community Structure**

The coral reef ecosystem of the Florida Keys outer reef tract has been particularly degraded over the last few decades, despite concerted efforts by several protection efforts, including the establishment of the Florida Keys National Marine Sanctuary (FKNMS). Whereas coral decline in some areas of the Caribbean has exhibited periods of moderate reversal, the Florida Keys coral cover has continuously declined (Gardner et al. 2003, Schutte et al. 2010). This decline occurred despite the relatively intact
herbivorous fish populations (Paddack et al. 2006, 2009). There has been little to no recovery of the Diadema antillarum urchin populations, however, an important algal grazer of Caribbean reefs (Chiappone et al. 2002). Bleaching, disease, and storm events have all contributed to hard coral decline in the Florida Keys (see Precht and Miller (2007) for a thorough review).

Conch Reef, an offshore fore- and mid-reef habitat in the upper Florida Keys has been the site of extensive research, including that of this study and many of the studies referenced herein. Conch Reef is designated a research only Special Protected Area within FKNMS and is closed to recreational diving or fishing and is representative of much of the Florida Upper Keys reef tract. Most offshore fore-reef communities now have less than 5% coral cover. Algal benthic coverage on Keys fore-reef communities frequently exceeds 70%, with the phaeophyte Dictyota spp. averaging 36% (Aronson et al. 1994, Beach et al. 2003). Dictyota spp. produce a number of powerful secondary metabolites that are known to inhibit (though not prevent) grazing by herbivorous fish (Hay et al. 1987), compounding impacts from the decline of Diadema populations. Sponge cover on these outer reefs can exceed 10%, with up to 5% comprised by a single species, the giant barrel sponge Xestospongia muta (Bertin and Callahan 2008). Populations of X. muta are increasing on the reef, with an average population density increase of 46% from 2000 to 2006 as measured by McMurray et al. (2010). Data regarding the change in abundance for other species of sponges are few, but it would not be unreasonable to speculate that the environmental conditions favorable to expansion of X. muta populations may also be favorable to other common sponge species.
The community at Conch Reef has an average of >2.6L m\(^{-2}\) of erect sponge species (Southwell et al. 1998, Martens et al. unpublished), along with extensive patches of encrusting sponge with differing morphologies. Boring sponge species are also prevalent and have been observed in living, dead, and rubble coral skeleton. Areal coverage by the common boring sponge *Cliona delitrix* exceeds 10% of the benthic habitat in fore-reef slope communities (Chiappone et al. 2007). The cryptic and endolithic nature of boring sponge cover can be difficult to measure, therefore their coverage values are almost certain to underestimate their true biomass. Numerous studies indicate that the abundance and bioerosion rate of boring sponges has been increasing, particularly with increasing reef ecosystem eutrophication (Holmes 2000, Rose & Risk 1985, Chazottes et al. 2002, Rützler 2002). The change in Florida Keys coral reef community structure has been extensive enough for some researchers to conclude that a phase-shift has already occurred in this system (Maliao et al. 2008).

**IMPACT OF SPONGE PUMPING**

Active pumping by sponges effects a number of ecosystem functions. Sponges are powerful filter feeders, with particle retention rates as high as 99% for some size fractions in the range of picoplankton (0.2-2.0\(\mu\)m) (Pile 1997, Lesser 2006, Hadas et al. 2009). Rates of pumping can reach 50,000 times the volume of the sponge per day in some species common to reefs throughout the Florida Keys and the Caribbean (Weisz et al. 2008). Calculations have shown that the abundance and activity of *X. muta* populations on Conch Reef is sufficient to filter a 11 m column of overlying water in a single day (Gibson et al., see chapter 3).
ROLE OF CORAL REEF SPONGES IN BIOGEOCHEMICAL TRANSFORMATIONS OF CARBON AND NITROGEN

The movement of water through chambers and across cell surfaces inside the sponge facilitates rapid exchange of nutrients and waste products and establishes chemical gradients. These gradients are conducive to complex biogeochemical processing by the sponge and symbiotic microbial communities associated with many sponge species (Wilkinson 1978a-c, Hentschel et al. 2006, Hoffman et al. 2008, Southwell et al. 2008). Sponges with large associated microbial communities (classified as High Microbial Abundance or HMA species) have been shown to host a number of important microbiotically-mediated biogeochemical processes including C fixation (Wilkinson 1983, van Duyl et al. 2008), DOC respiration (Reiswig 1974, Yahel et al. 2003, Gibson 2011, see chapter 2), N-fixation (Wilkinson & Fay 1979, Mohamed et al. 2008), nitrification (Corredor et al. 1988, Diaz & Ward 1997, Jimenez & Ribes 2007, Bayer et al. 2008, Southwell et al. 2008, Gibson 2011, see Chapters 3 and 4), and denitrification (Hoffman et al. 2009, Schlappy et al. 2010, Gibson 2011, see chapter 4). Two of these processes, namely DOC respiration and nitrification, helped to spur this investigation of a sponge-algal feedback loop on Florida Keys coral reefs where HMA species are particularly abundant (Southwell et al. 2008).

Respiration of POM and DOM by sponges will release remineralized waste products back into the ambient water column. Nitrogenous compounds may be released as either NH$_4^+$ or NO$_3^-$ depending on the extent of microbial nitrification hosted by the sponge (Southwell et al. 2008). Coral reefs are characterized by low concentrations of
available dissolved inorganic nitrogen (DIN) and efficient recycling of matter within their ecosystems (Odum & Odum 1955). The extent to which reef macroalgal communities benefit from inputs of DIN has been controversial and conflicting (Hatcher & Larkum 1983, Littler & Littler 1984, 2007, Lapointe 1997, 1999, Hughes et al. 1999, McCook 1999, Lirman 2001, McCook et al. 2001, Smith et al. 2001), yet there is little evidence that reef macroalgal population in Florida are presently limited by DIN (Miller et al. 1999, Szmant 2002, Beach et al. 2006, Gibson et al. 2008, Sotka & Hay 2009). Recent studies have indicated that while top-down grazing pressure effectively limits the coverage extent of benthic macroalgae in Florida, the grazer population is saturated and cannot reduce the current stock of macroalgae (Paddack et al. 2006). Any disturbance to the herbivore population may therefore allow the algal community to expand to a point beyond which it cannot be effectively cropped and thus reduce the amount of top-down control. Under these circumstances, which may already be present on Keys reef ecosystems, bottom-up forces such as nutrient limitation will take a greater role in dictating community structure. Thus, it is important to understand the controls on bottom-up drivers such as nutrient concentration and light availability for ecosystems in transition such as the Florida Keys. Any stimulation of primary production on the reef will increase the abundance of DOM and POM, and in turn stimulate the growth of heterotrophic ecosystem components such as sponges and bacteria. The putative feedback mechanism between autotrophic algal populations and heterotrophic sponge populations operates through the exchange of metabolic byproducts. This autocatalytic interaction could be particularly devastating to hard coral populations and reef carbonate structures,
especially if combined with the potential stimulation of bioeroding boring and encrusting HMA sponge populations.

TESTING FOR A FEEDBACK LOOP

A lab-based experiment was designed to investigate possible feedback pathways of matter exchange between algal and sponge populations. The purpose of our experimental design was to follow the path of C and N compounds as they are exchanged in a sponge-algal system. The central hypothesis postulates that: I.) autotrophic macroalgal populations fix inorganic C and N into organic matter that is released to the reef ecosystem, where; II.) sponge populations respire the organic C to CO$_2$ and remineralize organic N into bioavailable inorganic forms that, subsequently; III.) the reef macroalgal population utilizes for primary production and completes the feedback cycle (Fig. 6.1). The experiment progressed in three phases described in detail below. Essentially, fleshy macroalgae was grown in a seawater medium of inorganic, isotopically labeled C and N substrates to fix these tracers into biomass (Phase I). This labeled algae was placed in an aquarium with a reef sponge. The tank water was sampled over time to identify the presence of label in various constituents that indicate how it was processed (Phase II). The labeled algae was subsequently removed from the tank and fresh, unlabeled algae was added and allowed to grow (Phase III). This new batch of algae was sampled after a period of time to look for evidence of uptake of the label, essentially completing a matter exchange pathway. A number of replicates and controls, described in detail below, were conducted in order to constrain the possible mechanisms involved in these exchange processes.
The algae used in this study was *Dictyota spp.*, the most abundant macrophyte on Florida fore-reef communities. The algae is epilithic and epiphytic and can be found growing on almost any firm substrate on the reef including bare hard-bottom, living and dead corals, sponges, and calcareous algae (Herren et al. 2006). *Dictyota* has also been shown to leak a labile portion of its recently fixed organic carbon (Brylinsky 1977), a possible source of matter exchange in the system. *Ircinia strobilina*, the common stinker sponge, was selected for the sponge component of our model system. *Ircinia strobilina* is a common but not generally abundant sponge on fore- and mid-reef communities. It is an HMA species lacking association with symbiotic photosynthetic microbes, a known nitrifier (Southwell et al. 2008), and is known to meet the majority of its metabolic demand through respiration of DOC (Gibson 2011, see Chapter 2). *Dictyota* and *Ircinia* genera have broad distributions with representative species common to Caribbean and Indo-Pacific reefs.

**Methods**

**SPECIMEN COLLECTION**

Sponge specimens were collected from fore-reef communities in the upper Keys several days before the beginning of the experiment to allow recovery from collection. *I. strobilina* is extremely difficult to cut and is strongly attached to hard-bottom substrates on the reef. Therefore, specimens were collected by chiseling their attachment points so as to minimize disturbance to the sponge tissues. The remaining carbonate rock on the sponge was useful for handling and attaching the sponge during further manipulations. After collection sponge specimens were attached to a stable platform in 12 m of water for
at least three days. Only healthy looking sponges were selected for use in the aquarium experiment. The sponges were transported back to the lab within 1 hour in a 100 gallon aerated container full of ambient reef water.

*Dictyota* algae was collected by hand, removing the plant from the reef by its base, gently shaking off evident sediment and placing in zip-top bags. The algae specimens were transported back to land in the bags placed in an aerated cooler filled with ambient reef water. The bags of algae were left open while in the cooler to allow exchange with the aerated water. Algae specimens were collected on the same day of their use in the aquarium experiment.

**AQUARIUM EXPERIMENT—PHASE I: GROWING LABELED ALGAE**

*Dictyota* tissue was allocated into separate vexar mesh packets for later dispersal amongst various treatment tanks. These sample packets each contained approximately 50g (wet weight) of algae were placed in a 40 L glass aquarium outside and were shaded from direct sun exposure. The aquarium water was circulated with a spill pump and covered with polyethylene film to limit gas exchange. All subsequent experimental tanks were treated in the same manner. Seawater in the tanks was fresh offshore reef water filtered (1 µm) to remove most plankton from the water column. The *Phase I* treatment tank was amended with a 200 µM, or roughly 10%, DIC enrichment as NaH\(^{13}\)CO\(_3\). The aquarium DIN pool was enriched by 10 µM through the addition of \(^{15}\)NH\(_4\)Cl. Isotopically labeled C and N were purchased from Cambridge Scientific and featured isotopic ratio of >99% label. The isotopic treatment was intended to produce a significant label to ensure its signal could be followed over several subsequent
transformations throughout the experiment. The algae was grown in this medium for 39 hours under natural indirect sunlight (two daylight periods) to ensure new growth had occurred. At the end of the growth period, the algal envelopes were rinsed in fresh reef water to remove excess label and allocated to treatment aquaria for *Phase II* of the experiment.

**Aquarium Experiment—Phase II: Sponge respiration of algal organic matter.**

All tests were conducted concurrently in 40 L glass aquaria as described in *Phase I*. Each tank contained one *I. strobilina* and two packets of labeled *Dictyota* algae from *Phase I*. There were three replicates of the primary treatment design and four control treatments designed to constrain the mechanisms involved in nutrient cycling. Controls were conceived as baseline methodological tests and to isolate the biological effects of the three possible players in the experimental tanks, namely the sponge, the algae, and water column microbes. The control tests were aquaria established with *No Sponge* addition, *No Algae* addition, *No Treatment* addition where fresh unlabeled algae was added instead of the labeled batch, and a *Filtered* treatment where the tank water was repeatedly filtered throughout the experiment through a 0.2 μm hollow-fiber filter cartridge to remove microbial organisms from the water column. The control tanks were not replicated. All experiments were sampled over 48 hours until the end of *Phase II*.

**Aquarium Experiment—Phase III: Algal utilization of sponge derived DIN.**

At the end of *Phase II*, the labeled algae from *Phase I* was removed from all test aquaria. Freshly collected *Dictyota* was distributed in vexar mesh packets
(approximately 50g wet weight) to all treatment tanks from Phase II. The tanks then contained the metabolic products of the experimental sponge-algal system, including any isotopic label derived from Phase I algal organic matter. The Phase III Dictyota was grown in these aquaria for 46 hours and tested for uptake of isotopic label, which would only be available in the tanks as remineralized organic matter from Phase I.

Tissue Sampling and Analysis

Tissue samples for C and N stable isotope analyses were collected prior to experimental treatment of the sponge and algal specimens. Sponges were sampled by excising a small wedge of sponge tissue from the base of the sponge with a new razor blade. All samples included both pinacoderm and mesohyl layers but avoided excurrent oscula. Sponge samples were squeezed to remove as much water from the tissue as possible. Sponge cells are known to be highly mobile, moving freely within the mesohyl tissue matrix, and a sample need not be collected from an axial growth region to represent materials of recent assimilation (Hooper et al. 2002). Dictyota algal tissue was collected from each batch in duplicate. Sampling was repeated at the end of the entire experiment for I. strobilina and at the end of each experiment phase for the Dictyota. All tissue samples were immediately frozen in HDPE sample bottles and remained frozen until they could be lyophilized. Dry tissue samples were ground in a clean mortar and pestle and placed in combusted silver cups for solid phase combustion and GC-MS analysis of C and N stable isotopes. The silver cups were placed overnight in a vapor flux chamber containing 12 M HCl to remove inorganic carbon contamination and were subsequently
AQUARIUM WATER SAMPLING AND ANALYSIS

Water from the experimental aquaria was sampled eight times during Phase II of the experiment. Samples were collected for DOC, DIC isotopic ratio, and NO$_3^-$ isotopic ratio. All samples were collected with a clean, 3x sample rinsed 150 mL plastic syringe and sub-sampled immediately in the lab.

DOC samples were filtered through a pre-combusted GFF filter into duplicate pre-combusted 20 ml glass sample vials. Each sample vial was preserved with 100 µL 50% concentrated H$_3$PO$_4$ and kept at 4°C until analysis. Analysis was conducted by high temperature catalytic oxidation (HTCO) with non-dispersive infra-red detection (NDIR) on a Shimadzu TOC-5000 (see Chapter 2, this volume).

D$_1^{13}$C samples were collected by injection of 10 mL of sample water into 20 mL evacuated crimp-seal headspace vials and preservation with 100 µL concentrated HgCl$_2$. DIC samples were stored upside down at 4°C until analysis by GC-MS. DIC in the samples was converted to CO$_2$ by adding 100 µL 50% H$_3$PO$_4$ and shaking for 60 sec. The vial headspace was sampled via syringe and needle after addition of He to eliminate the vacuum. A time period of 60 sec was allotted for allowing gas equalization across the needle bore interface to prevent sample fractionation. The gas samples were injected by hand into the GC-MS sampling port.

$^{15}$NO$_3^-$ samples were dispensed into 60mL HDPE bottles and frozen until analysis. Samples were processed by the method of Sigman et al. (2001), involving
conversion of available NO$_3$ to N$_2$O gas by a culture of *Pseudomonas chlorophasis*. The bacterium *P. chlorophasis* is a denitrifying organism that lacks nitrous oxide reductase, the enzyme necessary to convert N$_2$O to N$_2$ gas and complete the denitrification pathway. The N$_2$O gas produced by the culture retains the isotopic ratio of water column NO$_3^-$ and is subsequently analyzed on a stable isotope GC-MS. Presence of labeled $^{15}$NO$_3^-$ in the water column would indicate nitrification of algal organic matter in *Phase II*.

**Results and Discussion**

**PHASE I: LABEL UPTAKE BY *DICTYOTA* ALGAE**

Algal tissue samples were analyzed for $\delta^{13}$C and $\delta^{15}$N, before and after treatment in labeled seawater. The initial, natural abundance $\delta^{13}$C and $\delta^{15}$N were -17.5‰ and 2.5‰ respectively. After growth in seawater labeled with NaH$^{13}$CO$_3$ and $^{15}$NH$_4$Cl for two daylight cycles, the algal tissue was labeled to 789.9‰ $\delta^{13}$C and 6637.5‰ $\delta^{15}$N. These values represent the final isotopic ratios for *Phase I* and the starting ratios for *Phase II*. The extreme values were desirable in order to follow the pathway of matter transfer through several successive processes and fractionation events.

**PHASE II: RESPIRATION OF ALGAL ORGANIC MATTER**

Tissue samples from *I. strobilina* at the initiation of the experiment had natural $\delta^{13}$C values ranging from -15.9‰ to -18.6‰. Nitrogen isotopic ratios were in the range of 1.9‰ to 4.3‰. At the end of the experiment, sponge and algal tissue were re-sampled. *Dictyota* tissue from *Phase I* became much lighter with respect to $\delta^{13}$C over the course of *Phase II*. The final (end of *Phase II*) $\delta^{13}$C for *Dictyota* ranged from 143.1‰ to 298.7‰.
This range does not include the value from the Filtered treatment due to significant fractionation of the C pool likely resulting from the filtration process. The δ\(^{13}\)C value of algal tissue in this tank at the end of Phase II was \(-2767.2\%\). Because of this effect, any isotopic results from the Filtered treatment must be discredited. The δ\(^{15}\)N of the Phase I algae also became lighter over the course of Phase II, indicating a replacement of much of the algal tissue grown in Phase I with tissue containing ambient natural DIN. The average change in the main treatment tanks was δ\(^{15}\)N value was \(-4364.2\%\) (SD=682.6). Once again, the Filtered treatment produced anomalous results, although not nearly as drastic as that of C, with a change of \(-5146.9\%\) of δ\(^{15}\)N. Interestingly the No Sponge treatment changed by only \(-3009.1\%\) in δ\(^{15}\)N, significantly less than that of the treatments with sponges. This could indicate less turnover of algal biomass and potential N limitation with lack of sponge-hosted nitrification.

Final sponge tissue ratios showed little change with respect to δ\(^{13}\)C. All experimental treatments (excluding Filtered, see above), including the No Treatment test became slightly heavier in δ\(^{13}\)C. The average shift in δ\(^{13}\)C of the three Main Treatment tanks was 0.31\%, while the No Treatment tanks changed by 0.20\%. The tank with No Algae addition changed by very little, only 0.08\%, perhaps indicating some interaction between algae and sponge affecting the sponge tissue δ\(^{13}\)C (Fig. 6.2). As these shifts are all very small and likely within the error of the instrument, all results are speculative. While changes in δ\(^{13}\)C of sponge tissue did not show a dramatic effect of interaction with labeled algae, the δ\(^{15}\)N signal does show an impact. Average sponge tissue δ\(^{15}\)N in the Main Treatment tanks changed by 2.2\% (SD=0.2) from start to finish. The tanks without labeled algae, i.e. No Algae and No Treatment, showed a shift of only 0.17\% and 0.18\%
respectively. Once again the Filtered tank was anomalous, with a $\delta^{15}$N shift of -1.13‰. The increase of sponge $\delta^{15}$N after interaction with labeled algae likely indicates assimilation of algal-derived DON. However, the small change relative to the amount of $^{15}$N label, combined with the negligible shift in $\delta^{13}$C indicates that the amount assimilated algal organic matter was very small. It is possible that the enriched $\delta^{15}$N signal is due to highly labeled PON or DON entrained but not assimilated by the sponge, effectively contaminating the tissue signal. Considering these confounding factors, isotopic results from sponge will not be considered a creditable indicator of DOM exchange.

**Phase II: DIC**

Results of $\delta^{13}$C sampling of water column DIC revealed a clear signal of respiration of algal organic matter in all labeled treatments (Fig. 6.4). The source of the respiration is, however, somewhat unclear. The sponge, water column microbes, and algae itself will all respire labile organic matter derived from the algae. While the experiment was designed to isolate the relative impact of these three system components on the DIC signal, variability in the response from the three replicate main treatments and lack of replication in control treatments obscure this finding. Particularly confounding is the high level of respiration in the No Sponge treatment tank, containing only the algae and water column microbial populations. One explanation for the strong signal in this tank is the filtering activity of the sponges in the other treatment tanks inhibiting the growth of the microbial population. Without the top-down pressure of sponge predation on the microbes, the community may have grown to large and diverse populations capable of respiring large amounts of DOC quickly. Additional complexity is added by
the possibility of microbial respiration and assimilation of labeled DOC followed by sponge feeding and respiration of now labeled microbes. While this possibility cannot be ruled out, the relevance to our hypothesis and the impact to the reef ecosystem would remain the same; sponges still respire algal derived DOM though may be doing so through intermediary water column microbial populations.

**PHASE II: DOC**

All experimental aquaria exhibited erratic DOC concentration changes. The values are suspect and we will not attempt to interpret them here. Field collected seawater samples analyzed at the same time feature a precision of <4.0 μM C, so instrumental or methodological factors are unlikely culprits for the inexplicable DOC concentrations observed (see Chapter 2). It is possible that a source of contamination was present in the polyethylene film covering the aquaria or that volatile organic carbons or hydrocarbon combustion byproducts were incorporated from extensive boating and boat-yard activity adjacent to the experimental location. Attempts to corroborate DOC results through mass balance with DIC is impossible, considering the open system. Future experiments of this design should consider glass-top or closed system tanks for more robust analyses.

**PHASE II: DISSOLVED $\delta^{15}$NO$_3^-$**

A key mechanism in the putative feedback loop between sponge and algal populations is the uptake of sponge derived DIN by macroalgal populations. Previous studies on Conch Reef have shown evidence of this process under natural and in situ
experimental conditions relying on the natural abundance of $\delta^{15}\text{NO}_3^-$ of sponge derived DIN (Silbiger and Lindquist, unpublished). The mechanism was investigated in our aquarium experiments by tracing the fate of $^{15}$N labeled algal organic matter from *Phase I* through the respiration pathway of *Phase II* and into the aquarium water column. The experimental tanks were sampled at three time points in order to monitor changes the abundance of any released $\delta^{15}\text{NO}_3^-$. A temporal increase would be indicative of nitrification of labeled algal organic matter by the sponge or water column microbial community. *Ircinia strobilina* is known to be an active nitrifier (Southwell et al. 2008), so this is a likely fate of the organic N introduced to the aquaria containing sponges in *Phase II*.

Steady increases in $\delta^{15}\text{NO}_3^-$ abundance occurred in the water samples from experimental tanks containing *I. strobilina* and labeled algae (Fig. 6.5). The two treatments that contained no labeled algal matter (*No Treatment* and *No Algae*) exhibited a significant reduction of the $\delta^{15}\text{NO}_3^-$ signal (Fig. 6.5). This dilution effect is expected due to the very light nature of NO$_3^-$ derived from sponge-hosted nitrification (Southwell et al. 2008b). The enrichment of $\delta^{15}\text{NO}_3^-$ in tanks containing $^{15}$N-labelled algae provides a pool of label that can be followed during the completion of the feedback loop back into algal biomass. Increases of the $\delta^{15}\text{NO}_3^-$ abundance in the *No Sponge* treatment tank also provided evidence of nitrification by the microbial communities living free in the water column or attached to the algal biomass. A small amount of nitrification (~9 μmol m$^{-3}$ d$^{-1}$) was observed during a contemporaneous *in situ* enclosure experiment on Conch Reef (Gibson 2011, see Chapter 5). The initial (immediately following algal addition) $\delta^{15}\text{NO}_3^-$ of *Phase II* aquaria receiving labeled algae was enriched over that of the aquaria
containing no labeled algae. The source for this $^{15}$N enrichment could be near instantaneous nitrification of leached algal DOM. Alternatively, there was a possible transfer of $^{15}$NO$_3^-$ produced in Phase I via water column microbial nitrification from the Phase I tank, even after rinsing the algae in clean seawater, or possibly contamination of the $^{15}$NH$_4$Cl with $^{15}$NO$_3^-$. 

**Phase III: Algal Tissue Samples**

Completion of the sponge-algal feedback loop relies on algal uptake of sponge derived NO$_3^-$ (and potentially CO$_2$, although this product should not limit algal growth) produced by its associated nitrifying microbes. We tested this mechanism by adding freshly collected Dictyota to the experiment aquaria at the end of Phase II. Water in these tanks contained the products of respiration carried out by components of the model system, whether sponge, algae, or microbial communities.

Algal tissue sampled from aquaria receiving labeled algae from Phase I was significantly enriched in $\delta^{15}$NO$_3^-$ at the end of Phase III. The initial algal $\delta^{15}$NO$_3^-$ value, reflective of the natural fore-reef environment, was 2.3‰. The mean $\delta^{15}$NO$_3^-$ of algal tissue from labeled tanks exceeded 50‰ (Fig. 6.3). Algae sampled from the No Sponge treatment also showed uptake of $^{15}$N label derived from microbial nitrification, although to a lesser extent than the treatments containing sponges. Tissue from the No Sponge tank had a final $\delta^{15}$NO$_3^-$ of 16‰ (Fig. 6.3). These data indicate that while the remineralization of algal organic matter can proceed through pathways mediated by epiphytic or water column microbial communities, the actively pumping HMA sponge
populations present on the reef accelerate the rate and extent of these biogeochemical mechanisms.

Conclusions

Sponge and macroalgal populations now occupy most of the available hard substrate on the offshore fore-reef community in the upper Florida Keys. One hypothesis for the relative success of these two ecosystem components is the presence of an autocatalytic feedback loop between sponge and algal populations. Our lab-based, aquarium experiments tested this hypothesis through the use of stable isotopically labeled C and N compounds as tracers during assimilation of algal-produced organic matter by sponges and algal uptake of DIN released from sponges (Fig. 6.1). Data on N matter exchange between sponges and algae directly demonstrated a feedback loop through assimilation by algae and remineralization by sponges. Results for the C exchange pathway were more ambiguous as differences between treatments and controls could not conclusively be reconciled. Indeed, with the notable exception of dissolved $^{15}$N$_2$O, little conclusive evidence was produced in the experiment to argue for sponge respiration of algal derived organic matter. Future experiments would benefit from better controls to account for all possible respiration pathways.

This study, as well as an in situ field study of the same area (Silbiger & Lindquist, unpublished), have generated direct evidence of N uptake by Dictyota and a consequent reduction in algal C:N ratio. However, previous studies by Miller et al. (1999) and Sotka & Hay (2009) found no significant uptake of N by Dictyota after fertilization experiments on the same reef ecosystem. Beach et al. (2006) conducted two experiments on Conch
Reef with *Dictyota* and found evidence of N limitation in one case but not the other. Contrasting results on evidence of N limitation in Dictyota implicates factors other than simply DIN concentration may be affecting algal N uptake. The effects of seasonality, depth and light availability, and history of exposure are all candidates for factors controlling *Dictyota* N uptake (Lirman & Biber 2000, Beach et al. 2006, Fong & Paul, 2011).

It is unlikely that the benthic cover of fleshy macroalgae on this Florida Keys reef is limited by nutrient availability (Miller et al. 1999, Beach et al. 2006, Gibson et al. 2008, Sotka & Hay 2009). The concentration of DIN rarely exceeds the putative 1 µM threshold (Boyer & Briceno 2010, Gibson unpublished data) of reef eutrophication proposed by Lapointe (1997) leading to algal dominance (Littler & Littler 1984). This is in spite of large fluxes of bioavailable DIN from allochthonous offshore tidal bore events (Leichter et al. 2003) and autochthonous nitrification by the prolific and expanding HMA sponge communities (Southwell et al. 2008, McMurray et al. 2010, Martens et al. unpublished). The lack of a persistent rise in DIN concentration on the reef implicates biological sinks or physical export mechanisms are balancing system inputs. Physical export from the reef system is unlikely to account for this apparent mass balance, as these mechanisms are not likely to change dramatically on the scale of a few decades that coincides with the system phase-shift. Recently our research team has directly measured sponge-hosted denitrification, a biological process that will act as a N sink within this system, however, the initial measured rates of denitrification are much smaller than coincident rates of nitrification in typical environmental conditions (Gibson 2011, see chapter 4 this volume). Therefore, it appears likely that the flux of DIN to the reef
ecosystem is balanced by biological assimilation and enrichment of organic matter in the reef ecosystem (Fabricius 2011). However, long-term water quality monitoring does not indicate eutrophication (Boyer & Briceno 2010), albeit the quarterly surface and bottle cast samples are not of sufficient resolution to capture reef temporal and spatial dynamics, particularly of benthic boundary layer processes. Perhaps the reef ecosystem, in its present state of sponge-algal dominance, has achieved efficiencies and interconnections sufficient to prevent or absorb geochemical imbalances. That is to say, perhaps this ecosystem has developed resilience.

Unlike many tropical reef ecosystems, the Florida Keys herbivorous fish population is not heavily fished (Bohnsack 2006) and populations of key herbivore species remain active as significant reef grazers (Paddack et al 2006, 2009). Paddack et al. (2006) determined that present populations of herbivorous fish in the upper Keys are capable of limiting algal populations to their current extent through grazing pressure, but are unable to reduce algal standing stocks. There is likely a threshold of algal benthic cover which, once achieved, overwhelms grazing pressure by herbivores such that they can no longer prevent expansion of algal populations. A study by Lirman (2001) concluded that common reef-building corals of the upper Florida Reef Tract were susceptible to mortality from algal overgrowth, noting that long-term survivorship of these coral was threatened unless algal populations could be reduced. Whether the excess of available food for herbivores will expand their populations or grazing rates remains to be seen, although Lirman & Biber (2000) found no evidence of a link between algal biomass and grazers or grazing rates on a Florida Reef system. This short-term study does not preclude long-term effect, however, the link grazing pressure and resource
supply is probably non-linear due to the defenses deterring herbivory that are present in the dominant macroalgal species (Hay 1991). Alternatively, the enriched algal biomass with a lower C:N ratio may become preferable to fish grazers, and its rate of consumption may increase (Boyer et al. 2004, Sotka & Hay 2009) and form a negative feedback with respect to the algae. And yet, this process would kindle further complexity as *Dictyota* is incredibly successful at fragmentative reproduction, a process stimulated by increased grazing by fishes (Harren et al. 2006). What would appear to be a control process on the growth of *Dictyota* could be further encouraging its spread and reinforcing the feedback loop we have described.

The ecological consequences of this feedback are broad and generally detrimental to the success or recovery of hard corals. Direct competition between macroalgae and hard corals will rarely result in coral mortality (McCook et al. 2001). Algal growth on coral reefs is generally considered to be not a cause but a consequence of coral mortality (of which Florida reefs have experienced plenty) (Aronson & Precht 2006). However, Lirman (2001) did observe coral mortality and dramatic reductions in growth rate in Florida reef building species when algae and coral interfaced, a result that intensified under reduced grazing pressure. The algae in this experiment did not directly overgrow live coral, but once established on available substrate the algae had significant effects on coral health (Lirman 2001). With average benthic coverage of 36% and maximum coverage exceeding 70% (Beach et al. 2003), *Dictyota spp.* are clearly established on Conch Reef, and, notably, have been for at least 20 years (Aronson et al. 1994). Established algal communities will impact reef structure through indirect mechanisms. Coral larvae cannot settle on macroalgae and thus a primary indirect effect will be the
suppression of coral recruitment (Hughes & Tanner 2000, McCook et al. 2001, Mumby et al. 2007). Algae may also impact live corals through the release of dissolved compounds that may stimulate detrimental microbial populations on corals (Kline et al. 2006, Smith et al. 2006) or trigger disease (Nugues et al. 2004; but see Vu et al. 2009). Additionally, as macroalgae may serve as a reservoir for coral pathogens (Nugues et al. 2004), increases in algal populations may lead to increased incidence of coral disease and yet another potential beneficial feedback to the algal community (Aronson & Precht 2006). The severity of these coral disease outbreaks is likely to be exacerbated by increased availability of nutrients (Bruno et al. 2003, Voss & Richardson 2006), possibly derived from the activities of sponge populations (Southwell et al. 2008a, Gibson 2011, see Chapter 4).

Sponges have also established themselves as principal components of the Conch Reef ecosystem. We spend much effort studying the ecological roles of conspicuous erect sponges populating the reef, but there are many cryptic, boring and encrusting sponge varieties that receive less attention. Boring sponge populations can threaten the structural stability of reef ecosystem (Neumann 1966, Rutzler 1975, Hallock & Schlager 1986, Glynn 1997). Sponges will compete directly with and overgrow corals with some success, but they can also undermine live coral structures through bioerosion (Holmes et al. 2000). These processes seem especially effective when corals are stressed (Rutzler 2002). Glynn (1997) notes that bioerosion will increase with both conditions causing coral tissue death and conditions favoring the growth of the bioeroder over that of calcifying species. Indeed, scientists have made observations of boring and encrusting sponges rapidly colonizing recently bleached corals (Aronson et al. 2002, Shonberg &
Ortiz 2008) and corals recently subjected to periods of thermal stress (Rutzler 2002). Boring sponge abundance and rate of bioerosion is increased by elevated nutrient conductions (Rose & Risk 1985, Holmes 2000, Holmes et al. 2000). With the losses of large coral communities, the calcification rates of the Florida Keys fore-reef may not be sufficient to keep up with the increasingly active bioeroding sponge community. Hallock (1988) contends that stimulation of bioerosion by eutrophication had resulted in “drowned reefs” in the geologic record. If the conditions supporting the growth and proliferation of macroalgal and erect sponge populations also benefits bioeroders (de Goeij et al. 2008), the Florida Keys reef may be such a drowning reef system.

Clearly, complexities inherent in nonlinear dynamics of adaptive systems make predictions of the trajectory of ecosystem state extremely difficult. While this study evinces a series of interconnected mechanisms that could build stability into a sponge-algal reef complex, uncertainties regarding the influence of other ecosystem components and their respective feedback mechanisms require further research.
Fig. 6.1. Conceptual diagram of putative sponge-algal autocatalytic (self-reinforcing) feedback loop.
Fig. 6.2. Sponge tissue C (top) and N (bottom) isotopic signals at the beginning and end of the experiment (Phase I through Phase III).
Fig. 6.3. Change in algal tissue C (top) and N (bottom) isotopic signatures from Phase III of the experiment. There is a clear signal of sponge derived DIN uptake by the algae. Data for the final 13C isotopic value of the Filtered treatment was excluded due to anomalous results (see text for details).
Fig. 6.4. $\delta^{13}C$ from water column DIC in experimental aquaria. Signal from algal matter respiration is present in all treatments receiving label. Variability in results prevents conclusive statements of direct sponge respiration of algal organic carbon.
Fig. 6.5. Trend of aquarium tank water $^{15}\text{NO}_3^-$ during the experimental period. Samples from aquaria receiving labeled algae show increasing $^{15}\text{NO}_3^-$, while those receiving no treatment see a depletion characteristic of sponge excurrent $^{15}\text{NO}_3^-$ natural abundance.
CHAPTER 7:
CONCLUSIONS AND FINAL THOUGHTS

This research investigated the role of sponge populations on the ecology of a coral reef ecosystem. Sponges are evolutionarily ancient animals that have come to inhabit nearly every marine (and some aquatic) benthic habitat on Earth. Coral reefs are among the most valuable ecosystems on the planet for the goods and services they provide that influence human welfare. The research findings presented here improve our knowledge of both sponge ecology and coral reef ecology and it is the hope of the author that these data will be built upon and inspire future research in a variety of fields and habitats.

This research found that coral reef sponge populations are capable of rapid and dramatic C and N transformations that impact reef ecosystem water quality. These transformations are the result of complex biogeochemical mechanisms carried out by the sponges and the associated microbial communities commonly found living in their tissues. Interactions between the sponges and other reef components including hard corals and fleshy macroalgae may be altering reef ecosystem structure and function, likely resulting in an increase in sponge and algal dominance at the further expense of hard corals. In the context of global coral reef decline, this research implicates sponge populations in benefiting from, and potentially contributing to, the decline of hard coral populations and an ecosystem phase shift. These results may be unique to the study system of the upper Florida Keys fore-reef community, though the mechanisms involved are potentially occurring in a number of similar systems.
Much of the data presented in this dissertation was only made possible through the use of novel *in situ* instrumentation. These devices facilitated investigations of nutrient cycling and benthic metabolism at scales and resolutions otherwise impossible. Creative use of advancing technologies for detecting the chemical and physical dynamics of marine ecosystems is likely to be a major focus of future research in marine science. These technologies are emerging for investigations at multiple scales: from microprobes for sub-millimeter spatial resolution of chemical gradients to instrument arrays, like those used here, for local scale observations to networks of ocean buoy sensors for basin scale parameterization of ocean dynamics to satellite systems for observations of global productivity and climate change. An important factor for maximizing the benefit of these technologies is the identification of critical interactions within and among ecosystems that operate a large amount of control on the system dynamics. These “hot-spots” and “hot-moments” exist at all scales and may not be obvious, yet their role becomes clear when investigations are conducted with a systems-based approach.

The body of work on sponge ecology provides an interesting example of the revelation of a critical ecosystem component and the utility of systems-based science. Although sponges have always been present on tropical coral reefs, they garnered little attention until the decline of hard coral populations revealed the relative health and abundance of sponges on many reef ecosystems. The historical dogma of sponge ecology expounds their roles as filter feeders working to “clean” the water column to provide a better habitat for the reef-building corals, and their capacity to bind reef rubble and stabilize vital hard substrate on the reef. As their associations with microbial communities became apparent, the range of functions carried out by sponges began to be
explored. It is now known that nearly every reaction in the complex N cycle can be carried out by sponges. Indeed, this biogeochemical faculty coupled with the incredible pumping capacity of these animals likely exerts a powerful regulatory mechanisms on the form and availability of N on the reef.

The discovery of coral reef sponge N processing, while certainly important on the reef, may find greater value when this knowledge is applied to other ecosystems where sponges are abundant. This study examined the Florida Keys coral reef, a fringing barrier reef on the Atlantic ocean side of the Keys archipelago. The reef ecosystem is relatively open and experiences exchange of water and material with the coastal ocean. Across the Keys island chain lies the shallow, largely confined estuary that is Florida Bay. The seagrass beds and mangrove islands that characterize this ecosystem are home abundant and diverse populations of sponges, animals with the same capacity for pumping and biogeochemical transformations as those on the neighboring coral reef. The difference lies in the greater ecosystem dynamics and the potential for autochthonous processes to dominate. Florida bay has far less water mass and hydrologic exchange than the offshore reef, and the relative importance of sponge processing may be greatly amplified in this system relative to the more open reef system. Indeed, the process of sponge-mediated nitrification/denitrification may play a critical role in balancing the N budget for Florida Bay and mediating N inputs from upstream land-based inputs.

This dissertation provides evidence that sponge populations are inheriting the reef from the scleractinian coral that built it. The implications of this shift are not yet known, nor do we have an understanding of the resilience of a sponge-dominated reef ecosystem. There is some evidence that the sponge populations are existing in autocatalytic feedback
with abundant fleshy macroalgal populations, a phenomena that would support the continued growth and development of these populations while hindering the recovery of hard corals on the reef. The emergence of sponges, algae, and soft corals as providers of reef structure is not an ecosystem capable of persisting in geologic time or surviving conditions of sea-level rise. Indeed, the Florida Keys reef appears to have “gone soft” and since it is unable to accrete significant new carbonate structure to match rising sea-level, it will likely become a drowned reef.
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