THE ROLE OF MARINE SPONGES IN CARBON AND NITROGEN CYCLES OF CORAL REEF AND NEARSHORE ENVIRONMENTS

Daniel Ryan Hoer

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Approved by:

Christopher S. Martens

Niels L. Lindquist

Carol Arnosti

Robert H. Byrne

Jaye E. Cable

ABSTRACT

Daniel Ryan Hoer: The Role of Marine Sponges in Carbon and Nitrogen Cycles of Coral Reef and Nearshore Environments
(Under the direction of Christopher S. Martens)

Sponges and their microbial consortia can alter the water quality of the surrounding environment through animal and hosted microbial chemical transformations resulting from their dynamic pumping, water filtration, and respiration processes. The goal of this dissertation was to quantify the role of these organisms in the cycles of carbon (C) and nitrogen (N) on reefs and representative environments of Florida Bay and describes five principle findings: 1) the abundant coral reef sponge *Xestospongia muta* satisfies the bulk of its respiration oxygen (O_2) demand through uptake of dissolved organic carbon, and this species removed C in excess of O_2 demand which is presumed to be reserved for cellular maintenance, growth, and the generation of reproductive materials. 2) Respiration activities in this species yielded a tremendous flux of dissolved inorganic nitrogen (DIN), and the rate of this N release appeared to be broadly conserved between Floridian and Bahamian reefs. 3) The magnitude and speciation of exhalent DIN from species tested in Florida Bay showed similar rates of N efflux as those on the reef, yet the remineralization of particulate organic matter appears to be the dominant feedstock for the observed N release. 4) The N released from these species represented a dominant source of N to a budget calculated for an offshore basin in Florida Bay (Mystery Basin). 5) Bloom conditions swept through Mystery Basin decimating sponge populations and water column N, and yielded significant and lasting changes to the chemical and ecological structure of the system. These

results indicated that sponges have the capacity to alter local water quality through the observed C and N transformations mediated by the holobiont (sponge and associated microbiome), and further suggests that they can drastically impact ecosystems where their populations dominate.

For Mom and Dad
You told me if I kept following my dreams, I would find something that I love to do.
You were right.

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LIST OF ABBREVIATIONS AND SYMBOLS

GF/F: Glass fiber filter; ~0.7 μm nominal pore size

DIN: Dissolved inorganic nitrogen; NH_4^+, NO_2^-, and NO_3^-

TN: Total nitrogen; all nitrogenous material which passes through a glass fiber filter (~0.7 μm nominal pore size; inorganic and organic compounds)

DOC: Dissolved organic carbon; operationally defined as organic carbon which passes through glass fiber filter

DON: Dissolved organic nitrogen; all nitrogenous material which passes through a glass fiber filter minus inorganic nitrogen (DON = TN – DIN).

DOM: Dissolved organic matter, all material that passes through a glass fiber filter inclusive of both carbon and nitrogen

HMA: High microbial abundance (*sensu* Hentschel et al. 2006), referring to species of sponges hosting large tissue microbial communities

LMA: Low microbial abundance (*sensu* Hentschel et al. 2006), referring to species with tissue microbial densities similar to surrounding seawater.

Δ[Analyte]: Refers to the excurrent concentration minus the ambient concentration of the stated analyte

NO_x^-: Combined concentrations of NO_3^- and NO_2^-

POC: Particulate organic carbon; operationally defined as all material which is retained on a glass fiber filter

POM: Particulate organic matter; all material retained on a glass fiber filter inclusive of both carbon and nitrogen

TOC: Total organic carbon; approximated as the sum of particulate and dissolved organic carbon (TOC = POC + DOC)

CHAPTER 1:

Introduction: Sponge Impacts on Coastal Chemical Cycles

Sponges are ubiquitous features of all marine environments. They form large and growing populations that dominate the benthic biomass in coastal ecosystems of South Florida (Butler et al 1995, Peterson et al. 2006, McMurray et al 2010). Sponges have large water filtering capacity, up to 50,000 times their own tissue volume each day (Weisz et al. 2008), and they dramatically alter the water quality of the surrounding environment through animal and hosted microbial chemical transformations directly associated with their pumping, water filtration, and respiration processes. The large heterotrophic capacity of these organisms and their ability to feed on both particulate and dissolved organic matter (POM and DOM), make them powerful drivers of organic matter cycling in coastal systems (Yahel et al. 2003, Gibson 2011, de Goeij et al. 2013); sponge populations in cryptic habitats on reefs are capable of consuming carbon (C) equivalent to the total fixed locally by primary productivity (de Geoij et al. 2008a). As a result of these respiration processes, the effluent jet of filtered water exhaled from these organisms is often characterized by high levels of dissolved inorganic nitrogen (DIN), making them important sources of remineralized nitrogen (N) as well as physical and chemical drivers of coupling between those processes occurring near the benthos and those which occur in the overlying water column (e.g., Corredor et al. 1988, Lesser et al. 2006, Southwell et al. 2008). Understanding the effect of localized, low-level nutrient enrichment from sponge effluent plumes on the chemical budgets of the surrounding benthos is critical given that these areas are important for chemical and energy exchange in coastal marine environments. The central goal of

my dissertation research was to quantify the importance of sponge-mediated C and N cycling processes in coral reef ecosystems and representative environments of Florida Bay where processes mediated by these organisms may be dominant drivers of nutrient cycling, productivity, and changes in water quality. The role of these processes in the tested ecosystems may directly translate to other coastal environments where sponges are abundant.

My first objective was to explore the respired C source of a common Caribbean reef sponge (*Xestospongia muta*) by determining the proportion of the metabolic oxygen (O_2) demand that is satisfied through respiration of dissolved and particulate organic matter. Based upon previous work with this species (Martens et al. unpublished data, Gibson 2011), I hypothesized that *X. muta* would satisfy the majority of its O_2 demand from DOM consumption with only a minor contribution from POM uptake. Sponges efficiently feed across a wide range of particle sizes (Reiswig 1971); however, even early studies of sponge energetics (Reiswig 1971, 1973, 1981) revealed an apparent discrepancy between their uptake of POM and metabolic C demands as indicated by their rates of O_2 utilzation. Sponges, particularly high microbial abundance (HMA) sponges, are thought to fill this metabolic C gap through utilization of dissolved organic carbon (DOC) (Yahel et al. 2003; de Goeij et al. 2013). Investigations of encrusting sponges commonly found in coral cavities showed that approximately 40% of the C uptake is accounted for by respiration; the remainder is hypothesized to be assimilated to account for rapid chaonocyte cell turnover in the sponge animal (de Goeij et al. 2008a, 2008b, 2009). Improved understanding of the C cycling mediated by these organisms and corresponding ecosystem impacts is critical to understanding trajectories of coral reef change, particularly in the Caribbean where sponges and seaweeds are maintaining a dominant and increasing presence on the benthos. Increased *in situ* studies with non-manipulated sponges will provide further insight into the

native behavior of these organisms, and will serve to advance the understanding of their role in coral reef ecosystems.

The product of this organic matter respiration is a large efflux of recycled N in the exhalent stream of sponges that has been observed to generate an ecologically relevant source of recycled N in coastal environments (e.g., Corredor et al. 1988, Jiménez and Ribes 2007, Southwell et al. 2008b, Fiore et al. 2013). How the processes mediated by these organisms are conserved across environmental gradients is only beginning to be understood. Consequently, I tested the degree to which the release of recycled N by the giant barrel sponge *X. muta* is conserved between the environments found on the Florida Keys reef tract and those found on the oligotrophic reefs of San Salvador, Bahamas.

Many studies focus on sponge-rich reef environments that were characterized by open exchange with the ocean (e.g., Corredor et al. 1988, Jiménez and Ribes 2007, Southwell et al. 2008b, Fiore et al. 2013). Florida Bay represents a similarly sponge-rich environment as some of the tested reefs, yet it is characterized by a shallow water column where physical transport is restricted due to abundant mud shoals which subdivide the bay into discrete basins (e.g., Phlips et al. 1995, Boyer et al. 1997, Fourqurean and Robblee 1999). Due to the physical restriction, these basins exhibit highly variable water residence times, and experience an augmented influence of local processes in chemical cycles (e.g., Fourqurean et al. 1993, Rudnick et al. 2005, Zhang and Fischer 2014). Sponge biomass survey results from Peterson and co-workers (2006) showed sponges at almost 75% of their surveyed sites, with biomass contributions of over 1400 g sponge dry weight m^{-2}; areal coverage of sponge biomass was focused on the hard-bottom areas along the southern edge of the bay as well as the eastern and western margins. With this expansive coverage and large biomass, sponge populations almost assuredly serve a critical role

in remineralizing organic matter and regenerating inorganic nutrients, yet species-specific biomass estimates will be required in order to determine the magnitude and potential ecological significance of this contribution. My objective was to determine if the sponge species found in the estuarine environment of Florida Bay exhibit similar behaviors to those species found on the reefs of the Florida Keys. Specifically, I aimed to determine if the species in FL Bay exhibited a dichotomy in exhalent DIN associated with the quantity of microbial biomass living in their tissues (e.g., Southwell et al. 2008b), if the exhalent DIN is a result of respiration of dissolved or particulate organic matter (DOM or POM), and if there is a divergence in respired organic matter associated with the presence or absence of microbial symbionts. I hypothesized that low microbial abundance species (LMA) would exhibit an effluent plume dominated by NH_4^+ and HMA sponges would exhibit large concentrations of NO_x^- in their exhalent jets (Jiménez and Ribes 2007, Southwell et al. 2008b, Bayer et al. 2008). Furthermore, I hypothesized that DOM will dominate organic matter metabolism of HMA sponges and be of negligible importance in LMA species (de Goeij et al. 2008; Gibson 2011). In order to test these hypotheses, the speciation and magnitude of DIN flux was determined for 11 dominant species using benthic chamber experiments across three sites exhibiting the range of benthic environments found in Florida Bay.

Bay-wide nutrient budgets constructed for this system (e.g., Rudnick et al. 1999, Boyer and Keller 2007) have shown that most N and P in the water column are in organic forms (Boyer et al. 1997, Boyer et al. 2006), and sources of these nutrients are often similarly dominated by organic matter (as much as 90% of influent N from the Everglades is as DON; Boyer et al. 1999, Childers et al. 2006). Consequently, local recycling processes have been found to regulate the supply of dissolved inorganic nitrogen (DIN) in many locations (Rudnick et al. 2005, Boyer and

Keller 2007, Boyer et al. 2009). For example, seagrass meadows have been shown to be particularly important sites of DIN generation through dissolved organic nitrogen (DON) remineralization (Yarbro and Carlson 2008). While sponges exhibit a dominant presence in this environment (Peterson et al. 2006) and have a demonstrated ability to influence local DIN concentrations with their remineralization processes (e.g., Corredor et al. 1988, Southwell et al. 2008b, Hoer 2015, this volume), the role of these organisms in Florida Bay N budgets has been largely unaddressed. We chose to explore the role of sponges on the N cycle of Florida Bay in a sponge-rich basin in the west-central region of the bay (Mystery Basin) where DIN contributed from the sponge population may serve an ecological role in buffering N limitation from influent marine conditions from the Gulf of Mexico (Lavrentyev et al. 1998). In order to quantify the role of sponges in this environment, sponge biomass was quantified through benthic surveys and the measured, species-specific efflux from the dominant species was applied to the surveyed biomass. The resultant contribution was compared to other fluxes for Florida Bay, and we hypothesized that sponge efflux would be a dominant source of recycled N to the system and would be critical to meet the N demand from predominantly seagrasses. Additionally, we hypothesized that this N budget would vary spatially based on the local contributions of various processes, and a simple model was constructed to evaluate the dominant processes throughout the tested basin. However, the hypothesized dominance of sponge efflux on the N cycle in Florida Bay is subject to considerable temporal variability given the susceptibility of these organisms to rapid, mass mortality events (Butler et al. 1995, Peterson et al. 2006).

Florida Bay has experienced a series of large ecological disturbances which intensified in the late 1980s with a mass mortality event observed in the dominant seagrass, *Thalassia testudinum* (Fourqurean and Robblee 1999). Contemporaneously, a series of intense and

persistent phytoplankton blooms were observed throughout the Bay (Phlips and Badylak 1996, Phlips et al. 1999). These dense blooms are hypothesized to have precipitated rapid sponge die-offs (Butler et al. 1995, Wall et al. 2012); in two consecutive bloom events in central Florida Bay (1991-1992 and 1992-1993) locally dominant species (*S. vesparium*, *Iricina* sp., and *Spongia* sp.) experienced mortalities ranging from 40 to 100% (Butler et al. 1995). Despite temporal overlap, a direct, causal link between blooming *Synechococcus* and sponge mortality remains unclear (Butler et al. 1995, Lynch and Phlips 2000, Peterson et al. 2006). Yet, the ephemeral nature of intense blooms and their often sudden onset makes observation of such impacts difficult. In early September 2013, bloom conditions were found in Mystery Basin, a small, offshore basin located just north of the Arsnicker Keys that was the target site of a recently constructed N budget. The bloom was first noted by fishermen at locations in Rabbit Key Basin just to the north of Mystery Basin prior to our observations. Site descriptions by both our group and reports from fishermen to the Florida Fish and Wildlife Conservation Commission indicated that the bloom appeared to be expanding southward from a point of origin north or northeast of Rabbit Key. We had generated significant data characterizing this location prior to the onset of bloom conditions (extensive water quality sampling and surveyed sponge biomass) that enabled a unique examination of the ecosystem response of a sponge-rich basin throughout a cyanobacterial bloom and following its dissipation.

The research presented in this dissertation comprises a significant contribution to the understanding of the role of sponges in the coastal cycles of C and N. Particularly, we highlighted the ability of these organisms to represent a dominant driver in local chemical cycles, particularly where their populations are large. Furthermore, these processes were tested across environmental gradients within Florida Bay as well as within the wider Caribbean in an effort to

understand how the behavior of these organisms changed in response to varying habitats. As a result, the findings presented herein may have applicability to a broad suite of habitats where the tested sponges are found.

CHAPTER 2:

Majority of Respiration Demand of a Common Caribbean Sponge Met by Dissolved Organic Carbon Consumption

Introduction

Hard coral cover has declined from an average nearing 50% to less than 10% on Caribbean reefs between 1977 and 2001 (Gardner et al. 2003). Overall scleractinian cover on Caribbean reefs remains suppressed, with most reefs exhibiting less than 20% total cover (Green et al. 2008, Perry et al. 2013). Concomitant with this decline, sponge density began increasing, and sponges now dominate benthic biomass on some reef ecosystems (Aronson et al. 2002, McMurray 2010). Shallow water Caribbean sponge communities have large water filtering capacity (Corredor et al. 1988, Weisz et al. 2008) and their exhaled water carries with it the imprint of a wide variety of biogeochemical transformations mediated by the sponge holobiont (sponge animal plus associated microbial and macrofaunal communities) (Southwell et al. 2008, Hoffmann et al. 2009, Maldonado et al. 2012). Many sponges host vast consortia of microbes within their tissues (termed high microbial abundance (HMA) sponges, Hentschel et al. 2006) that drive diverse nutrient element transformations. In contrast, sponge species with low numbers of associated microorganisms (called low microbial abundance (LMA) sponges, Hentschel et al. 2006) produce effluent with a chemical signature dominated by the products of animal-based metabolism (Southwell et al. 2008, Webster and Taylor 2012). The cycling of carbon (C) and nitrogen (N) by HMA sponges is of critical importance for the health of reef ecosystems because of the large and increasing populations of this group of sponges and their large capacity to pump

and filter water. The relative abundance of HMA sponges represented 50% or more of the total sponge biomass below 15 m water depth in 13 of 15 benthic surveys of reefs throughout the Caribbean between 1978 and 2011; of the 8 studies during this period reporting trending in sponge populations, 6 showed an increasing trend in HMA cover below 15 m (Pawlik et al. 2015 and citations therein).

Sponges efficiently feed across a wide range of particle sizes (Reiswig 1971); however, early studies of sponge energetics (Reiswig 1971, 1973, 1981) revealed an apparent discrepancy between their uptake of particulate organic carbon (POC) and metabolic C demands as indicated by their rates of oxygen (O_2) uptake. Sponges, particularly HMA sponges, are thought to fill this metabolic C gap through utilization of dissolved organic carbon (DOC) (Yahel et al. 2003; Gibson 2011). Investigations of encrusting sponges commonly found in coral cavities showed that ~40% of the C uptake is accounted for by respiration; the remainder is hypothesized to be assimilated to account for rapid chaonocyte cell turnover in the sponge animal (de Goeij et al. 2008a, 2008b, 2009). At the reef scale, this process is thought to represent a C uptake rate of a similar magnitude as the rate C fixation due to gross primary productivity (de Goeij et al. 2013). Non-encrusting species of sponge have also been shown to have the capacity to utilize DOC (Yahel et al. 2003, Gibson 2011, and Mueller et al. 2014), but compared to cryptic species, evidence of DOC uptake in satisfaction of their metabolic C requirements is limited. In this study we tested for direct DOC uptake and respiration by the HMA sponge *Xestospongia muta* (Demospongiae), commonly called the giant barrel sponge, to quantify the relative importance of DOC and POC to the metabolic C demand of a non-cryptic, non-excavating species on Caribbean reefs. Sponge pumping rate as well as removal of DOC, POC, and O_2 were measured

in situ in undisturbed individuals attached to their original substrate in order to minimize any physiological changes resulting from physical manipulation.

Methods

Study animal

X. muta is an important component of total benthic biomass on reefs throughout the Caribbean (Büttner 1996, Armstrong et al. 2006), and especially on the Florida Keys reef tract where it can represent as much as 65% of total sponge biomass with population densities as high 0.2 sponges m^{-2} (Southwell et al. 2008, McMurray et al. 2008, 2010). *X. muta* is an HMA sponge, with tissue bacterial densities of up to 8×10^9 microorganisms per gram of sponge wet weight (Hentschel et al. 2006). This species has been previously shown to absorb DOC (Gibson 2011), making it an ideal candidate for exploration of the role of DOC in sponge metabolism. In order to assess potential C allocation towards growth, growth rates for the tested *X. muta* individuals was calculated using the Tanaka growth rate model from McMurray et al. (2008); *X. muta* has been shown to grow relatively quickly in the Conch Reef environment, with particularly strong growth rates observed during the summer months (McMurray et al. 2008).

Sample collection

Water samples were collected in August 2011 on Conch Reef (24° 57.62' N, 80° 26.82' W) in the Florida Keys. Conch Reef is the location of the Aquarius Reef Base (ARB), a saturation-diving laboratory within a Special Protection Area of the Florida Keys National Marine Sanctuary. This designation assigns a no-take status and closes the area to all activities apart from permitted research activities. Bottom cover is characterized by sponges, soft corals, and benthic macroalgae (Stokes et al. 2011) with a minor and declining contribution from hard corals (Gardner et al. 2003). Two healthy-looking *X. muta* individuals were chosen for *in situ*

instrumentation and water sample collection. Two large specimens were used due to limited *in situ* instrumentation as well as to maximize the temporal coverage of sampling for the tested sponges. This species' large size and barrel morphology featuring a single, large osculum facilitated *in situ* instrument deployment and the collection of water samples for chemical analyses. The selected individuals were 3 meters apart at a depth of ~18 meters. The dimensions of the sampled sponges, henceforth referred to as sponges 1 and 2, were measured by divers on SCUBA in order to calculate sponge volumes without destroying the animal. The presented sponge volumes were calculated using the formula $V_{sponge} = 28.514*\text{osculum diameter}^{2.1}$ (McMurray et al. 2010), which compared well with our volumes generated using geometric approximations of the sponge.

Sponge excurrent and ambient water samples were collected by divers on SCUBA working out of ARB from August 9 to 16, 2011. Ambient waters near the exterior walls of the sponge (<20 cm from the outer wall of the sponge) and excurrent waters exiting the sponge as a coherent jet were collected in triplicate at each sampling period. Ambient and excurrent samples were collected within 10 minutes of each other. These temporally-paired water samples allowed for quantification of chemical transformations mediated by the sponge and sponge associated microbial consortia (Yahel et al. 2005, Southwell et al. 2008). Samples were collected at three time points every day, 07:00, 12:00, and 17:00, to reflect any changes from morning to evening resultant from light-associated alterations in the sponge holobiont behavior as *X. muta* hosts dense populations of cyanobacteria in its ectoderm (Erwin and Thacker 2007). Each water sample was simultaneously collected and filtered (Whatman GF/F; 0.7 μm nominal pore size) in a 60 mL polypropylene syringe connected to a 3-way polycarbonate stopcock with one arm of the stopcock having an in-line filter and 10 cm of small-diameter, high-density polyethylene

tubing that allowed sample collection with minimal disturbance to the excurrent water jet and no disturbance of the sponge. A new pre-combusted, 25 mm Whatman GF/F filter was used for to filter each sample. Whatman GF/Fs were selected for filtration due to their suitability for pre-combustion and use in prior studies of DOC uptake by Caribbean sponges (e.g. Yahel et al. 2003, Gibson 2011). During sample collection, the syringe, filter, and sample collection tubing were rinsed 3x by pulling filtered target water into the syringe and then pushing the filtered water out of the stopcock arm not capped with the filter and tubing. The fourth and final water sample was drawn slowly into the syringe (< 2 mL sec^{-1}) so as to ensure the collected samples were representative of the desired water mass. Upon completion of sample collection, the stopcock was closed and the sample was stored in an ice bath in ARB until taken to the surface for transport to shore for subsampling and preservation (less than 8 hours from collection to preservation). At the shore-based lab, samples were immediately divided into triplicate borosilicate glass scintillation vials. Vials were first rinsed with the sample water, then were filled with 20mL of sample water, and 100 µL of 50% H_3PO_4 was added. After the acid addition, the sample was stored at 4°C until analyzed.

In situ samples were also collected to examine particulate organic matter (POM) uptake by one of the two tested barrel sponges. POM samples were collected from the same individual (sponge 2) daily during the mission using a passive *in situ* vacuum filtration apparatus (N. Lindquist personal communication 2007, Monismith et al. 2010). The system pulls water through a 0.7 µm GF/F (Whatman, 47 mm) using the pressure differential between the atmosphere and our water sampling depth. Flow rates were controlled to draw approximately 6 L hr^{-1} to match the sampling flow rate for water sampling by syringe. Samples were collected simultaneously from ambient and excurrent water masses giving a 2 hour, time-integrated

sample of 13 L of filtered water. Filters were frozen after collection in combusted foil until analysis. Ambient and excurrent sample inlets were covered with a polypropylene mesh pre-filter (pore-size: ~100 μm) to exclude particles larger than those thought to be efficiently retained by sponges (Reiswig 1971, Pile et al. 1996, Yahel et al. 2003). Pre-filters were replaced daily. These POM samples were compared to 12 paired ambient and excurrent samples collected on Conch Reef using identical methodology as part of a wider survey of particulate carbon demand for *X. muta*. The samples were collected from several haphazardly selected healthy-looking *X. muta* individuals with sampling performed over several days in July, September, and October 2007.

All plastics utilized in sample collection and processing (including syringes, stopcocks, tubing, filter holders, and collection vial lids) were composed of polypropylene, high-density polyethylene, or polycarbonate and all were soaked in a 0.1M HCl bath for at least 12 hours and rinsed 6 times with 18.2 MΩ type I water prior to use and between each sampling. Borosilicate scintillation vials used for sample collection were subjected to the same washing procedure, followed by combustion at 450°C for >6 hours to remove any residual DOC. Combusted glassware was stored in combusted foil and bagged to minimize outside contamination prior to use. Filters were combusted at 450°C for >6 hrs.

Sample Analysis

DOC samples were analyzed using high-temperature catalytic oxidation (HTCO) and non-dispersive infrared spectroscopy (NDIR) using a Shimadzu TOC-L CPH/CPN organic carbon analyzer. Analysis standards were diluted from a lab-prepared stock solution of potassium hydrogen phthalate (KHP) (Sigma-Aldrich 96148) and acidified with 100μL 50% H_3PO_4 per 20mL of prepared volume. Lab prepared carbon standards were batch checked

against commercially produced stock solutions (La-Mar-Ka Chemical Company) to ensure accuracy. Calibration curves were closely monitored during analysis and were remade and rerun if the correlation coefficient was found to be less than 0.995. Additionally, standards were interspersed with samples. Each sample or standard was transferred to duplicate, combusted analysis tubes to isolate instrument variability from collection variability. Further quality control was ensured by reserving a single sample from each triplicate set for separate analysis to confirm the obtained values from the other two samples; all samples from a triplicate set were analyzed, yet not contemporaneously, to isolate for any variability in instrument performance. Samples were bubbled with commercially-produced, CO_2-free, Zero-Grade air at 80mL per minute for 10 minutes to ensure all inorganic C and volatile organic compounds were purged prior to sample injection; therefore, the values obtained are most accurately characterized of Non-Purgeable Organic Carbon (NPOC). We assume a negligible contribution to DOC from volatile organics, and henceforth the obtained values will be simply referred to as DOC. Each analysis tube was injected a minimum of 3 times, and a maximum of 5, depending upon whether the resultant peaks fell within user-provided statistical boundaries (Standard Deviation < 0.100 and Coefficient of Variance < 2.0%**)**. Therefore each reported concentration represents an average of N=18-30 individual measurements of DOC. The average difference between replicate measurements was 2.3 μmol C L^{-1} (N=224 replicate pairs), which is interpreted as the approximate analytical precision.

POM samples were analyzed via flash combustion and thermal conductivity detection using a Carlo Erba NA 1500 elemental analyzer. The collected filters were lyophilized to remove any residual water on the filter. After lyophilization, filters were folded onto themselves four times and exposed to concentrated HCl vapor overnight in a closed vessel. Acid flushed

filters were then dried at 80°C for one hour and pulverized. Pulverized samples were placed into combusted foil boats and analyzed for C and N composition.

In situ Instrumentation

The water pumping speeds of the two selected *X. muta* were continuously measured between May 25 and August 17, 2011 using Nortek Vector acoustic Doppler velocimeters (ADVs). The ADVs were deployed on tripod stands built to minimize sensor movement during field deployments and were oriented such that their sampling volumes were within the center of the effluent jet halfway down the interior of the oscular cavity. The deployment locations were checked by divers using fluorescein dye injections to confirm placement in the center of the excurrent jet. The ADVs and Aanderaa Data Instruments (AADI) SeaGuard systems were cabled to the ARB Life Support Buoy (LSB) that supported the ARB with air, power, and communications. These data were transmitted wirelessly to onshore computers for logging and real-time monitoring. The ADVs collected data in 30 second bursts, every 5 minutes, for 85 days. ADV data collected during DOC sample collection from August 9 to 16 was averaged based upon hour-long sample blocks corresponding to the dates and time periods during which discrete samples were collected: 07:00-08:00, 11:30-12:30, and 17:00-18:00. Additionally, a nighttime sample block was generated (23:00 to 0:00) in order to assess any differences between daytime and nighttime behavior. Each sampled sponge had 26 sample blocks (19 daytime, 7 nighttime) in which data were averaged; each block of data represented 12 sampling events containing approximately 1000 individual measurements of vertical fluid speed. Prior to averaging, the measured vertical velocities from the ADVs were "despiked" to remove spurious data points, which can occur as a result of measured velocities exceeding the user-defined nominal velocity range or reflection of Doppler pulses off of boundaries (Goring and Nikora

2002). These spikes are common to ADV measurements in natural environments, and the despiking process was performed using the Tukey 53H method as described in Goring and Nikora (2002). After removal, the data spikes were replaced by interpolating the data between the beginning and the end of the removed spike. The vertical fluid speed determined by ADV was used to calculate a volumetric flow associated with these individuals. This volumetric flow was calculated as a product of the planar area of the oscular opening and the measured vertically-directed fluid speed ($V = pA_{osc}V_{exc}$; where V is the volumetric flux, p is the proportion of the oscular area characterized by a discrete jet, A_{osc} is the oscular area, and V_{exc} is the measured excurrent velocity). Oscular openings for both sponges were approximately elliptical, but only a fraction of the measured planar area exhibits flow speeds as high as those measured via *in situ* velocimetry. The proportion of planar area characterizing the excurrent jet was determined by analyzing video-recorded fluorescein dye releases across multiple *X. muta* oscula as described in Weisz et al. 2008. The jet was characterized as the area where the dye front moved directly upward, and these dimensions were used to generate a percentage of the planar area of the osculum represented by the jet.

To measure O_2 uptake by the sponges, a Aanderaa Data Instruments (AADI) SeaGuard system equipped with a ten sensor, digital optode string for continuous measurements of dissolved oxygen (DO) was deployed simultaneously with the ADVs. Pairs of O_2 optode sensors were positioned to simultaneously sample ambient and effluent waters of five *X. muta* individuals, including the two that were the focus of this study's water sampling and ADV monitoring, in order to determine O_2 drawdown and calculate respiration rate of the sponge holobiont. Depths of the five *X. muta* individuals ranged from 17 to 19 meters. Each of the O_2 sensors collected DO concentrations every 30 seconds for the duration of the three month

deployment. These data were treated identically to the ADV data in that they were subsampled in blocks based on the dates and times that discrete sample collection occurred. In the same manner as the ADV data, a nighttime block was analyzed to assess the role of phototrophic symbionts in the oxygen cycling of *X. muta*. The sample blocks for each sponge represented approximately 120 paired ambient and excurrent data points.

Results

Sponge volumes and growth rates

The oscula of the sponges were approximately elliptical, therefore sponge volumes were separately calculated using the major and minor axis length in place of the osculum diameter, and the resultant values were averaged. This leads to a calculated volume for sponge 1 of 150 ± 30 L and 48 ± 5 L for sponge 2, where the uncertainties represent the range between calculated values from the major and minor axes. The volumes obtained were in agreement with values calculated through geometric approximations of the shape of the sponge (126 L and 42 L for sponges 1 and 2, respectively).

The average, observed growth rate for *X. muta* on Conch Reef was approximately 2000 cm^3 $sponge^{-1}$ yr^{-1}, determined from 104 individuals spanning a range of initial volumes (McMurray et al. 2008). This average value agrees well with the calculated annual growth rate for our test sponges (2700 and 1800 cm^3 $sponge^{-1}$ yr^{-1} for sponges 1 and 2, respectively), determined using the Tanaka growth rate model from McMurray et al. (2008). Assuming this growth rate, an average tissue density of 0.62 g cm^{-3} for *X. muta* (Fiore et al. 2013), and an average C content of *X. muta* tissue of 16% (Martens et al. unpublished data), we calculated a potential tissue generation rate for our tested sponges of 180 and 270 g C $sponge^{-1}$ yr^{-1} for sponges 1 and 2, respectively ($C_{growth} = G_{rate} * d_{tissue} * C_{tissue}$; where C_{growth} is the C allocated for

growth, G_{rate} is the growth rate, d_{tissue} is the tissue density, and C_{tissue} is the carbon content of sponge tissue).

Pumping Velocity

The two *X. muta* individuals showed significantly different average excurrent flow rates over the measured period (paired t-test). Sponge 1 produced an average excurrent flow rate of 5.3 ± 0.9 cm sec^{-1} (mean ± 1 SD, N = 19, P = 0.0001), and sponge 2 showed an average excurrent jet flow rate of 4.3 ± 0.9 cm sec^{-1} (mean ± 1 SD, N = 19, P = 0.0002). The nighttime excurrent flow rates did not differ significantly from the daytime values (4.9 ± 1.4 and 4.3 ± 0.9 cm sec^{-1}, mean ± 1 SD, sponges 1 and 2, respectively). Video-recorded dye flow measurements characterized the excurrent jet in this species as approximately 40% (SD = 9, N = 21) of the planar area of the sponge osculum, and this proportion was applied to the analyzed individuals to generate volumetric fluxes ($V = 0.4A_{osc}V_{exc}$). Additionally, the dye flow videos generated pumping velocities that corroborated the pumping velocities measured by ADV (N. Lindquist unpublished data). Sponge 1 had a planar area of 2787 cm^2 (jet area = 1143 cm^2) and this generated an average volumetric flow rate of 6 L sec^{-1}, while sponge 2 had a planar area of 929 cm^2 (jet area = 381 cm^2) and an average volumetric flow rate of 2 L sec^{-1}. For sponges 1 and 2, these flow rates are equivalent to filtering water more than 5,000x and 3,500x their body volume daily. The water pumping rates measured for *X. muta* agree well with previous assessments for this species (Weisz et al. 2008, Fiore et al. 2013) as well as for other HMA sponge species (Weisz et al. 2008, de Goeij et al. 2008 and citations within).

DOC Uptake by X. muta

The average DOC concentration in ambient water surrounding the test sponges was 89 ± 5 μmol C L^{-1} (mean ± 1 SD) with no significant difference between sponges 1 and 2. Both *X.*

muta showed significant uptake of DOC from paired sample collections (one sample t-test versus 0) (Figure 2.1). Of the collected 38 ambient/excurrent pairs, 33 pairs showed DOC uptake greater than the 2.3 μmol C L^{-1} estimated analytical precision, 3 pairs showed an increase of DOC, and 2 pairs showed no significant difference. From this dataset, four paired samples were detected as outliers and removed from the dataset using the modified z-score method ($M_i \geq 3.5$, Iglewicz and Hoaglin 1993). Sponge 1 showed a DOC uptake of 13 ± 5 μmol C L^{-1} from ambient water (mean ± 1 SD, Wilcoxon Signed-Rank test, N = 18, P = 0.0002) while sponge 2, exhibited a DOC uptake of 12 ± 6 μmol C L^{-1} (mean ± 1 SD, Wilcoxon Signed-Rank test, N = 16, P = 0.0006). This yielded an average DOC uptake for the two analyzed individuals of 13 ± 5 μmol C L^{-1} (mean ± 1 SD, Wilcoxon Signed-Rank test, N = 34, P < 0.0001). There were no significant differences in DOC uptake between the two test sponges or between collection times (07:00, 12:00, and 17:00). The obtained uptake values were converted to fluxes using the volumetric pumping rates determined by the ADV measurements and normalized to calculated sponge volumes. This yields DOC uptake fluxes of 1.8 ± 0.9 mmol C hr^{-1} L_{sponge}^{-1} for sponge 1 and 1.5 ± 0.8 mmol C hr^{-1} L_{sponge}^{-1} for sponge 2 (mean ± 1 SD).

POC Uptake by X. muta

Ambient POC concentrations were found to be 2.1 ± 0.7 μmol C L^{-1} (mean ± 1 SD), a substantially lower concentration than found for DOC. The measured POC content represents only 2% of the total organic carbon (TOC; 91 ± 5 μmol C L^{-1}; mean ± 1 SD) in ambient reef water, where TOC is defined as the sum of POC and DOC (Yahel et al. 2003).

The sampled individual (sponge 2) showed significant uptake of POC. A single sample pair was removed as an outlier (Modified Z-Score ≥ 3.5, Iglewicz and Hoaglin 1993). The remaining samples showed a mean uptake of 0.43 ± 0.11 μmol C L^{-1} (mean ± 1 SD, Wilcoxon

Signed-Rank test, N = 4, P = 0.05). As with the DOC, the POC values were converted to uptake fluxes using the volumetric flow rate and the calculated sponge volume yielding a POC uptake flux of 0.05 mmol C hr^{-1} L_{sponge}^{-1}. Comparatively, the 12 sample pairs from 2007 showed a mean ambient POC content that was slightly higher than was observed at the tested individuals in 2011 (3.9 ± 1.3 μmol C L^{-1}; mean ± 1 SD). The tested sponges also retained slightly more POC from the filtered water (0.96 ± 0.28 μmol C L^{-1}; mean ± 1 SD).

O_2 *Uptake by X. muta*

The O_2 concentration in the ambient water surrounding the sponges was 180 ± 10.5 μmol O_2 L^{-1} (mean ± 1 SD), with no significant difference between the two sampled sponges. Both individuals showed significant drops in O_2 concentration from ambient to excurrent water masses (Figure 2.2). Sponge 1 showed an average O_2 uptake of 10 ± 2 μmol O_2 L^{-1} (mean ± 1 SD, Wilcoxon signed-rank test, N = 17, P = 0.0003), and sponge 2 showed an average O_2 uptake of 10 ± 3 μmol O_2 L^{-1} (mean ± 1 SD, Wilcoxon signed-rank test, N = 17, P < 0.0003). These results yielded an average O_2 uptake for the two sponges of 10 ± 3 μmol O_2 L^{-1} (mean ± 1 SD, Wilcoxon signed-rank test, N = 34, P < 0.0001). There were no significant differences among any of the sampled times (07:00-08:00, 11:30-12:30, 17:00-18:00, and 23:00-0:00). Sponge 2 showed a single period where there was a significant reduction in pumping rate (0.9 cm sec^{-1}, 75% reduced from the average, one-sample t-test vs. mean pumping rate, P < 0.0001) and the O_2 uptake during this time was significantly greater than the average, (120 μmol O_2 L^{-1}, 10x average O_2 uptake, one-sample t-test vs. mean O_2 uptake, P < 0.0001). This time point was detected and removed from the record for sponge 2 as an outlier for both DOC and DO uptake. Including this anomalous point, 4 time points were removed as outliers in a similar fashion to the POC and DOC datasets (Modified Z-Score ≥ 3.5, Iglewicz and Hoaglin 1993). As with the DOC and

POC uptake values, the O_2 uptake values were also converted to fluxes using the volumetric flow rate and calculated volume. Sponge 1 showed uptake of 1.3 ± 0.5 mmol O_2 hr^{-1} L_{sponge}^{-1} and sponge 2 showed uptake of 1.3 ± 0.5 mmol O_2 hr^{-1} L_{sponge}^{-1} (mean ± 1 SD). Nighttime oxygen flux did not differ significantly from daytime values for either tested sponge (1.2 ± 0.4 and 1.2 ± 0.4 mmol O_2 L_{sponge}^{-1} hr^{-1}; mean ± 1 SD; two-sample t-test, P = 0.80 and 0.70; sponges 1 and 2, respectively).

Respiration balance for X. muta

The measured DOC uptake was greater than the measured O_2 demand for both sampled sponges and for their average, but the observed difference was not significant for sponge 2 (Figures 2.3 and 2.4). Assuming a respiratory quotient of 1 (mole $C_{respired}$ mole O_2^{-1}), the accumulation of non-respired C was approximately 0.6 and 0.3 mmol C hr^{-1} L_{sponge}^{-1} for sponges 1 and 2, respectively. The average non-respired C retained by these sponges equates to accumulating approximately 9500 g C $sponge^{-1}$ yr^{-1} for sponge 1 and 1500 g C $sponge^{-1}$ yr^{-1} for sponge 2.

Discussion

Our results showed that dissolved organic matter is the dominant organic C source for the giant barrel sponge *X. muta* (Table 2.1) and that metabolic O_2 demand by *X. muta* can be accounted for exclusively by DOC-fueled respiration (Figure 2.3). This lends further support to the growing body of direct measurements showing that C utilized by sponges and their associated microbial consortia for respiration and growth comes predominantly from the dissolved organic pool (e.g., Yahel et al. 2003, de Goeij et al. 2013, and Mueller et al. 2014). If the tested individuals removed all the POC available in the ambient reef water they pumped through their tissues, the C obtained would only account for 25-33% of their respiratory O_2

demand (Table 2.1). This would generate a discrepancy between absorbed C and respiratory O_2 demand of similar magnitude to that observed by Reiswig (1981). This disparity strongly suggests that these sponges utilize DOC as their primary C source. While DOC represents a wide spectrum of elemental composition, structural diversity, and biological lability (Carlson 2002, Nebbioso and Piccolo 2013), the benthic boundary waters on Conch Reef likely contain concentrations of labile DOC produced by abundant soft corals and macroalgae (de Goeij et al. 2013, Mueller et al. 2014). Organic matter sourced from the local production on the reef benthos has been previously implicated through analysis of $\delta^{13}C$, $\delta^{15}N$, and fatty acid biomarkers as the primary source of nutritive organic matter absorbed by Caribbean sponges (van Duyl et al. 2011). Moreover, the quantity of DOC removed by the test sponges is well within the portion of the measured ambient DOC pool which is assumed to represent the labile fraction on the reef (19 ± 7 μmol C L^{-1}, 14 – 30% of reef DOC is considered "labile"; van Duyl and Gast 2001). However, without rigorous structural characterization of the DOC pool, the suggestion that sponges are using exclusively the labile fraction of available DOC is purely conjectural. These direct measurements of predominant C uptake and utilization from the DOC rather than POC pool by multiple HMA species contradicts a great many previous studies indicating that POC is the dominant form of C utilized by sponges and that sponge growth can be limited by available levels of POC (reviewed by Pawlik et al. 2015).

Our measured DOC uptake rates agree well with published values for non-manipulated "InEx" ΔDOC sampling (Yahel et al. 2003, Gibson 2011, Mueller et al 2014, Table 2.1). Gibson (2011) measured similar DOC uptake by *X. muta* in a broader survey of metabolic activity of sponges across Conch Reef, illustrating that this behavior is likely conserved within this species at the reef scale and is not unique to our sampling period or individual sponges. Low POC

uptake in our 2007 survey of particulate uptake in this species further suggests relatively conserved metabolic behavior in this species at the reef scale. Ambient POC and ΔPOC were higher in 2007 than 2011, yet the tested sponges in 2007 removed the same proportion of filtered POC (~20%) and even at elevated ambient POC concentrations particulate removal alone is insufficient to account for the observed metabolic O_2 demand of the tested individuals. Our ΔDOC results agree with the *in situ* work of Mueller et al. (2014), which may indicate physiological similarities between *X. muta* and excavating species; however without normalizing to sponge biomass, it is difficult to further compare the observed fluxes. Our volume-normalized DOC removal rates were significantly lower than those from de Goeij et al. (2008a) and lower than the ^{13}C-labeled DOC respiration and assimilation rates of de Geoij et al. (2008b, 2013). Assessments of DOM uptake performed by de Goeij et al. (2008a, 2008b, 2013) employed chamber methodologies for which the sponges were removed from their original substrate and enclosed in chambers for monitoring. This experimental manipulation potentially has large impacts on sponge behavior as sponges have been previously shown to be sensitive to environmental and physical stressors (e.g., Gerrodette and Flechsig 1979, Tompkins-MacDonald and Leys 2008). Additionally, the ^{13}C labeled incubations of de Goeij et al. (2008b, 2013) were performed with elevated concentrations of labile, diatom DOM which would be expected to accelerate the apparent rate of DOC uptake relative to natural composition DOC if the labile fraction of DOM is the primary target of sponge respiration. The sponges tested in this study were undisturbed so as to remove any potentially confounding factors resultant from individual or environmental manipulations. Independent of methodological differences, physiological differences between encrusting species studied by de Geoij et al (2008a, 2008b, 2013) and non-

encrusting species like *X. muta* could also be cause for the observed disparity, but the specific differences between these species are unknown.

Yahel et al. (2003) provided the only other instance in the literature of non-manipulated, simultaneous "InEx" ΔDOC and ΔO_2 measurements on a marine sponge. The work of Yahel and co-workers revealed a slight difference between DOC uptake and O_2 uptake for the Red Sea sponge *Theonella swinhoei*, and their measured C and O_2 fluxes are similar to those presented here for *X. muta*. Yahel et al. (2003) attributed the observed difference between O_2 and DOC uptake to O_2 production by phototrophic symbionts of *T. swinhoei*. *X. muta* also showed a discrepancy between DOC and O_2 uptake (Figure 2.3), and like *T. swinhoei*, possesses phototrophic organisms in its ectoderm (Erwin and Thacker 2007). In order to examine a potential role of phototrophic associates in the balance between DOC and O_2 demand, nighttime oxygen fluxes were calculated and compared to daylight values. Surprisingly, observed nighttime O_2 uptake fluxes were not significantly different from daylight hours for either individual (nighttime O_2 Flux: 1.2 ± 0.4 and 1.2 ± 0.4 mmol O_2 L_{sponge}^{-1} hr^{-1}; Daytime O_2 Flux: 1.3 ± 0.5 and 1.3 ± 0.5 mmol O_2 hr^{-1} L_{sponge}^{-1}; mean ± 1 SD; two-sample t-test, P = 0.8 and 0.7 for sponges 1 and 2, respectively). Assuming C uptake was also equal overnight, we hypothesized O_2 uptake from sponge filtered water would increase in the absence of symbiont photosynthetic O_2 production, yet this was not observed. The absence of this increased oxygen uptake overnight could indicate internal consumption of photosynthetic O_2 during daylight hours, potentially through respiration of "new" photosynthetically-fixed C by the sponge holobiont or through phagocytosis of microbial biomass by sponge cells. These internal processes would go undetected by our "InEx" sampling because both the O_2 and C being respired are sourced within the sponges' tissues and not from filtered water. Minimizing the role of O_2 from symbiont

photosynthesis in the respiration of absorbed DOC would suggest that C taken up in surplus of observed O_2 demand is being utilized for organism growth and cell regeneration. Future overnight collections of DOC and POC could further elucidate the diurnal role of photosynthesis in these organisms.

The measured DOC uptake was greater than the measured O_2 demand for both sampled sponges and for their average, but the observed difference was not significant for sponge 2 (Figures 2.3 and 2.4). The observed O_2 demand for these sponges falls within the range of previously reported respiration rates of undisturbed sponges *in situ.* Reiswig (1974, 1981) showed a range from 0.5 to 4.7 mmol O_2 L_{sponge}^{-1} hr^{-1} for *Tethya crypta* and *Verongia fistularis* respectively, and Yahel et al. (2003) reported a rate of 1.38 ± 0.78 mmol O_2 L_{sponge}^{-1} hr^{-1} for *T. swinhoei*. Additionally, these values agree with respiration demand for an encrusting sponge, *Halisarca caerulea,* analyzed by incubations (de Goeij et al. 2008a). de Goeij and co-workers (2008a) reported a large difference between observed O_2 demand and observed DOC uptake in these incubations, indicative of only 39 - 45% of acquired organic C being respired. The remaining 55 - 61% was posited to be allocated to rapid turnover and expulsion of sponge biomass, confirmed later as rapid turnover and shedding of choanocytes (de Goeij et al. 2009). *X. muta* showed 76 ± 13% of total C acquired could be accounted for in respiration, assuming a respiratory quotient of 1 (mol $C_{respired}$ mol O_2^{-1}) (Figure 2.4). The utilized respiratory quotient is a conservative estimate assuming highly labile organic matter respiration, but is subject to change with changes in food composition and physiological state (Maldonado et al. 2012). A lack of additional information regarding effluent CO_2 concentrations precludes direct calculation of the respiratory quotient; a value of 1 will be used in this discussion. Considering the average of the two sampled individuals, the non-respired C acquired represents 0.4 mmol C L_{sponge}^{-1} hr^{-1}

(Figure 2.4). This calculated C accumulation rate should be considered a conservative estimate as it does not account for the C fixed by photoautotrophic production via associated microorganisms. Nevertheless, the observed C accumulation is much less than the quantity of C retained by *H. caerulea* for choanocyte turnover and shedding (13.8 mmol C L_{sponge}^{-1} hr^{-1}, de Goeij et al. 2009) which may suggest a lower rate of cell turnover and shedding in *X. muta*, particularly if C allocation for organismal growth is considered. The growth of cryptic species was assumed to be zero (de Goeij et al. 2008a) whereas the same assumption cannot be made for *X. muta* which shows considerable annual growth (McMurray et al. 2008). Sponges in cryptic habitats may be severely space limited and, by consequence, could be driven to allocate more energy towards functions other than growth, but *X. muta* individuals are not likely to experience the same degree of spatial pressure. Nevertheless, estimated C retained for growth only accounts for ~3% of the observed C accumulation; in the case of sponge 1, approximately 9500 g C $sponge^{-1}$ yr^{-1} of non-respired C is retained with an estimated 270 g C $sponge^{-1}$ yr^{-1} of the total being allocated to organismal growth. This reserves roughly 97% of non-respired C for other organismal functions such as the renewal of sponge pumping cells or the production of reproductive materials that are exported from the sponge. For *X. muta*, reproduction involves the exudation of a mass of sticky mucus within which are embedded numerous small embryos that develop though early larval stages in mucus that spreads over substrates adjacent to the spawning sponge (Ritson-Williams et al. 2005, McMurray et al. 2008).

Sponge population densities at Conch Reef increased by up to 46% from 2000 to 2006 (McMurray et al. 2010), with *X. muta* among the species showing the greatest increases. In 2006, *X. muta* abundance was found to range between 0.134 and 0.227 sponge individuals m^{-2} at our Conch Reef study site, with the mean sponge volume being approximately 1500 cm^3 m^{-2}

(McMurray et al. 2010). DOC removal from reef water due to *X. muta* would have been approximately 60 ± 40 mmol C m^{-2} day^{-1} in 2006, based on a range of biomass estimates by McMurray et al. (2010). This population flux suggests that DOC uptake by a single species of sponge could be equivalent or greater than the daily DOC released by benthic photosynthesis (20 to 50 mmol C m^{-2} day^{-1} as DOC); estimates of gross primary productivity for reef environments are approximately 200 – 500 mmol C m^{-2} day^{-1}, with conservative evaluations suggesting 10% of C fixed by macroalgal photosynthesis is exuded as DOC (B. Hatcher 1990, Haas et al. 2011). If all non-encrusting HMA sponge biomass on Conch Reef absorbs DOC at the same rate as *X. muta*, the total community flux is approximately 130 mmol C m^{-2} day^{-1} (using mean HMA biomass data from Southwell et al. 2008). This rough estimate of C flux approaches total gross primary productivity for the reef, and shows that the non-encrusting HMA sponge community has the potential to remove DOC as efficiently as in coral cavity environments (de Goeij et al. 2013). The ecosystem implications of the observed sponge utilization of dissolved organic matter are still uncertain, yet *X. muta* has been shown to produce large quantities of dissolved inorganic nitrogen (DIN) as a result of organic matter remineralization (Corredor et al. 1988, Southwell et al. 2008, Fiore et al. 2013). Both Southwell et al. (2008) and Fiore et al. (2013) showed that these fluxes of DIN can contribute a significant amount of N to the community within the near-bottom waters of the benthic boundary layer. This bioavailable N contribution may result in enhanced photosynthetic production thereby increasing DOC production and enhancing the sponge's primary C feedstock. Additionally, this uptake of DOC by sponge populations has been hypothesized by de Goeij et al. (2013) to beneficially impact higher trophic levels through sponge-produced detritus, forming a putative "Sponge Loop" on reef systems. Our POC measurements did not provide evidence for the export of particulate C concomitant to

the import of DOC to confirm participation of *X. muta* in the sponge loop. This disparity may be due to a lack of efficient export of detrital material in the excurrent jet, temporally limited POC collections, or to enhanced production of detritus by sponges in chamber environments rich with labile DOC (de Goeij et al. 2013) as compared to natural DOC composition on reefs (van Duyl and Gast 2001, Tanaka et al. 2011). We also did not observe the production of detritus by *X. muta* in the more extensive POC collection campaign conducted in 2007, which argues against temporally-limited sampling as the cause for the lack of detrital export. Additionally, Mueller et al. (2014) did not show quantitative detrital production in *C. delitrix* or *Siphonodictyon* sp. and could not definitively confirm their participation in this aspect of the sponge loop. However, detrital production in *X. muta* could also be limited by allocation of retained C towards organism growth, although this impact was calculated and determined to be minimal, or to the production of reproductive materials. *X. muta* has been shown to spawn twice annually, in spring and late summer (McMurrray et al. 2008), which may have contributed to the rates of C retention observed in the tested individuals.

The presented values for C and O_2 flux represent the collective impact of the sponge holobiont, making it difficult to establish the relative importance of animal versus microbial processes in organic matter transformations in *X. muta* tissues. The role of associated microorganisms in the metabolism of DOC is suggested frequently in bulk C assessments of sponge metabolism, beginning with Reiswig's work (1971, 1981), and has been further implicated in experiments using labeled C substrates (de Goeij et al. 2008b). Recent work by Mueller et al. (2014) provides *in situ* evidence of DOC uptake by a non-manipulated LMA sponge *Cliona delitrix*, which would imply an enhanced role for the sponge animal in the direct uptake and utilization of DOC (assumed LMA at the genus level based upon *Cliona varians*,

Gloeckner et al. 2014). It is important to note that LMA sponges are not without bacterial biomass in their tissues, but rather they have bacterial populations approaching seawater concentrations, which may still serve a functional role in the processing of DOC (Hentschel et al. 2006). The combination of animal and microbial processes in *X. muta* and other HMA sponges may provide a competitive advantage in oligotrophic systems by supplying metabolic access to a wider range of C sources than would be assessable without associated microorganisms (van Duyl et al. 2008, de Goeij et al. 2008b and citations therein).

DOC represented more than 90% of the TOC removed from filtered water by the tested *X. muta* individuals, and therefore likely constitutes the dominant C source for this species. In the tested individuals, 76 ± 13% of the TOC absorbed is accounted for by observed O_2 demand, suggesting substantial assimilation, but to a much lesser degree than in the previous studies of cryptic species from coral cavities (e.g. de Goeij et al. 2008b). This less pronounced role of C assimilation during the utilization of DOC by *X. muta* could lead to a reduction in the detrital flux from cell shedding, potentially decoupling part of the "sponge loop" hypothesized by de Goeij et al. (2013). The observed C cycling by *X. muta* serves to illustrate the fate of a large proportion of DOC available in reef waters; preliminary calculations suggest the *X. muta* population on Conch Reef can remove a large proportion of the DOC produced through reef primary productivity. This behavior further demonstrates a symbiont-based metabolic adaptation that allows some sponge species to utilize a resource likely unavailable to LMA sponge species. Improved understanding of the C cycling mediated by these organisms and the corresponding ecosystem impacts is critical to understanding trajectories of coral reef change, particularly in the Caribbean where sponges and seaweeds are maintaining a dominant and increasing benthic presence. Increased *in situ* studies with non-manipulated sponges will provide further insight

into the native behavior of these organisms, and will serve to advance the understanding of their role in coral reef ecosystems.

Table 2.1: Comparison of published directly measured in situ carbon uptake and respiration activity for sponge species (mean ± SD). Results of studies conducted in chambers are not included due to possible manipulation artifacts. Listed flux measurements were normalized to L of sponge biomass.

Species	**ΔDOC (µmol C L^{-1})** ***DOC Flux*** ***(mmol C L^{-1} sponge hr^{-1})***	**ΔPOC (µmol C L^{-1})** ***POC Flux*** ***(mmol C L^{-1} sponge hr^{-1})***	**ΔO_2 (µmol C L^{-1})** ***O_2 Flux*** ***(mmol O_2 L^{-1} sponge hr^{-1})***	**Source**
Xestospongia muta	12.6 ± 5.0 *1.63 ± 0.83*	0.4 ± 0.1 *0.05 ± 0.01*	9.9 ± 2.5 *1.28 ± 0.51*	This study
Xestospongia muta		0.96 ± 0.28		This study (2007 samples)
Teonella swinhoei	10 ± 8 *1.56 ± 1.1*	2.1 ± 1.0 * *0.24 ± 0.18 **	9 ± 5 *1.38 ± 0.78*	Yahel et al (2003)
Siphonodictyon sp.	13 ± 17	3 ± 1		Mueller et al (2014)
Cliona delitrix	10 ± 12	3 ± 1		Mueller et al (2014)

**POC represents LvPOC, or living particulate organic matter*

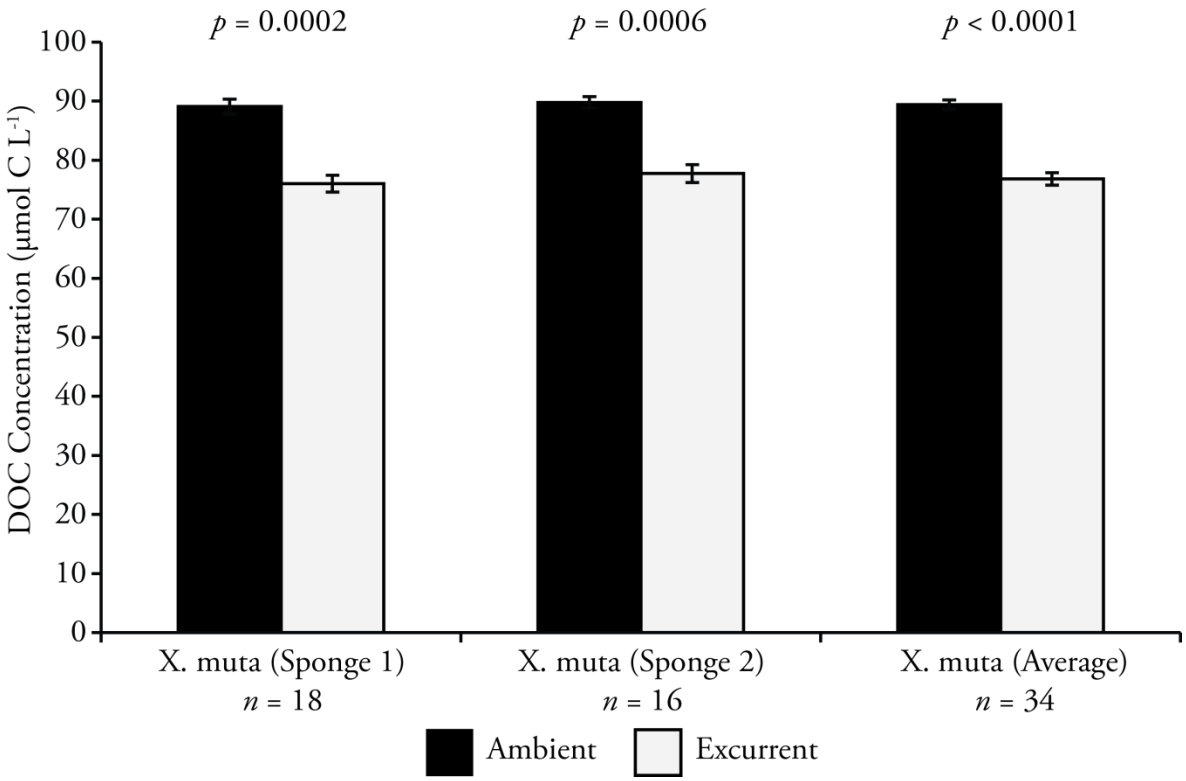

Figure 2.1: Average DOC concentration in the ambient and excurrent waters of two *Xestospongia muta* (sponges 1 and 2) and the overall average. Error bars are 1 SE and N equals the number of paired ambient-excurrent water collections. P values (Wilcoxon signed-rank test) indicate the level of significance of the difference between the ambient and excurrent DOC concentrations.

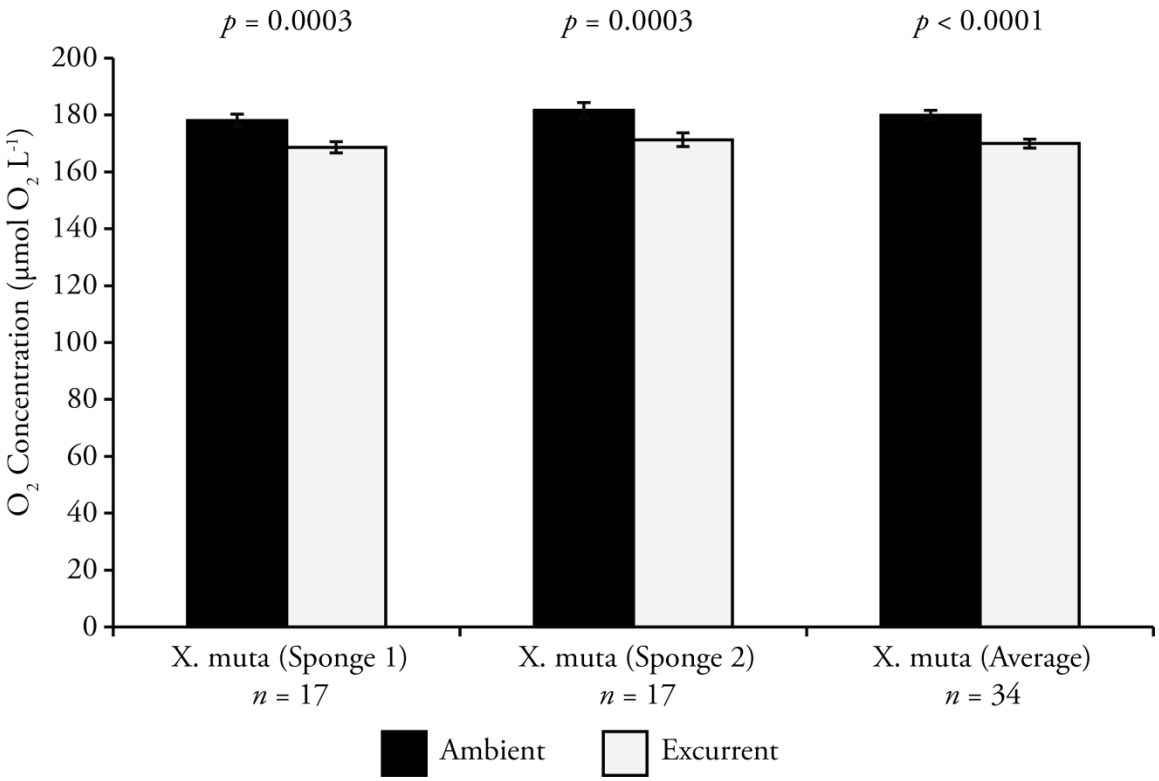

Figure 2.2: Average O_2 concentration in the ambient and excurrent waters of two *Xestospongia muta* (sponges 1 and 2) and the overall average. Error bars are 1 SE and N equals the number of averaged sample periods. P values (Wilcoxon signed-rank test) indicate the level of significance of the difference between the observed difference between ambient and excurrent O_2 concentrations.

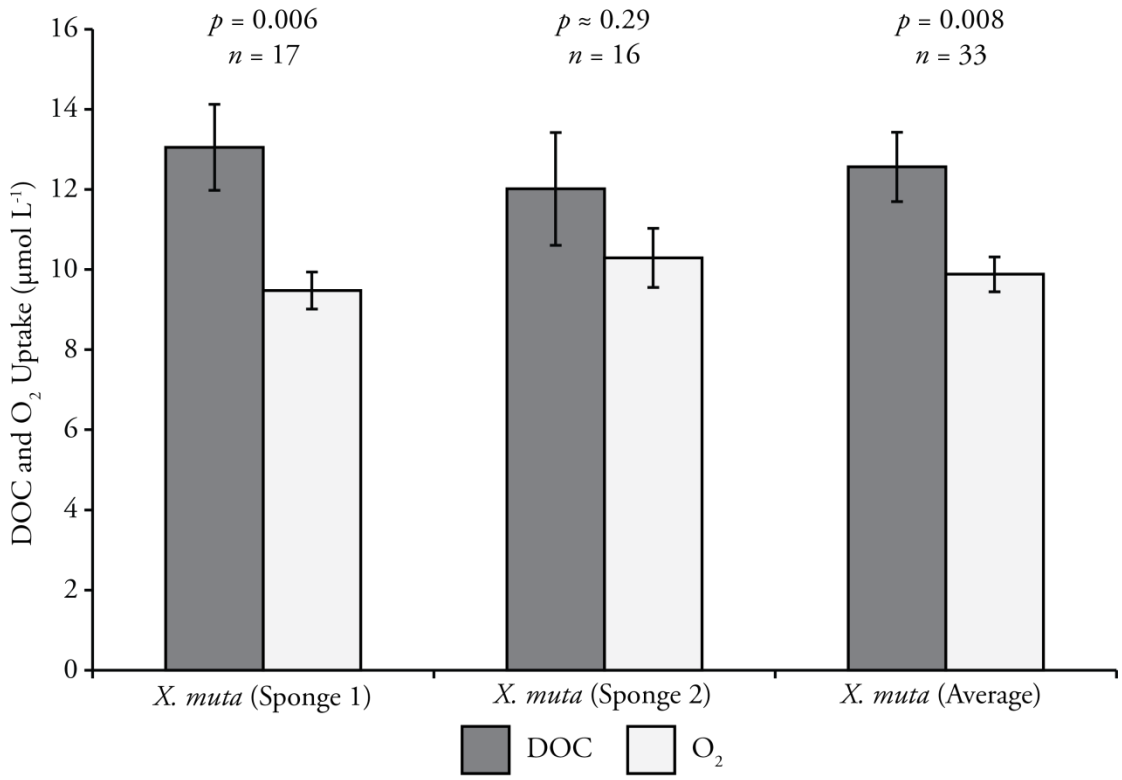

Figure 2.3: Average uptake of O_2 and DOC for the two tested sponges and the overall average. The difference between ΔDOC and ΔO_2 was analyzed to assess stoichiometric balance between respiration O_2 demand and DOC uptake. Error bars are 1 SE, N equals the number of paired water collections and averaged O_2 measurements, and P values (two-sample t-test) indicate the level of significance of the difference between the observed change in DOC and O_2.

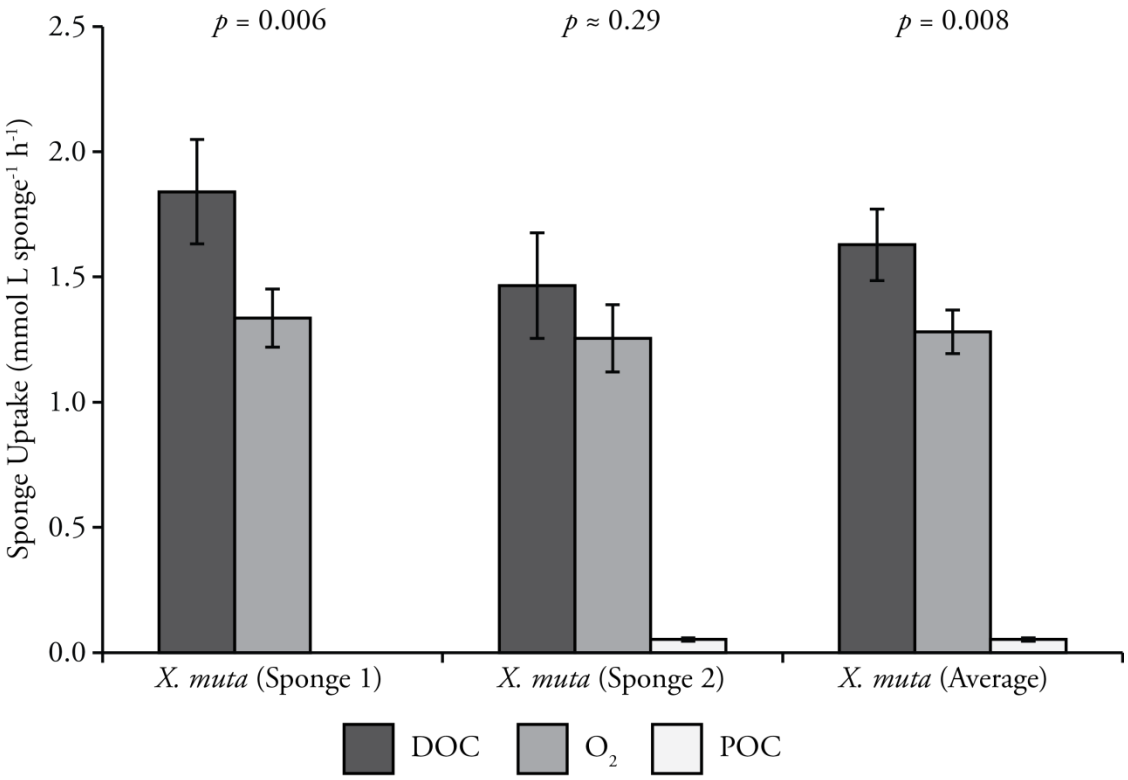

Figure 2.4: Average uptake fluxes of DOC, O_2, and POC for the two tested sponges and the overall average. POC was only sampled for sponge 2. Flux calculations were performed using the average, volumetric pumping rate for each individual and the average uptake of DOC, O_2, and POC. These values were then normalized to the volume of the tested sponge. Error bars represent 1 SE and P values (two-sample t-test) indicate the level of significance of the difference between DOC and O_2 fluxes.

CHAPTER 3:

Xestospongia muta as a Significant Source of Recycled Nitrogen to Floridian and Bahamian Reefs

Introduction

Declining coral cover throughout the Caribbean between 1977 and the present (Gardner et al. 2003, Green et al. 2008, and Perry et al. 2013) has been accompanied by a corresponding increase in sponges and macroalgae, and these organisms now dominate many reef environments (Aronson et al. 2002, McMurray et al. 2010). Sponges actively pump water through their tissues as a means to gather resources from the surrounding environment and expel waste products. As such, these organisms exhibit large filtration rates (Corredor et al. 1988, Weisz et al. 2008), and their pumping activities generate physical connectivity coupling processes occurring near the reef hardbottom and those in the overlying water column (e.g., Lesser 2006, Southwell et al. 2008b, Keesing et al. 2013). These organisms feed across a wide particle size range (Reiswig 1971) and have been implicated in the respiration of dissolved organic matter (DOM; Yahel et al. 2003, de Goeij et al. 2008, Hoer 2015, this volume). As a result of these respiration processes, the exhalent water of marine sponges is often enriched in dissolved inorganic nitrogen (DIN) making sponges potentially potent local drivers of nutrient recycling.

The efflux of remineralized nitrogen (N) observed from marine sponges contains ammonium (NH_4^+) and nitrate plus nitrite ($NO_3^- + NO_2^-$; henceforth NO_x^-) at levels significantly higher than those found in surrounding seawater (e.g., Corredor et al. 1988, Jiménez and Ribes 2007, Southwell et al. 2008b, Hoer 2015, this volume). The speciation of DIN in sponge effluent

is dictated by a presence or lack of microbial processes occurring within the tissue of the tested sponge (Diaz and Ward 1997, Jiménez and Ribes 2007, Hoffmann et al. 2009, Hoer 2015, this volume). Some sponge species host large microbial populations within their tissues, and the microbial consortia living within these high microbial abundance sponges (HMA *sensu* Hentschel et al. 2006) often represent as much as 40% of total sponge volume (Freeman and Thacker 2011). The microbial community hosted by these sponges drives diverse nutrient element transformations including nitrification (Southwell et al. 2008a, Hoffmann et al. 2009, Fiore et al. 2013), and may provide metabolic access to a wider range of organic substrates (Pawlik et al. 2015 and references therein). Sponge species with low numbers of associated microorganisms (termed low microbial abundance, LMA, sponges, Hentschel et al. 2006) produce ammonium-rich effluent with a chemical signature characteristic of animal-based metabolism (Southwell et al. 2008b).

The relative abundance of HMA sponges represents >50% the total sponge biomass below 15 m water depth on reefs throughout the Caribbean, and many reefs are characterized by a trend of increasing HMA sponge populations below this depth (Pawlik et al 2015). These expanding populations, the ability of HMA sponges to consume a wide range of particulate and dissolved organic matter (POM and DOM; Yahel et al. 2003, de Goeij et al. 2013, Hoer 2015, this volume), and the consequent efflux of reminieralized N (Corredor et al. 1988, Southwell et al. 2008b, Hoer 2015, this volume) make these organisms of critical importance to the cycling of carbon (C) and N at the reef scale. Yet, the extent to which the processes mediated by these organisms are conserved across environmental gradients is only beginning to be understood.

In this study, we tested the degree to which the release of recycled N by the giant barrel sponge *Xestospongia muta* (Demospongiae) is conserved between the environments found on the

Florida Keys reef tract and those found on the oligotrophic reefs of San Salvador, Bahamas. *X. muta* was chosen as a target species because it is a conspicuous and important component of total benthic biomass on reefs throughout the Caribbean (H. Büttner 1996, Armstrong et al. 2006), and is particularly prevalent on the Florida Keys reef tract where it can represent as much as 65% of total sponge biomass (Southwell et al. 2008b, McMurray et al. 2008, 2010). Further, *X. muta* is an HMA sponge, with tissue bacterial densities approaching 8 x 10^9 microorganisms per gram of sponge wet weight (Hentschel et al. 2006), and thus is expected to be able to exploit a range of organic substrates for nutritive gain and exhibit significant nutrient element transformations in both environments (Southwell et al. 2008b, Fiore et al. 2013, Hoer 2015, this volume). This species has been previously shown to exhibit temporally and spatially variable DIN efflux (Southwell et al. 2008, Gibson 2011, Fiore et al. 2013) making it an ideal candidate for exploration of behavioral stability across an environmental gradient. Sponge pumping rate and manipulations of DIN in filtered water were measured *in situ* using undisturbed individuals attached to their original substrate. This *in situ* approach allows sponge processes to be observed in the absence of environmental manipulations that may impact the natural behavior of the animal (e.g, Gerrodette and Flechsig 1979, Tompkins-MacDonald and Leys 2008, Fan et al. 2013).

Methods

Sample collection

Water samples were collected from *X. muta* individuals on Conch Reef (24°57’0.27”N, 80°27’12.21”W) in the Florida Keys and five reef sites around the island of San Salvador, Bahamas (Snow Bay: 23°56’38.06"N, 74°30'24.20"W; French Bay: 23°56'59.02"N, 74°32'8.11"W; Sandy Point: 23°56'18.50"N, 74°33'40.97"W; Hall’s Landing: 24° 0'24.31"N,

74°32'22.89"W; Cockburn Town: 24° 1'34.49"N, 74°31'50.65"W). Water depth ranged from 8 to 31 m and the sampled individuals inhabited regions that spanned a variety of environmental conditions from the reef flat to the outer reef wall. *X. muta* is an excellent species for quantitative measures of sponge-mediated biogeochemical transformations and pumping velocity as the species is characterized by a barrel morphology featuring a large, central osculum. This morphological feature facilitated non-disruptive sampling of the excurrent jet and allowed for precise quantifications of excurrent flow speed using the dye-front method and acoustic Doppler velocimetry.

Dissolved inorganic nitrogen samples on Conch Reef were collected from the individuals measured in the work of Hoer (2015, this volume). The samples discussed here were collected contemporaneously using identical methodology (Hoer 2015, this volume). Site descriptions, morphometric measures of the tested sponges, and sampling methodologies are presented in detail in Hoer (2015), and therefore these will be only briefly discussed here. Sample collection in San Salavador, Bahamas was performed using healthy-looking sponges which were randomly selected by divers on SCUBA across a depth gradient at the chosen various sites. Prior to sampling, each individual was confirmed to be pumping with fluorescein dye. Similar to the sponges in Florida, the dimensions of the tested individuals were measured at the time of sample collection in order to calculate sponge volumes without necessitating harvest of the animal. However, organism volumes were calculated using geometric approximations rather than the formula presented by McMurray and co-workers (2010). For consistency, the sponges chosen in Florida were geometrically approximated and their volumes were recalculated using the full set of previously collected dimensions.

Sponge water sampling was performed by divers on SCUBA using the "InEx" method (Yahel et al. 2005); collections in the Florida Keys were performed from August 9 to 16, 2011 and those on San Salvador were performed from March 2 to 9, 2013. Temporally paired, triplicate samples of ambient water near the exterior walls of the sponge (<20 cm from the outer wall of the sponge) and excurrent waters exiting the sponge as a coherent jet were collected during each sampling period. Ambient and excurrent pairs were collected within 10 minutes of one another, and each water sample was collected and simultaneously filtered with an in-line filter attached to a 60 mL polypropylene syringe by a polycarbonate stopcock. Attached to the filter and syringe was a 10 cm piece of small-diameter, high density polyethylene (HDPE) tubing which allowed samples to be collected with minimal disturbance to the target individual. A new pre-combusted, 25 mm Whatman GF/F filter was used to filter each water sample; GF/Fs were selected for filtration due to their suitability for combustion (baking at 450°C for >6 hours) and use in prior studies of DIN production by Caribbean sponges (e.g. Diaz and Ward 1997, Southwell et al. 2008b). During sampling, the entire collection apparatus (syringe, tubing, filter, and stopcock) was rinsed 3x by pulling filtered target water into the syringe, and the rinsing water was expelled out of the open stopcock arm. The fourth and final sample was carefully drawn into the syringe (< 2 mL sec^{-1}) in order to ensure that the collected sample was representative of the desired water mass. Upon completion, the stopcock was closed and the sample was stored in an ice bath prior to subsampling and preservation (less than 8 hours from collection to preservation). At the lab, samples were immediately divided for different analyses (NO_x^- and NH_4^+). Nitrate and Nitrite (NO_x^-) samples (20 mL volume) were placed into sample-rinsed, borosilicate glass scintillation vials and frozen until subsequent analysis. Ammonium (NH_4^+) samples (20 mL volume) were placed into sample-rinsed amber HDPE bottles.

Ammonium samples from Florida were analyzed immediately to reduce the potential impact of degradation on sample integrity but, due to logistical limitations, NH_4^+ samples from San Salvador were frozen and analyzed upon return to North Carolina. For each time point, the sampled parameters were collected in triplicate for further quality assurance and control.

All plastics utilized in sample collection and processing (including syringes, stopcocks, tubing, filter holders, sample bottles, and collection vial lids) were composed of polypropylene, HDPE, or polycarbonate and all were soaked in a 0.1M HCl bath for at least 12 hours and rinsed 6 times with 18.2 MΩ type I water prior to use and between each sampling. Due to limited access to HCl in the Bahamas, sample collection plastics were not acid washed between uses and were only rinsed with copious amounts of 18.2 MΩ type I water. Borosilicate scintillation vials used for sample collection were subjected to the same washing procedure, followed by combustion at 450°C for >6 hours to remove any residual nitrogenous material. Combusted glassware was stored in foil and bagged to minimize outside contamination prior to use. Filters were combusted at 450°C for >6 hrs. Amber HDPE bottles used for ammonium samples were acid washed and rinsed following the aforementioned protocol, and after this procedure small aliquots of the o-phthaladehyde working reagent was added to the bottles and allowed to react for 24 hours to ensure remove any residual ammonium from the sample bottle prior to use for standards or samples.

Sponge pumping rates

Sponge pumping rates were quantified using two methodologies, acoustic Doppler velocimetry and dye-front speed. The tested individuals on Conch Reef were continuously measured between May 25 and August 17, 2011 using Nortek Vector acoustic Doppler velocimeters (ADVs; Hoer 2015, this volume). Due to contemporaneous collection of DIN and

the DOM samples (Hoer 2015, this volume), the ADV data presented therein was utilized for these collections. Detailed methodology for the deployment of instruments and the treatment of the resultant data (binning of sample blocks, despiking, etc.) is presented there. Pumping rates were determined for the sponges sampled around San Salvador by measuring the speed at which pulses of fluorescein dye were ejected from the osculum of the target individual (Weisz et al. 2008). A diver positioned a ruler 15 cm into the osculum and release discrete puffs of dye ($n \geq 40$), measuring the time for the dye plug to travel to the rim of the sponge osculum. The average time required for the puffs to traverse this distance was taken to represent the vertical velocity of the excurrent jet (cm sec^{-1}). Streams of dye were also released across the planar area of the osculum in order to determine the radial distribution of the exhalent stream velocities.

Sample Analysis

Ammonium analyses were performed using the method of Holmes et al. (1999). Sampled volumes were reacted with 5mL of o-phthaladehyde working reagent in amber, HDPE sample bottles and allowed to develop at room temperature for 2.5 hours. After the incubation period, samples were analyzed using a Turner Designs TD-700 laboratory fluorometer equipped with an ammonium optical kit (Turner Designs 10-303). The method detection limit was determined to be 10 nmol L^{-1} by repeated standard measurements. Standards were prepared daily at the point of use by serial dilution of a purchased stock solution (Ricca Chemical Company 693-16), and analyzed with the prepared samples. NO_x^- discrete samples were analyzed using Spectrophotometric Elemental Analysis System (SEASII-NO_x) autoanalyzers configured for benchtop use (Steimle et al. 2002; Adornato et al. 2005, Adornato et al. 2007 for detailed descriptions of similarly utilized instrumentation). During analysis, the instrument measured the combined concentrations of NO_3^- and NO_2^-, or NO_x^- ; both NO_2^- in the water

sample and NO_2^- produced from cadmium reduction of NO_3^- were determined spectrophotometrically utilizing the Griess method (Adronato et al. 2007). The deployed instruments used 15 cm optical pathlengths which increased sensitivity and reduced the method detection limit to 25 nmol NO_x^- L^{-1} (determined by repeated analysis of standard solutions). Standards for NO_x^- measurements were prepared by dilution of a purchased stock (SPEX Certiprep AS-NO39-2Y and ASNO29-2Y), and analyzed daily with collected samples.

Results

Pumping Velocity

The two *X. muta* individuals showed significantly different average excurrent flow rates over the measured period (paired t-test). The sponge 1 *X. muta* produced an average excurrent flow rate of 5.3 ± 0.9 cm sec^{-1} (mean ± 1 SD, N = 19, P = 0.0001), and sponge 2 showed an average excurrent jet flow rate of 4.3 ± 0.9 cm sec^{-1} (mean ± 1 SD, N = 19, P = 0.0002). Video-recorded dye flow measurements characterized the excurrent jet in this species as approximately 40% (SD = 9, N = 21) of the planar area of the sponge osculum, and this proportion was applied to the analyzed individuals to generate volumetric fluxes ($V = 0.4A_{osc}V_{exc}$; where V is the volumetric flux, 0.4 is the proportion of the oscular area characterized by a discrete jet, A_{osc} is the oscular area, and V_{exc} is the measured excurrent velocity). Additionally, the dye flow videos generated pumping velocities that corroborated the velocities measured by ADV (N. Lindquist unpublished data). Sponge 1 had a planar area of 2787 cm^2 (jet area = 1143 cm^2) and this generated an average volumetric flow rate of 6 L sec^{-1}, while sponge 2 had a planar area of 929 cm^2 (jet area = 381 cm^2) and an average volumetric flow rate of 2 L sec^{-1}. For sponges 1 and 2, these flow rates are equivalent to filtering water more than 5,000x and 3,500x their body volume daily (Sponge 1: 130 L; Sponge 2: 42 L, based on geometric approximations).

The sponges measured in San Salvador had somewhat higher excurrent velocities than those measured on Conch Reef. Yet, the Bahamian sponges tended to have smaller oscular openings and, therefore, the calculated volumetric flowrates for these organisms were comparable to the smaller of the two individuals sampled on Conch Reef. The average excurrent flow rate was 7.7 ± 2.9 cm sec^{-1} (mean ± 1SD; n = 18 individuals), for individuals ranging from 14 to 160 L_{sponge}. For these individuals, the excurrent jet represented approximately the same proportion of the planar area as those in Florida, yet rough surface conditions and surge near the bottom clouded these results. Consequently, the proportions from individuals measured in the Florida Keys were applied to these organisms when generating volumetric flow rates. The average volumetric flow rate was 1.6 ± 1.0 L sec^{-1} (mean ± 1SD; n = 17 individuals) for the tested organisms. The water pumping rates measured for *X. muta* in both environments agree with previous assessments for this species (Weisz et al. 2008, Fiore et al. 2013) as well as for other HMA sponges (Weisz et al. 2008, de Goeij et al. 2008 and citations within).

DIN Release Rate

The average DIN concentrations in ambient water masses surrounding the tested sponges were similar at both sites, although NO_x^- appeared to be somewhat elevated in the Florida Keys. Ambient NH_4^+ and NO_x^- concentrations on Conch Reef were 0.22 ± 0.13 and 0.62 ± 0.43 μmol N L^{-1}, respectively (mean ± 1SD; n = 36 and 27 for NH_4^+ and NO_x^-, respectively) and on San Salvador they were 0.15 ± 0.11 and 0.29 ± 0.21 μmol N L^{-1} for NH_4^+ and NO_x^-, respectively (mean ± 1SD; n = 20). DIN release or uptake was determined as the difference between the concentration of the target analyte measured in ambient water and in the excurrent jet (Yahel et al. 2005; $\Delta[Analyte] = [Analyte]_{excurrent} - [Analyte]_{ambient}$). Therefore, only samples collected contemporaneously from the same individual were considered. Of the 44 paired ambient and

excurrent collections of NO_x^- concentration across the tested environments, 38 showed significant release of NO_x^-, 4 showed NO_x^- uptake, and 2 showed no significant difference between ambient and excurrent water. Sponges in Florida showed average NO_x^- release of 0.55 ± 0.15 μmol N L^{-1} (mean ± 1SE; n = 27; Wilcoxon Signed-Rank test, p = 0.0004; Figure 3.1) and the average value for sponges in the Bahamas was slightly higher at 0.92 ± 0.15 μmol N L^{-1} (mean ± 1SE; n = 20; Wilcoxon Signed-Rank test, p < 0.0001; Figure 3.1). Behavior of the individuals with respect to NH_4^+ concentrations was slightly more variable, particularly at sites in the Bahamas. However, the variability in samples from Bahamian reefs may be due to low concentrations found in oligotrophic waters and the extended preservation time necessitated by international transport (1-2 weeks from collection to analysis). Of the 42 paired collections, 29 showed NH_4^+ uptake, 12 showed NH_4^+ production, and 1 showed no significant difference between ambient and excurrent collections. The average of these paired collections for individuals in Florida showed NH_4^+ uptake of 0.06 ± 0.03 μmol N L^{-1} (mean ± 1SE; n = 36; Wilcoxon Signed-Rank test, p = 0.0001; Figure 3.2) whereas individuals in the Bahamas showed no significant change from ambient waters (0.02 ± 0.04 μmol N L^{-1}; mean ± 1SE; n = 20; Wilcoxon Signed-Rank test, p = 0.23; Figure 3.2).

The obtained values for "InEx" (*sensu* Yahel et al. 2005) difference were converted to fluxes using the volumetric pumping rates determined from dye-flow and ADV measurements and then were normalized to the volume of the analyzed sponge. For 3 individuals sampled in the Bahamas, pumping velocity could not be obtained (morphological issues, excessive surge, etc.) and therefore flux data are not presented for these organisms. This yields average NO_x^- release rates of 81 ± 17 μmol NO_x^- hr^{-1} L_{sponge}^{-1} for individuals in Florida (mean ± 1 SE; n = 27; one sample t-test vs. 0; p < 0.0001; Figure 3.3) and 66 ± 11 μmol NO_x^- hr^{-1} L_{sponge}^{-1} for

individuals in the Bahamas (mean ± 1 SE; n = 27; one sample t-test vs. 0; $p < 0.0001$; Figure 3.3). Similarly, this calculation was performed for NH_4^+ difference and yielded an average uptake flux of 13 ± 6 μmol NH_4^+ hr^{-1} L_{sponge}^{-1} for individuals in Florida (mean ± 1 SE; n = 27; one sample t-test vs. 0; $p = 0.04$; Figure 3.3) and an average flux in the Bahamas which was not significantly different from zero (1.5 ± 2.7 μmol NH_4^+ hr^{-1} L_{sponge}^{-1}; mean ± 1 SE; n = 27; one sample t-test vs. 0; $p = 0.59$; Figure 3.3). No significant difference in DIN production or uptake was observed between the collection times at either location (unpaired t-test; $p > 0.10$).

Discussion

Sponge-mediated nitrification has been shown to be a potentially important source for bioavailable N to organisms in a variety of environments (e.g., Corredor et al. 1988, Jiménez and Ribes 2007, Hoer 2015, this volume), and our observations represent further evidence of this process occurring in undisturbed individuals in their natural habitat (e.g., Southwell et al. 2008b, Gibson 2011, Fiore et al. 2013, Hoer 2015, this volume). The average NO_x^- fluxes measured for *X. muta* in the two tested environments (75 ± 11 μmol NO_x^- hr^{-1} L_{sponge}^{-1}; mean ± 1SE; n = 44) are in very good agreement with *in situ* autoanalyzer deployments conducted on this species on Conch Reef (80 ± 40 μmol NO_x^- hr^{-1} L_{sponge}^{-1}; Gibson 2011), but are lower than previously conducted paired syringe samplings on this species (170 ± 40 μmol NO_x^- hr^{-1} L_{sponge}^{-1}; Southwell et al. 2008b). While the exact reason for this difference is unknown, the DIN concentrations measured during the work of Southwell et al. (2008b) were appreciably higher than those measured during our observations, and this may have contributed to the elevated efflux of NO_x^- calculated from their measurements. Furthermore, discrete collection methodologies only provide brief (10s of minutes) observations of sponge-mediated processes and temporal variability in organism behavior is expected. This temporal variability could be cause for the

disparity between our observations and those of Southwell et al. (2008b), and this dynamic behavior would be expected to cause increased uncertainty as these values are extrapolated to longer timescales. However, long-term, *in situ* observations like those performed by Gibson (2011) should be a more accurate reflection of organism behavior, particularly at the timescale of hours and days, and the similarity of our results to these prior data is a promising indication that obtained discrete samples may be representative of long-term organism behavior. Our rates of NO_x^- release are also lower than those observed by Fiore et al. (2013) across a similar environmental gradient as was tested here. However, Fiore and co-workers (2013) overestimated the volumetric flow rate through the sponges they measured by assuming the vertical excurrent velocity was representative of the full planar area of the osculum. If we expand our flux by assuming that the excurrent velocity includes the entire planar area, our values are very similar to the NO_x^- efflux measured by Fiore et al. (2013) on Rock Bottom Reef in the Cayman Islands.

The observed production of NO_x^- is in stoichiometric excess of the nitrification rate that would be supported by NH_4^+ uptake from filtered water, which indicates direct, internal connectivity between the respiration of absorbed organic matter and the subsequent nitrification of the produced NH_4^+ (Corredor et al. 1988, Southwell et al. 2008b, Fiore et al. 2013, Hoer 2015, this volume). Therefore, this respired organic matter is expected to be the primary N feedstock for nitrification processes mediated by these organisms. Further, investigations of this species on Conch Reef indicated that the primary source of respired organic matter is from the dissolved pool (Hoer 2015, this volume). This likely holds true for the sponges sampled on the oligotrophic environments of San Salvador, but the absence of direct quantifications preclude us from asserting this definitively. Using average respired DOM by these organisms (1.3 ± 0.5 mmol C hr^{-1} L_{sponge}^{-1}; mean ± 1SE; Hoer 2015, this volume) we calculated that the organisms

also respired approximately 0.1 ± 0.04 mmol N hr^{-1} L_{sponge}^{-1} (mean ± 1SE), using the average C:N content of DOM in the surface ocean (C:N ~ 13.6; Benner 2002). This estimated quantity of oxidized dissolved organic N is remarkably close to the quantity of released DIN (0.062 ± 0.013 mmol DIN hr^{-1} L_{sponge}^{-1}; mean ± 1SE), further implicating it as the probable feedstock for the released inorganic nitrogen. This rapid remineralization of dissolved organic matter may represent an ecologically relevant source of DIN on nutrient-poor reefs by rapidly recycling nutrients bound in organic compounds into more bioavailable inorganic forms (e.g., Diaz and Ward 1997, Ribes et al. 2005, de Goeij et al. 2013).

We observed no significant difference between the rate of NO_x^- production in the Florida Keys and that calculated for sponges in the Bahamas (unpaired t-test; n = 44 collections; p = 0.49; Figures 3.1 and 3.3). However, individuals in Florida were observed to utilize more NH_4^+ from filtered water than organisms tested on Bahamian reefs (Figures 3.2 and 3.3). The observed similarity between the rates of DIN release measured in the tested environments was unexpected given the metabolic reliance of these organisms on organic matter from the dissolved pool and the presumed increase in the recalcitrant fraction of this pool on San Salvador reefs as compared to those in Florida. A significant proportion of reef DOM is considered to be biological labile (30%; van Duyl and Gast 2001), however, the sampled reef waters of San Salvador are consistently bathed in oceanic water with a DOM signature that is likely characterized by a reduced contribution of bioavailable compounds (Benner 2002, Carlson 2002, Nebbioso and Piccolo 2013). This posited decrease in DOM bioavailability was predicted to reduce the rate that the tested sponges could release reminieralized N. The lack of this hypothesized disparity may indicate that the DOM pool is characterized by similar compounds in both environments, or that sponges have metabolic access to compounds to which they are exposed, including those

regularly exposed to open oceanic water. However, the suggestions regarding the fraction of the DOM pool utilized by the sponge community are purely conjectural. Future work describing the ability of sponges to respire DOM on oligotrophic reefs, and molecular characterization of available DOM in these environments may provide some insight into these hypotheses.

The similarity between the results in these two environments contrasts with the results of Fiore and co-workers (2013) that showed profound differences between fluxes observed on Conch Reef and those on Lee Stocking Island, Bahamas. The *X. muta* individuals they sampled on Conch Reef showed a broad trend of NO_x^- uptake and measurements for sponges on Bahamian reefs exhibited significant NO_x^- efflux (Fiore et al. 2013); NO_x^- uptake accounted for 27% of all their measured flux and 67% of their flux measurements in the Florida Keys. Uptake of NO_x^- was observed in 4 of the 44 total collections performed during our assessments, and these events represented approximately 9% of our total observations and ~15% of observations in the Florida Keys. The sparse occurrence of these events in our dataset may be indicative of reduced temporal variability in the dominant microbial process occurring within the tissue of the host sponge relative to observations of Fiore et al. (2013). Yet, neither the degree of temporal variability nor the dominance of NO_x^- absorption from ambient water seen in the work of Fiore et al. (2013) was observed in our measurements , nor was it observed in long-term *in situ* deployments of autoanalyzers on *X. muta* in this environment (Gibson 2011). Fiore et al. (2013) also noted a much higher flux of NH_4^+ than NO_x^- for many of the sampled sponges, which was not observed in any of the 22 individuals that we tested across a similar environmental gradient (Figures 3.1, 3.2, and 3.3). However, the highlighted differences do not preclude the occurrence of temporal variability of the magnitude presented by Fiore et al. (2013), particularly given the demonstrated ability for these organisms to mediate processes that consume NO_x^- (i.e.

denitrification; Gibson 2011). Rather, this disparity raises questions as to what environmental or organismal factors precipitated this drastic change between the observed DIN fluxes in this species, and what facilitated a dominant presence of N reduction pathways. All NO_x^- uptake events in our tested individuals occurred in specimens sampled on Conch Reef, yet these events did not occur contemporaneously in both tested organisms, indicating the potential for an individualized organism alteration in the absence of a clear environmental trigger. Interestingly, at the time of these apparent shifts in the N metabolism of the holobiont, sponge pumping rate and respiration oxygen demand were no different than those at other times during the record, and syringe collected dissolved organic matter showed a clear signal of normative uptake (Hoer 2015, this volume).

Nevertheless, our results indicate the potential for *X. muta* to represent a large source of remineralized N on both Floridian and Bahamian reefs. The lack of difference between the observed N release rates in these locations indicates the potential for nitrification behavior in this species to be somewhat conserved across a variety of environments. This conservation may allow the use of N release rates calculated in these environments to predict the impact of these organisms on reefs throughout the Caribbean. Yet, given previously presented environmental variability, this idea warrants future exploration. The net DIN ($NO_x^- + NH_4^+$) efflux from the tested organisms was 62 ± 13 μmol DIN hr^{-1} L_{sponge}^{-1}, which at the measured range of densities of *X. muta* on Conch Reef (1.3 – 2.8 L m^{-2}; McMurray et al. 2010, Gibson 2011) equates to a DIN flux of 1.9 ± 0.4 mmol N m^{-2} day^{-1} to 4.1 ± 0.9 mmol N m^{-2} day^{-1} from this species alone. DIN areal fluxes from this species are comparable to previously calculated values from sponge communities in the Caribbean and Mediterranean (e.g. Diaz and Ward 1997, Jiménez and Ribes 2007) and are higher than what was observed in Florida Bay and the western coast of Australia

(Hoer 2015, this volume; Keesing et al. 2013). On Caribbean reefs, this sponge-mediated efflux of DIN may benefit benthic algal biomass which dominates reef primary productivity (Hatcher 1990). Rates of net primary production on the reef benthos range between 200 and 500 mmol C m^{-2} day^{-1} (Hatcher 1990), and this primary productivity drives an N demand of 13 – 33 mmol N m^{-2} day^{-1}, assuming the C:N ratio of a dominant macroalgal species (*Dictyota* sp., C:N ~15; Beach et al. 2006, Silbiger 2009). Based on these approximations, a single sponge species can supply between 6 and 30% of the areal N demand from net primary productivity. Assuming all sponge biomass surveyed by Southwell et al. (2008b) releases DIN at the rate of *X. muta*, the sponge associated N efflux could account for as much as half of the estimated N demand (3.6 L_{sponge} m^{-2}; 5.4 ± 1.1 mmol N m^{-2} day^{-1}). Therefore, it is plausible that these organisms represent a significant source or recycled N on reefs where populations of these sponges are large, particularly in the oligotrophic marine conditions of San Salvador. Furthermore, as sponge populations expand in habitats throughout the Caribbean (Aronson et al. 2002, McMurray et al. 2010), a concomitant increase in the contribution of DIN from these organisms would be expected. This is especially true given the N release rates calculated here as well as in other studies (e.g., Corredor et al. 1988, Southwell et al. 2008b, Hoer 2015 this volume), and the potential for these rates to be conserved under varying environmental conditions.

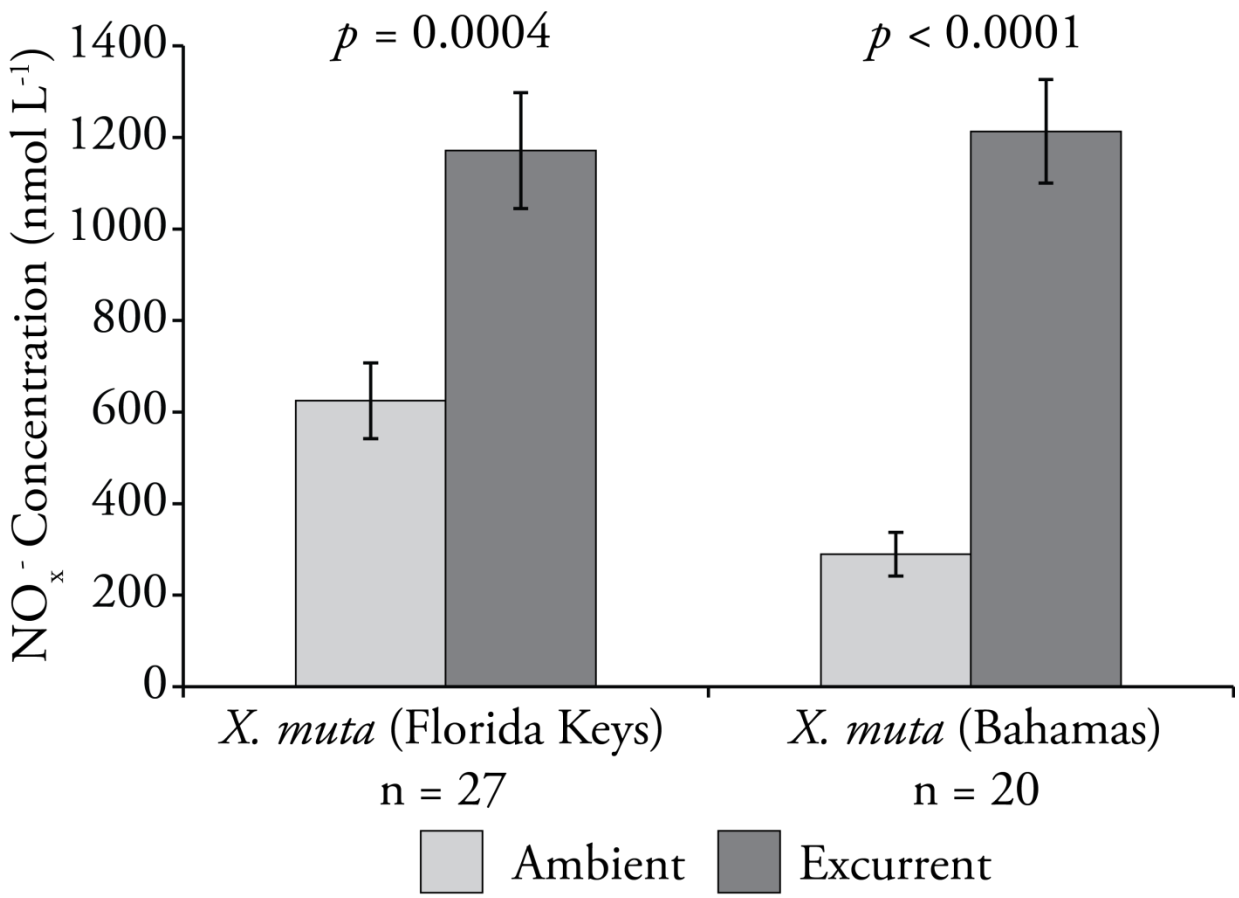

Figure 3.1: Average NO_x^- concentration in the ambient and excurrent waters of *Xestospongia muta* individuals tested in the Florida Keys and the Bahamas. Error bars are ± 1SE and n equals the number of paired ambient-excurrent collections. P values (Wilcoxon signed-rank test) indicate the level of significance between the ambient and excurrent NO_x^- concentrations.

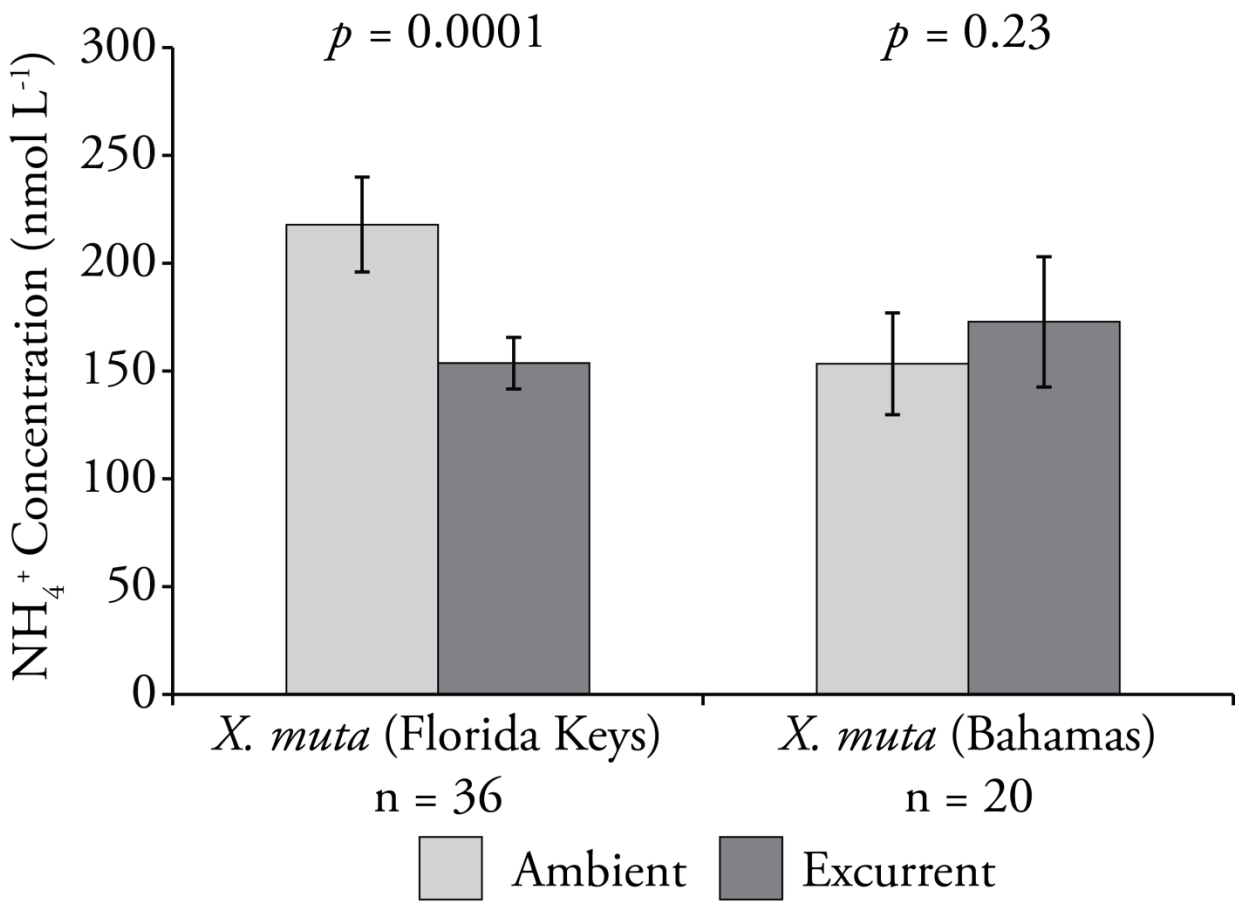

Figure 3.2: Average NH_4^+ concentration in the ambient and excurrent waters of *Xestospongia muta* individuals tested in the Florida Keys and the Bahamas. Error bars are ± 1SE and n equals the number of paired ambient-excurrent collections. P values (Wilcoxon signed-rank test) indicate the level of significance between the ambient and excurrent NH_4^+ concentrations.

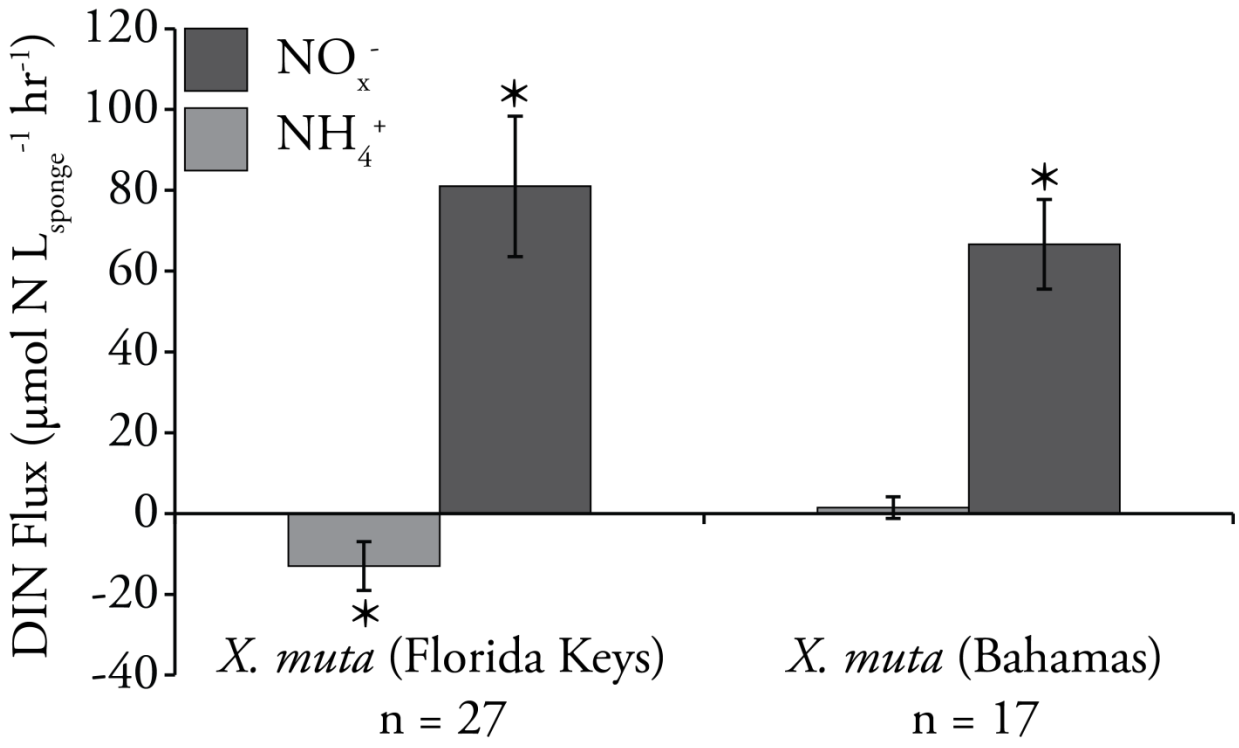

Figure 3.3: Average fluxes of NO_x^- and NH_4^+ for the tested sponges from Florida and the Bahamas. Flux calculations were performed using the average, volumetric pumping rate for each individual and the average uptake or production of NO_x^- and NH_4^+. These values were then normalized to the volume of the tested sponge and averaged for each tested site. Error bars represent ±1SE, n represents the number of paired collections, and asterisks (*) over the displaced column represents significance of the flux (one-sample t-test vs. 0).

CHAPTER 4:

Efflux and Speciation of Dissolved Inorganic Nitrogen (DIN) from Ecologically Relevant Sponge Species in Florida Bay

Introduction

Florida Bay is a lagoonal estuary whose western boundary is open to the Gulf of Mexico; it is bordered on the north by peninsular Florida and on the south and east by the Florida Keys archipelagic island chain. The bay is a shallow (< 3 m water depth), oligotrophic system and dissolved organic matter represents the majority of water column nitrogen (N) and phosphorus (P) (Boyer et al. 1997, Boyer et al. 2006). N supply to the bay is similarly dominated by organic matter with as much as 90% of sourced N from the Everglades entering as dissolved organic nitrogen (Boyer et al. 1999, Childers et al. 2006). This dominant presence of organic nutrients suggests an exceedingly important role of the microbial loop or other means of organic matter remineralization in the sourcing of inorganic nutrients to photosynthetic organisms (Yarbro and Carlson 2008). The extensive seagrass beds throughout Florida Bay have been highlighted as potentially important sites of dissolved organic nitrogen (DON) remineralization (Yarbro and Carlson 2008). Sponges, known as an important source for dissolved inorganic nitrogen (DIN) derived from particulate and dissolved organic matter (e.g., Corredor et al. 1988, Jiménez and Ribes 2007, and Southwell et al. 2008b) are also abundant components of benthic biomass (Peterson et al. 2006). The restricted oceanic exchange in this environment allows local processes to dominate chemical cycling (e.g. Fourqurean et al. 1999, Peterson et al. 2006, Zhang

and Fischer 2014) and these systems may be characterized by an enhanced, yet previously unaddressed, importance of sponge recycling processes.

An extensive survey of sponge biomass in Florida Bay revealed their near ubiquitous presence with populations with densities between 0.02 and 22 individuals m^{-2} at more than 70% of 207 sites surveyed (Peterson et al. 2006). Their abundant presence (Peterson et al. 2006) and efficiency as filter feeders (e.g. Reiswig 1971, Weisz et al. 2008) make the sponge population a dominant benthic heterotroph in this ecosystem. The grazing pressure from sponges is enhanced by the ability to source metabolic substrates from both dissolved organic matter (DOM) as well as particulate organic matter (POM) (e.g., Resiwig 1974, van Duyl et al. 2008, Pawlik et al. 2015). As a result of this dietary plasticity, the sponge community may represent an important pathway for DOM remineralization in Florida Bay, given the metabolic reliance of some sponges on DOM in reef ecosystems (de Goeij et al. 2008, Mueller et al. 2014, Hoer 2015, this volume) and the large sourcing of dissolved organics to this environment from the Everglades wetlands (e.g., Fourqurean et al. 1999, Boyer et al. 2006, Childers et al. 2006).

Sponges feed by actively pumping large volumes of water through their tissues with their exhalent jet being expelled into the overlying water column. As a result, they are important physical and chemical drivers of coupling between processes occurring near the benthos and those in the water column (e.g., Lesser 2006, Southwell et al. 2008a, Keesing et al. 2013). Although they are distributed non-uniformly, sponges have the potential to represent the most important source of recycled N in Florida Bay, particularly considering previously measured rates of DIN release from two ecologically important species in this system, *C. nucula* and *Ircinia* sp. (Corredor et al. 1988, Diaz and Ward 1997, Southwell et al. 2008b). The fluxes of remineralized N that have been observed from sponge communities in tropical and temperate

ecosystems are characterized by both ammonium (NH_4^+) and nitrate plus nitrite ($NO_3^- + NO_2^-$; NO_x^-) (e.g., Corredor et al. 1988, Jiménez and Ribes 2007, and Southwell et al. 2008b). The speciation of DIN in sponge effluent is known to be dictated by occurance or absence of microbial processes within the sponge tissues (Diaz and Ward 1997, Jiménez and Ribes 2007, Hoffmann et al. 2009). Large microbe populations live within the tissue of high microbial abundance (HMA) sponges (Hentschel et al. 2006), often representing as much as 40% of total sponge tissue volume (Freeman and Thacker 2011). Microbial metabolism occurring within HMA sponges drives diverse nutrient element transformations, including nitrification (Southwell et al. 2008a, Hoffmann et al. 2009), potentially providing increased diversity in metabolically accessible organic matter (Pawlik et al. 2015 and references therein). Conversely, sponge species with low numbers of associated microorganisms (termed low microbial abundance (LMA) sponges, Hentschel et al. 2006) produce ammonium-rich effluent with a chemical signature characteristic of animal-based metabolism (Southwell et al. 2008b).

Many studies reporting DIN release by sponges (e.g. Corredor et al. 1988, Jiménez and Ribes 2007, Hoffmann et al. 2009) utilize methodologies that involve significant physical disturbances of the sponge animal or altered environmental conditions. This experimental manipulation may have large impacts on sponge behavior as they have been previously shown to be sensitive to environmental and physical stressors (e.g., Gerrodette and Flechsig 1979, Tompkins-MacDonald and Leys 2008). Here we present the results of *in situ*, underwater DIN flux measurements from undisturbed individuals representing 11 important species (Peterson et al. 2006) found in typical "hardground" areas of Florida Bay. The flux measurements were made by briefly enclosing untouched sponges representing each of these species attached to their original substrate in well-oxygenated chambers. The results allow a quantitative assessment of

their potential role in nitrogen cycling in Florida Bay. We hypothesize a continued differentiation between the speciation of DIN in exhalent waters of LMA and HMA sponges, with NH_4^+ and NO_x^- dominating their respective exhalent jets (Jiménez and Ribes 2007, Southwell et al. 2008b, Bayer et al. 2008). We also sought to quantify the relative importance of dissolved and particulate organic matter for sponge metabolism in Florida Bay, and further hypothesize that DOM should be an important metabolic substrate for HMA sponges and be of negligible importance in LMA sponges (Southwell et al. 2008b, de Goeij et al. 2008; Gibson 2011). We hypothesize that the sponge community has the potential to be an important driver of local chemical cycling that will be an important factor in balancing the N budget in Florida Bay.

Methods

Sample Sites

Three shallow sites (water depth < 3m) which exemplify varying environments that can be found within the Florida Bay ecosystem were sampled. The first site was a dock located at Florida Fish and Wildlife Conservation (FWC) Commission South Florida Regional Laboratory in Marathon, FL (Site ID: FWC Dock, 24°42'45.31" N, 81°5'54.89" W). The dock site was conveniently assessable and is characterized by a thin (approximately 20 cm) layer of carbonate mud overlying Pleistocene limestone, and is densely populated with benthic macroalgae. The dock itself is stabilized by a rock jetty that has been colonized by a variety of boring and encrusting sponge species, primarily *Chondrilla nucula*, with minimal contribution from non-boring and non-encrusting species. It has a large oceanic influence due to its proximity to an opening in the Florida Keys island chain between Marathon and Big Pine Key, which results in high currents during tidal exchanges with the ocean and corresponding fluctuations in water quality parameters (D. Hoer unpublished data). The second site was located within a semi-

protected cove 13 km northeast of the FWC dock site (Site ID: Burnt Point, 24°45’24.6” N, 80°58’55.0” W). The Burnt Point site has a thinner veneer of carbonate mud (5 to 10 cm) overlying the limestone than the dock site, and a diverse sponge population that included large populations of non-boring and non-encrusting species such as *Ircinia campana* and *Spongia spp.*, in addition to seagrasses and macroalgae. This site had a lower degree of tidal connectivity with the ocean, partially due to sheltering land projections and a greater distance from the nearest passage to the ocean. The third site is a small offshore basin, 13 km north of Long Key, FL, located within the Everglades National Park boundaries (Site ID: Mystery Basin, 24°56’36.6” N, 80°49’32.8” W) and almost completely isolated by an outer rim of shallow banks that shoal during low tides (Figure 4.1). The Mystery Basin site featured the thinnest veneer of carbonate sand and mud overlying the limestone hard-bottom averaging less than 5 cm thickness, and was characterized by a diverse population of sponges including large populations of *Sphesiospongia vesparium* and *Ircinia variabilis*. The shoals forming the outer rim of the basin restrict water exchange with the surrounding areas (Peterson et al. 2006) and are covered by seagrasses rooted in carbonate mud.

Sample Collection and Chamber methodology

Eleven sponge species were examined in this study: *Haliclona sp., Halichondria melanodocia, Cinachyrella* sp., *S. vesparium, I. variabilis, I.campana, Spongia graminea, Spongia Barbara, Geodia gibberosa,* and *C. nucula.* These species were chosen as they dominate sponge biomass at our studied sites and the whole of Florida Bay (Peterson et al. 2006, B. Peterson personal communication, January, 2011). Many of the sponge species that inhabit FL Bay are characterized by a multitude of small, diffuse oscula, each of which would necessitate individual measurements via the “InEx” method described by Yahel et al. (2005).

Consequently, a chamber incubation method was developed to determine fluxes and chemical speciation of released DIN. A simple benthic chamber was constructed from a 20L polypropylene drinking water jug with its bottom removed. The bottom edge of the modified jug was weighted with a fabric skirt filled with lead beads. The resulting chamber could be placed directly over a target sponge, still attached to the surrounding substrate, without touching the tested animal. The final chamber volume was 16.5 L after modifications, sufficiently small to allow observation of DIN concentration increases over periods of 30 to 150 minutes which could be used to calculate net DIN fluxes. The chamber (Figure 4.2) had inlets in place for water sample collection, air introduction from a SCUBA regulator, and an optical probe (HACH LDO101) for monitoring dissolved oxygen concentration in the chamber in real time. Using pumping function confirmed by fluorescein dye as a proxy for fitness, healthy sponge individuals were randomly selected by divers on SCUBA. After a targeted individual was confirmed to be pumping, the sponge was isolated from the surrounding substrate by slipping slit-collared polypropylene plastic sheet around the base of the sponge to seal off sediment contact with water inside the chamber. An ambient water sample was collected, ambient dissolved oxygen concentration was noted, and then the chamber was carefully lowered over the individual. Triplicate samples were taken every 30 minutes for 2.5 hours, and the chamber oxygen concentration was monitored constantly with air being slowly bubbled into the chamber whenever the oxygen concentration fell below 75% of ambient levels. One-way check valves were put in place to ensure that gas was able to escape during bubbling, and to allow ambient water inflow during sample collection. After the final sample was collected, the chamber was removed and the sponge was again checked with dye to ensure continued pumping activity. The sponge was then harvested for volume determination; sponge volumes were measured by water

displacement, with each sponge being measured 3 times for quality control. A minimum of 3 replicate individuals per species were analyzed to ensure reproducibility of any observed phenomenon, and 3 chamber replicates without a sponge were performed to observe any environmentally mediated phenomena in the absence of a sponge. Chamber experiments were performed during field excursions in May, July, and September 2013 and July 2014.

Samples were collected in triplicate 60 mL polypropylene syringes connected to polycarbonate stopcocks, which interfaced syringes directly to the outlet on the chamber. Samples were filtered *in situ* using pre-combusted, 25 mm Whatman GF/Fs that were plumbed in-line during sampling. Whatman GF/Fs were selected for sample filtration and POM collection due to their ability to be combusted prior to use and in order to coordinate with other reports of carbon and nitrogen cycling by Caribbean sponges (e.g., Diaz and Ward 1997, Yahel et al. 2003, Southwell et al. 2008b). Prior to sample collection, the syringe, filter, and connected fittings were rinsed once with sample water. Sample rinses were limited in order to minimize dilution of chamber water with ambient water introduced as sample was drawn out of the chamber. Syringes were immediately placed in an ice bath for transport to shore for subsampling and preservation (less than 8 hours from collection to preservation or analysis).

Upon return to shore, samples were immediately divided for dissolved inorganic nitrogen (DIN; NH_4^+ and NO_2^-+NO_3^- (NO_x^-)) as well as dissolved organic matter (total nitrogen (TN), and dissolved organic carbon (DOC)); DON was determined as the TN content less DIN. Dissolved organic matter samples were placed into three replicate borosilicate glass scintillation vials. Vials were rinsed with sample, filled with 20mL of sample water, and 100 µL of 50% H_3PO_4 was added. After the acid addition, the sample was stored at 4°C until subsequent analysis. Nitrate and Nitrite (NO_x^-) samples (20 mL volume) were placed into sample-rinsed, borosilicate

glass scintillation vials and frozen until subsequent analysis. Ammonium (NH_4^+) samples (20 mL volume) were placed into sample-rinsed amber HDPE bottles. Ammonium samples were analyzed immediately to reduce the potential impact of degradation on sample integrity. For each time point, the sampled parameters were collected in triplicate for quality assurance and control.

Particulate organic matter samples were collected from ambient water masses at our 3 study sites in order to assess the availability of particulate C and N. POM samples were collected daily at one or more of the sites during each of three field excursions (May, July, and September 2013) at three time points, 08:00, 12:00, and 16:00, in an attempt to estimate any diurnal variability in particulate loading. Mystery Basin and FWC Dock sites were sampled during May 2013, Burnt Point was sampled during July 2013, and the FWC Dock was sampled during September 2013. The FWC Dock site was sampled during 2 field seasons (May and September 2013) to assess long term variability. Peristaltic pumps were set up to pump ambient water (from approximately 30 cm off the bottom) through high-density polyethylene tubing to a shipboard 0.7 μm GF/F (Whatman, 47 mm). Flow rates were set to 20 mL min^{-1} by adjusting pump speeds at the beginning of each filter collection to ensure accurate collections. Samples were collected to give a 3 hour, time-integrated sample of 3.6 L of filtered water. Sample inlets were covered with a mesh pre-filter (polypropylene; pore size ≈ 100 μm) to exclude particles larger than those thought to be efficiently retained by sponges (Reiswig 1971, Yahel et al. 2003). Pre-filters were replaced daily.

All plastics utilized in sample collection and processing (including the incubation chamber, benthic isolation plastic, syringes, stopcocks, tubing, filter holders, and collection vial lids) were composed of polypropylene, high-density polyethylene, or polycarbonate and all were

soaked in a 0.1 mol L^{-1} HCl bath for >12 hours and triple rinsed with 18.2 MΩ type I water prior to use, and between each sampling in the case of sample collection plastics. Borosilicate scintillation vials used for sample collection were subjected to the same washing procedure, followed by combustion at 450°C for > 6 hours to remove any residual DOC. Combusted glassware was stored in combusted foil and bagged to minimize outside contamination prior to use. Utilized filters were combusted at 450°C for >6 hrs and stored in combusted foil. Amber HDPE sample bottles used for ammonium samples were acid washed and rinsed following the aforementioned protocol. Additionally, small aliquots of the o-phthaladehyde working reagent were added to HDPE bottles and allowed to react for 24 hours to ensure remove any residual ammonium from the sample bottle prior to use for standards or samples.

Sample Analysis

Ammonium analyses were performed by fluorescence using the method of Holmes et al. (1999). Immediately after subsampling, 20mL volumes were reacted with 5mL of o-phthaladehyde working reagent in 30 mL amber, HDPE sample bottles for 2.5 hours. After the incubation period, samples were analyzed using a Turner Designs TD-700 laboratory fluorometer with an ammonium fluorescence optical kit (Turner Designs 10-303). The method detection limit of the utilized method was 10 nmol L^{-1}, determined by repeated standard measurements. Standards were prepared daily in reacted sample bottles by serial dilution of a purchased stock solution (Ricca Chemical Company 693-16), and analyzed with the prepared samples. Nitrate plus nitrite (NO_x^-) samples were analyzed using Spectrophotometric Elemental Analysis System (SEASII-NOx) autoanalyzers (Adornato et al. 2005 and references therein) configured for bench-top use. NO_x^- analysis with SEASII was accomplished with cadmium reduction of nitrate to nitrite followed by detection methodology based on the Griess Reaction.

The method detection limit for this protocol was 25 nmol L^{-1}, and was determined by repeated analysis of standard solutions.

DOC and TN samples were analyzed simultaneously with a Shimadzu TOC-L/TNM-L organic carbon and total nitrogen analyzer. Samples were analyzed using high-temperature catalytic oxidation (HTCO) and subsequent detection of DOC via non-dispersive infrared spectroscopy (NDIR) and TN with chemiluminescence. The carbon values obtained are more accurately characterized as values of Non-Purgeable Organic Carbon (NPOC) due to the purging of volatile organics by vigorously bubbling during instrumental analysis. We assume a negligible contribution to DOC from volatile organics, and henceforth the obtained values will be simply referred to as DOC.

POM samples were analyzed via flash combustion and total conductivity detection using a Carlo Erba NA 1500 elemental analyzer. The collected filters were lyophilized to remove any residual water on the filter. After lyophilization, filters were folded onto themselves four times and exposed to concentrated HCl vapor in a closed vessel overnight. Acid flushed filters were then dried at 80°C for one hour and pulverized. Pulverized samples were placed into combusted foil boats and analyzed for C and N composition.

Results

Dissolved inorganic nitrogen flux

DIN concentrations in the sponge incubation chambers increased linearly with time during each experiment, and did not appear to diminish towards the end of the experiments (Figure 4.3). Therefore, rates of DIN release (mol L_{sponge}^{-1} hr^{-1}) could be directly calculated as the linear regression of the concentration time-series data and the sponge volume measurements made after each incubation experiment. The significance of the calculated rate for each chamber

incubation was determined from one-way analysis of variance (ANOVA) of the linear trend in concentration over time, as well as the significance of the average calculated release rate for a given species (mol L_{sponge}^{-1} hr^{-1}) versus zero (one-way t-test vs. 0). Significant rates of DIN release noted in Figure 4.4 fulfilled both criteria at the 95% confidence interval. The reported N release rate for each species represents the average of the volume-normalized slopes from each of the replicate chambers. Errors in these measures were calculated based upon the deviation between replicates in the volume-normalized rate of N release (N = 3 - 5). Calculated rates of DIN release from replicate chambers for each species were checked against one another for quality control, and outliers were detected and removed from the dataset using the modified z-score method ($M_i \geq 3.5$, Iglewicz and Hoaglin 1993); this quality control led to the removal of 6 of the 96 measurements of DIN release. The control chambers performed without sponge biomass showed no significant linear trend in DIN over the sampled time period (ANOVA, $p = 0.7$ and $p = 0.4$ for NO_x^- and NH_4^+ respectively), and therefore any trend observed in the chambers containing sponge was determined to be the result of the sponge holobiont (sponge animal and associated microbial biomass).

Seven of the 11 sampled species exhibited significant trends in either NH_4^+ or NO_x^- over time (ANOVA, $p < 0.05$) and significance of the calculated rate of N release (one-sample t-test versus 0, $p < 0.05$) (Figure 4.4). Five of these 7 generated significant, mean rates of N release (one-way t-test versus 0; $p \leq 0.05$) indicative of NO_x^- production, with *C. nucula* and *G. gibberosa* showing the highest nitrification rates per liter of sponge biomass. NO_x^- release rates ranged from 25 ± 7.6 to 170 ± 37 µmol NO_x^- hr^{-1} L_{sponge}^{-1} with an average of 44 ± 13 µmol NO_x^- hr^{-1} L_{sponge}^{-1} across all sampled species and chambers (Mean ± 1 SE; Table 4.1). Significant ammonium release was characteristic of only two species, *Haliclona sp.*and *S. vesparium*, which

showed rates of 52 ± 7 and 9 ± 2.2 μmol NH_4^+ hr^{-1} L_{sponge}^{-1}, respectively. Ammonium release rates averaged 14 ± 5 μmol NH_4^+ hr^{-1} L_{sponge}^{-1} across all species and chambers (Table 4.1).

Dissolved organic matter fluxes

Two of the eleven sampled species exhibited a significant linear trend in DOC concentration over the chambered period (ANOVA, $p < 0.05$), and none of the species exhibited significant DON uptake or production at the 95% confidence level. DOC concentrations significantly decreased in the presence of *G. gibberosa* and *S. barbara*, suggesting significant uptake rate for both species (Figure 4.5 1.0 ± 0.17 and 0.22 ± 0.07 mmol C hr^{-1} L_{sponge}^{-1} for *G. gibberosa* and *S. barbara*, respectively; mean ± 1 SE; one sample t-test versus 0, $p < 0.05$). The observed trends were best characterized by linear fits, however exponential models were also tested (de Goeij et al. 2008a and de Goeij and van Duyl et al. 2007), with no significant improvement in uncertainties. Rates of change in dissolved organic matter were subject to the same quality control procedures as the DIN release rates, which lead to the removal of 7 apparent outliers from the total of 96 measurements of DOC and DON change. The reported values represent the average of replicate chambers and the associated deviation between tested individuals.

Particulate organic matter loading

The average POC content at each site was 14 ± 1, 23 ± 5, and 8 ± 1 μmol C L^{-1} (mean ± 1 SE, N = 35, 10, and 16) for FWC Dock, Mystery Basin, and Burnt Point respectively. The average PON content at the tested sites was 2.3 ± 0.3, 3.2 ± 0.6, and 1.2 ± 0.1 μmol N L^{-1} (mean ± 1 SE, N = 35, 10, and 16) for FWC Dock, Mystery Basin, and Burnt Point respectively. There was no significant variability observed at the FWC Dock site when comparing the different

months sampled (paired t-test, $P > 0.05$) nor was there significant diurnal variability at any of the tested sites (paired t-test, $P > 0.05$).

Discussion

Seven of the 11 chambered species exhibited significant DIN efflux (one-sample t-test vs. 0, $P < 0.05$, Figure 4.4), with the largest rates of DIN production occuring as accumulations of NO_x^-, similar to the observations of Diaz and Ward (1997). Three of the tested species were determined to be LMA sponges (*Haliclona sp.*: Sipkema et al. 2009; *H. melanodocia*: Weisz et al. 2008; *Cinachyrella* sp.: genus level distinction based on Gloeckner et al. 2014), while the remaining 8 were classified as HMA sponges (*S. vesparium*: Weisz et al. 2008; *Ircinia sp.*: genus level distinction based on Gloeckner et al. 2014; *Spongia sp.*: genus level distinction based on Ereskovsky et al. 2005 and included references; *H. lachne*; Ereskovsky et al. 2004; *G. gibberosa*: genus level distinction, Hoffmann et al. 2009; *C. nucula*: Hill et al. 2006). The chosen species provided further evidence of the differences in the speciation of effluent DIN between HMA and LMA sponges (Jiménez and Ribes 2007, Southwell et al. 2008b, Bayer et al. 2008; Figure 4.4). *Haliclona sp.* was the only sampled LMA species to exhibit significant flux of DIN (one-sample t-test vs. 0, $P < 0.05$), while *H. melanodocia* approached statistical significance ($P = 0.06$). DIN release rates associated with these species was of modest magnitude, as compared to some HMA individuals, and was exclusively NH_4^+ (Table 4.1, Figure 4.4). This production of ammonium is likely due to respiration processes and ammonification of organic matter mediated by the sponge animal (Diaz and Ward 1997). The mesohyl, or internal tissue between the outer body and inner cavity, of LMA sponges is characterized by a microbial community whose abundance and diversity is much lower than that of HMA species and approaches the community found in

seawater (Hentschel et al. 2006). As a result, the exhaled water mass carries with it a dominant signature of sponge-animal metabolism rather than microbial processes.

The incubated HMA species exhibited significant production of DIN, primarily in the form of NO_x^-; five of the 8 sampled HMA sponges exhibited DIN release in the form of NO_2^- + NO_3^-, and only *S. vesparium* exhibited a significant production of ammonium (Table 4.1, Figure 4.4). The produced NO_x^- is likely the result of microbially mediated nitrification occurring within the tissue of the sponges (e.g. Bayer et al. 2008 and citations within, Hoffmann et al. 2009, Schläppy et al. 2010). The spectrum of nitrification rates observed in the tested species agrees with the wide range results of other incubation-style measurements for variety of sponges in the Caribbean and Mediterranean Seas (Jiménez and Ribes 2007, Southwell et al. 2008b, Schläppy et al. 2010). The rates of nitrification observed for *C. nucula* are in good agreement with a previous assessment of this species by Diaz and Ward (1997), but are much higher than reported by Corredor et al. (1988) (Table 4.1). This discrepancy is likely due to alleviating the inhibiting effects of chamber volume observed in the work of Corredor and co-workers (1988) through the use of a larger incubation vessel (16.5L vs 2.25L) (Diaz and Ward 1997). Our oxygenation of the vessel to maintain consistent O_2 concentrations, or our slightly shorter incubation times could have also played a role (Diaz and Ward 1997). The oxygenation of the chamber allowed respiration to continue without initiating hypoxic stress, but allowed continual carbon dioxide (CO_2) addition via respiration. This added CO_2 likely reduced the pH of the chamber considerably over the course of the incubation (Gibson 2011), but the impact of this reduced pH on the behavior or the holobiont is unknown and warrants future examination. The chamber inhibition discussed by Diaz and Ward (1997) was likely a factor for other, larger species examined in the present study (e.g., *Spongia spp.*, *S. vesparium*, *H. melanodocia*), and

therefore the observed rates for these species should be considered underestimates of their DIN release due to the size of the chamber relative to the sponge. In addition to chamber effects, our calculated nitrification rates are probably underestimated due to the potential for microbial utilization of nitrite and nitrate produced within the chamber (Diaz and Ward 1997).

Our results for *G. gibberosa* are much lower than the results Hoffmann et al. (2009) obtained using a *Geodia* species common to the North Atlantic. This difference is most likely due to the amended ammonium prior to their incubation (12 μmol L^{-1} NH_4^+; Hoffmann et al. 2009). The resultant initial chamber NH_4^+ concentration was an order of magnitude larger than the ambient NH_4^+ measured at the start time of our chambers, significantly increasing the available DIN feedstock for nitrification, and thereby likely enhancing the nitrification rate.

S. vesparium is an oddity among HMA sponges as it produces exclusively NH_4^+ and yields the lowest volume-normalized DIN efflux of all the sampled species (Figure 4.4). *S. vesparium* has previously been shown to behave in marked contrast to other HMA species in both DIN speciation and elevated pumping rates (Southwell et al. 2008a, Weisz et al. 2008). There have been several conflicting reports (e.g., Poppell et al. 2014 and Gloeckner et al. 2014) about the microbial density (low versus high community density) within the tissues of this putative HMA species, subsequent to the initial classification by Weisz et al. (2008). Poppell et al. (2014) refuted the previous HMA designation (Weisz et al. 2008) and classified *S. vesparium* as a LMA species based on scanning electron microscopy of samples collected on the bay side of Summerland Key, Florida. Altering the classification of *S. vesparium* could help to rectify aforementioned idiosyncrasies for this species such as the observed production of NH_4^+ versus NO_x^- (this study, Southwell et al. 2008a), and its accelerated pumping rate relative to other HMA species (Weisz et al. 2008). Conversely, Gloeckner et al. (2014) corroborated the previous

designation of Weisz et al. (2008) and determined *S. vesparium* to be a HMA species based on transmission electron microscopy of samples collected off Exuma Cay, Bahamas. The environmental conditions in these studies differ considerably, and minor microbial community variability has been previously shown within a species found across different environments (Taylor et al. 2005). Yet, the differences between the results of Gloeckner et al. (2014) and Poppell et al. (2014) are much greater than those presented by Taylor et al. (2005), which complicates an attempt to attribute the observed differences to environmental variables. Nevertheless, it is plausible that *S. vesparium* exhibits a greater degree of plasticity than has been previously recognized in sponge-microbe symbioses, and this possibility should be considered in the context of the apparent dichotomy in effluent N from LMA and HMA sponges. The environmental conditions for our tested *S. vesparium* closely resemble those presented by Poppell and co-workers (2014), and may have contributed to the apparent differences between the behavior of this species as compared to other HMA species.

The ammonification rates determined for *I. campana* differ significantly from the results of Southwell et al. (2008b), yet there is no significant difference between the nitrification rates (Table 4.1). The difference in ammonium production may be due methodological deviations or to variable rates of ammonium oxidation mediated by the holobiont under contrasting environmental conditions. Southwell and co-workers used small sponge cuttings in shore-based incubations, a different experimental approach than the *in situ* incubations with whole, attached individuals in the current study. Sponges are sensitive to physical and environmental stressors, with changes in environmental conditions significantly altering the function of the sponge-microbe holobiont (e.g. Fan et al. 2013); this effect would be particularly pronounced in HMA sponges whose DIN production is thought to be largely influenced by the activity of their hosted

microbial consortia. Additionally, variable ammonium excretion with relatively constant NO_x production has been shown to occur during seasonal transitions in an HMA sponge native to the Mediterranean (Bayer et al. 2008). Large differences in the factors hypothesized to precipitate this change (temperature, salinity, dissolved oxygen, and ammonium availability) can be found when comparing the sites tested in Southwell et al. (2008b) to those in FL Bay (e.g. Boyer et al. 1999, Stokes et al. 2011); this environmental divergence may contribute to the observed intraspecific variability in ammonium oxidation.

The sponges that hosted active nitrifying bacteria (those with significant, positive release of NO_x^-) had a greater total DIN production than those without active populations of nitrifiers in their tissues (all other species, two-sample t-test, $N = 6$, $P < 0.05$), where total DIN released was taken as the sum of the produced NH_4^+ and NO_x^-. We hypothesize that this increase in total DIN efflux is a result of the slower pumping rates in HMA sponges hosting active, nitrifying microbes relative to other sponges. The reduced pumping rate increases tissue residence time in these individuals, and this increased residence time, coupled with the high surface area to volume ratio of the aquiferous structures in HMA sponges (Weisz et al. 2008), may serve to enhance the rate of nitrification in these sponges. To test this hypothesis, the pumping rates for species thought to host active nitrifiers (*C. nucula, H. lachne,* and *S. barbara*) were compared to those species, both HMA and LMA, without evidence for actively nitrifying microbes in their tissues (*S. vesparium, I. variabilis, H. melanodocia, Haliclona* sp., and *Cinachyrella* sp.). Pumping rates were determined by video-recording fluorescein dye movement as it is released in the excurrent jet of sponge oscula (N. Lindquist unpublished data, methodology following Weisz et al. 2008). The pumping rate was found to be significantly slower for species hosting active nitrifying populations than that for those species without (two-sample t-test, $N = 7$, $P = 0.04$; N. Lindquist

unpublished data). While the available data are limited, they preliminarily indicate that the increased tissue residence time of filtered water may allow for increased chemical exchange and reaction of dissolved constituents in species thought to be hosting active nitrifying microbes. Further exploration of this hypothesis may help to elucidate the mechanism behind the subtle differences observed between sponge species.

The observed DIN release at our Florida Bay sites suggest that sponges may be important agents of N cycling, particularly in benthic environments with large populations of HMA sponges (Diaz and Ward 1997). These ubiquitous holobionts represent sites of rapid organic matter remineralization potentially able to generate localized hotspots of elevated DIN concentrations and altered NH_4 to NO_x ratios (Diaz and Ward 1997, Ribes et al. 2005). Because the largest reservoirs of N in Florida Bay are present as particulate and dissolved organic matter (Boyer et al. 2006), this newly identified source of recycled, bioavailable DIN should be ecologically important, especially in the western areas of Florida Bay where N is thought to be limiting to primary production (Fourqurean et al. 1993, Phlips et al. 1999). Inorganic N production by these species is dependent upon heterotrophic conversion of amino nitrogen from respired organic compounds to NH_4^+ coupled to subsequent nitrification yielding NO_x^- (Corredor et al. 1988). The accumulation of NO_x^- does not appear to trail ammonium production in those species hosting nitrifying populations (Figure 4.3), thereby providing further evidence for rapidly coupled ammonification of organic matter and nitrification within sponge tissues which has been observed in non-incubation sampling (Southwell et al. 2008b). Due to the different metabolic capabilities demonstrated by sponges in other environments (e.g., Resiwig 1974, van Duyl et al. 2008, Pawlik et al. 2015), we hypothesized that the tested sponges would exhibit divergent behavior in their organic matter preference with HMA species consuming primarily DOM and

LMA species feeding primarily from POM, as has been demonstrated in other environments. Surprisingly, observed DOM utilization was minimal. *G. gibberosa* showed a DOC uptake rate that was similar to other HMA species found on reefs (e.g., Yahel et al. 2003, de Goeij et al. 2008, Hoer 2015, this volume) while *S. barbara* was much lower (Figure 4.5. The tested *G. gibberosa* individuals were often found with macroalgal epibionts which were gently removed prior to incubation. Macroalgae is recognized to release a significant proportion of photosynthetically fixed C as DOC into the surrounding water (Haas et al. 2011). The close association of this species with a source of fresh DOM could contribute to the observed DOC uptake as it is regularly exposed to a labile C source. Similarly, *C. nucula* is commonly found in Florida Bay within seagrass beds, often growing attached to seagrass leaves, and as a result would be expected to be consistently exposed to labile C exuded by the seagrasses (Ziegler and Benner 1999); the tested individuals suggested the ability of this species to utilize DOC, yet the observed flux was not significant at 95% confidence level (Trend: ANOVA; $p = 0.06$; average DIN release rate: one sample t-test vs. 0; $p = 0.06$). The potential for HMA sponges to feed from both the particulate and dissolved pools, and the regular exposure of these species to labile DOC either from neighboring or epibiotic primary producers may predispose these species to fulfill their metabolic C demand from the labile pool of exuded C. Conversely, *S. barbara* does not exhibit a direct association with a DOM source, and why this behavior was observed in this species and not others tested is unknown. This species was shown to have the slowest volumetric pumping rate of any of the tested species (N. Lindquist unpublished data), which would generate increased tissue residence times, potentially rendering the available DOM more accessible to the sponge holobiont (Weisz et al. 2008).

This notable lack of feeding from the dissolved organics, coupled with the observed large rates of DIN production suggests that the dominant sponge species in Florida Bay feed primarily on POM. The apparent absence of DOM uptake by the HMA sponges tested in this environment may suggest either the lack of sponge-hosted microbial communities to feed on dissolved organics or a degree of dietary plasticity which allows the same species in different locations to shift food sources depending upon availability or palatability. Gibson (2011) did not observe DOC uptake in *S. vesparium* on ocean-side reefs of the Keys, confirming our observations, but there is no further evidence in the literature to confirm or refute the observed behavior in the remainder of the tested species. Despite being classified as an oligotrophic system (Childers et al. 2006, Boyer et al. 2006), POM availability in Florida Bay is relatively high as compared to that observed on the neighboring reef tract; the ambient POC and PON concentrations at our tested sites were significantly higher than has been observed on the reef side of the Florida Keys archipelago (Trussel et al. 2006, Hoer 2015, this volume; two sample t-test, $P < 0.0001$). In addition to the elevated abundance observed in Florida Bay relative to the reef, the POM measured at our sites exhibited a C:N ratio that was not significantly different from Redfield (6.5 $\pm$ 0.1; mean $\pm$ 1 SE; one sample t-test; $P > 0.5$), indicating that it represented primarily living planktonic biomass or freshly produced detrital material (Tanaka et al. 2011). The elemental signature of active and abundant photosynthetic biomass found in ambient POM may suggest a particulate pool with a high degree of palatability for sponges that have been shown to feed somewhat selectively on live planktonic biomass (Yahel et al. 2005, Hanson et al. 2009). This putative contribution of freshly produced POM and the elevated abundance of particulates may contribute to the decreased reliance on DOM by sponges in Florida Bay as opposed to reef ecosystems. The availability of fresh detrital material and live planktonic biomass would be

expected to vary seasonally (Phlips et al 1999), and sponges may alter their nutritive dependence on DOM as the availability of POM waxes and wanes temporally. Further sampling of these species under seasonally different POM loads or in different environments will be required to test this hypothesis.

A stoichiometric imbalance exists between ambient PON content and the quantity of produced DIN for many of the tested species; at the most extreme, ambient PON available at the site represented a third of the produced DIN. This imbalance is likely due to an underestimation of the ingested organic matter, both dissolved and particulate, by the tested sponges (Jiménez and Ribes 2007). The observed imbalance could be due to consumption of organic matter fixed by photosymbionts internal to sponge tissue or due to consumption of sponge associated microbes by the sponge animal (Jiménez and Ribes 2007 and references therein). While both of these processes likely contribute minimally to the overall budget of the tested species (Jiménez and Ribes 2007), they are not responsible for the imbalance observed with the LMA species *H. melanodocia* and *Haliclona* sp., neither of which harbors significant microbial consortia or photosymbionts (Erwin and Thacker 2007, Gloeckner et al. 2014). The most parsimonious explanation for the observed imbalance is methodological limitations. The POM collections represent the ambient concentrations at the benthos of the sampled site. Without intermediate sampling specific to each incubation, the rate at which the sponges consume the available POM is unknown. Additionally, the seal between our incubation chambers and the plastic sheeting covering the benthos likely allowed some introduction of ambient water periodically throughout the experiment due to influent ambient water replacing collected sample volumes or due to displacement and subsequent replacement associated with air additions to maintain oxygen content. The signal of dilution from influent ambient water is not seen in the DIN plots (Figure

4.3), but chamber DIN enrichment was relatively extreme, such that dilution from ambient water would likely be within the uncertainty of the method. Similarly, even a small uptake of DON would account for this imbalance (less than 20% of ambient DON), and influent ambient water may have diluted the observed change in dissolved organics. Nevertheless, the results presented here, utilizing a method which involves minimal disturbance of the target organism, represent further evidence of large DIN flux from sponge communities which are in agreement with past studies of sponge-recycled N (Diaz and Ward 1997, Southwell et al. 2008a,b).

Field surveys from Peterson and co-workers (2006) showed sponges at almost 75% of the sites analyzed, with biomass contributions of over 1400 g sponge dry weight m^{-2}; areal coverage of sponges was focused on the hard-bottom areas along the southern edge of the bay as well as the eastern and western margins. Using our species-specific rates of DIN production and the surveyed biomass from Peterson et al. (2006) of the 11 incubated species, preliminary estimates of areal recycled N fluxes from the sponge community were calculated. The eastern and southwestern margins hosted the largest values of sponge-community DIN production, as predicted by the aforementioned peaks in sponge population density (Peterson et al. 2006). The largest calculated flux of recycled N was found at a site in the northeastern corner of the bay, and it represented an N source of 640 ± 140 μmol N hr^{-1} m^{-2}. This flux of remineralized N is roughly comparable to previous estimates of DIN contribution from the sponge community on Caribbean reefs (Corredor et al. 1988, Diaz and Ward 1997, Southwell et al. 2008b), and many of the calculated fluxes exceed other sources of remineralized N in the system (15 ± 11 μmol N hr^{-1} m^{-2}; Yarbro and Carlson 2008). Yet, this N source had significant spatial heterogeneity and its role in N budgets will be highly dependent upon spatial scale variations in the sponge community, so this estimate should be considered preliminary and viewed cautiously. Nevertheless, our results

reflect the considerable capacity for N recycling mediated by natural sponge communities in Florida Bay. While the areal flux is patchy and largely dependent upon community composition and density, the recycled N flux associated with sponge biomass has the potential to rival all other sources of DIN to the shallow Florida Bay water column, and thus may provide a large proportion of the photosynthetic N requirement of the expansive sea grass population. With the expansive coverage and large biomass, sponge populations almost assuredly serve a critical role in remineralizing organic matter and regenerating inorganic nutrients on a local scale in Florida Bay, yet species-specific biomass estimates will be required in order to determine the magnitude and potential ecological significance of this contribution.

Table 4.1: Inorganic nitrogen production rates from the sampled species, including previously published values. Reported values are normalized to sponge volume and represent the mean ± 1 SE. Approximate sponge volumes were calculated for Corredor et al. (1988) and Diaz and Ward (1997) based on a volume to dry-weight ratio calculated from *C. nucula* individuals used in chamber incubations (N. Lindquist et al. unpublished).

Species	N	NH_4^+ Flux (μmol L^{-1} sponge hr^{-1})	NO_x^- Flux (μmol L^{-1} sponge hr^{-1})	Source
Control	3	-0.9 ± 0.2	-0.9 ± 3	This study
Haliclona sp.	3	50 ± 7 ‡	0.3 ± 0.8	This study
H. melanodocia	3	40 ± 10	-0.7 ± 1	This study
Cinachyrella sp.	3	-5 ± 2	10 ± 1	This study
S. vesparium	4	9 ± 2 ‡	-0.9 ± 0.1	This study
I. variabilis	4	40 ± 10	10 ± 20	This study
I. campana	5	10 ± 6	60 ± 20 ‡	This study
I. campana		220 ± 50	90 ± 20	Southwell et al. (2008b)
S. graminea	5	30 ± 10	30 ± 8	This study
S. Barbara	5	4 ± 5	50 ± 4 ‡	This study
H. lachne	5	-2 ± 1	80 ± 20 ‡	This study
G. gibberosa	4	1 ± 1	120 ± 40 ‡	This study
C. nucula	4	-5 ± 6	170 ± 40 ‡	This study
C. nucula		---	30 ± 7	Corredor et al. (1988)
C. nucula		---	*Minimum*: 50 ± 10 *Maximum*: 130 ± 130	Diaz and Ward (1997)

‡ Indicates calculated fluxes with both trend significance in chambered concentration over time (one way ANOVA) and significance of average DIN production (one-way t-test vs. 0, $P \leq 0.05$).

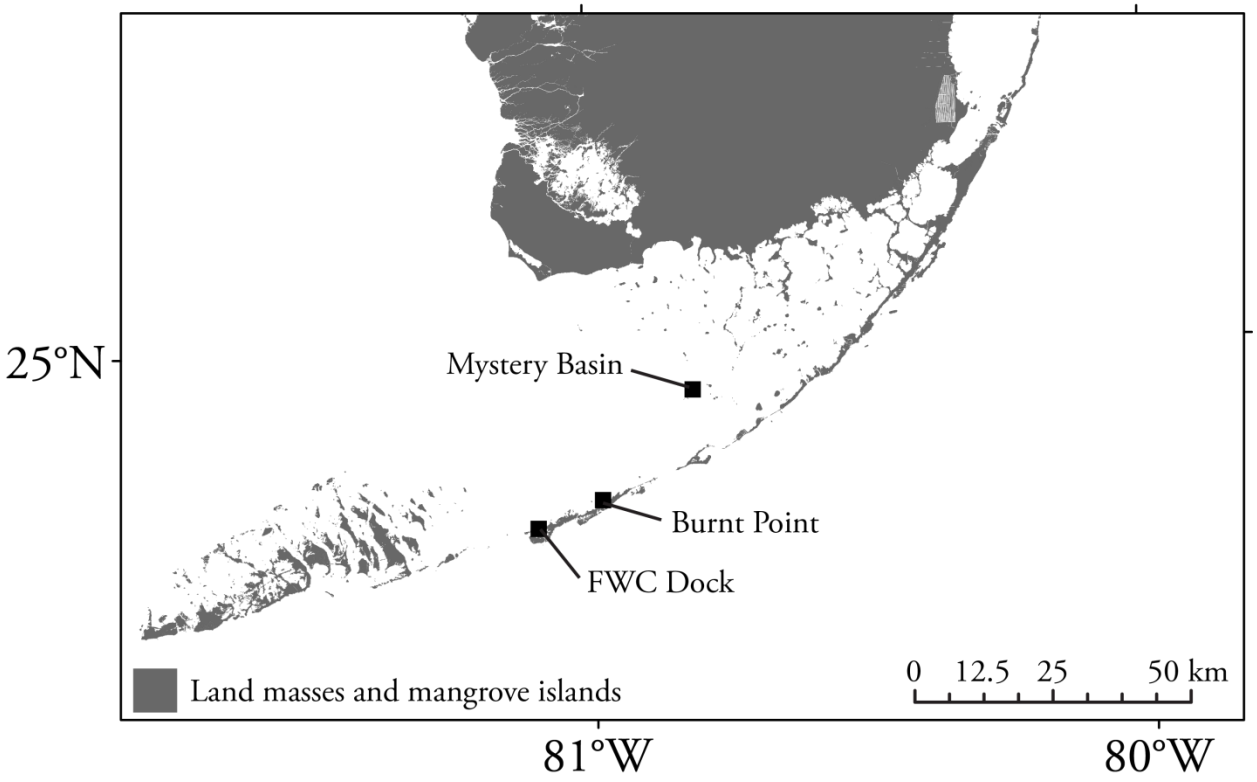

Figure 4.1: Map of Florida Bay. Squares indicate locations where chamber experiments were performed.

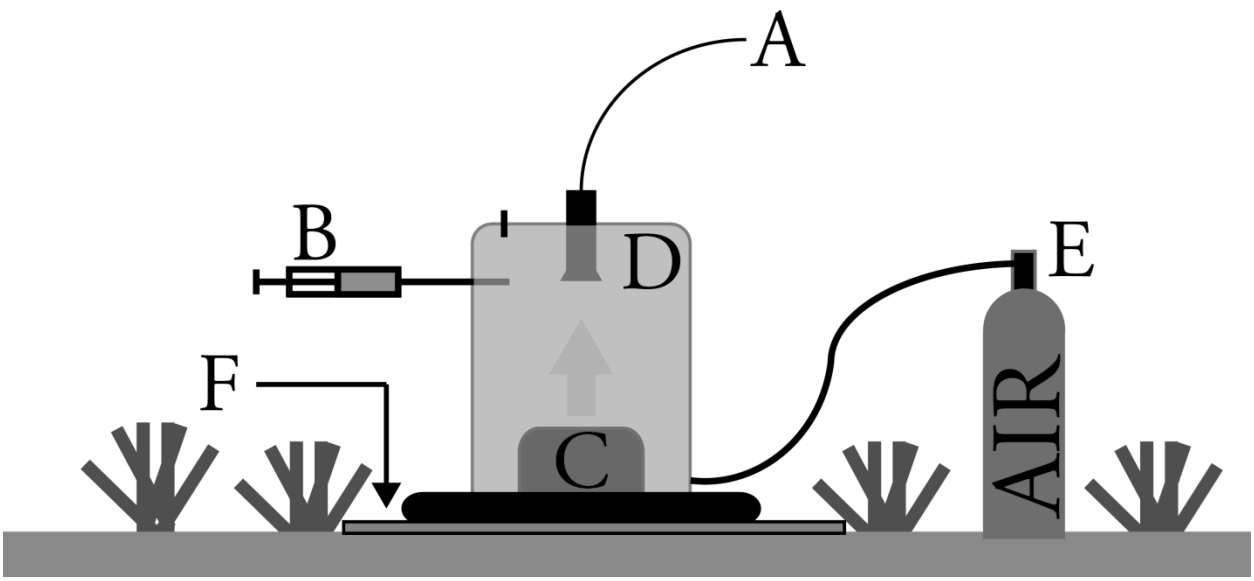

Figure 4.2: Schematic representation of the benthic chamber utilized for determining sponge DIN production *in situ*. A. Oxygen optical probe with cable to surface datalogger; B. syringe outlet for discrete sample collection; C. enclosed sponge individual; D. polypropylene benthic chamber; E. SCUBA cylinder for aerating the chamber; F. Plastic sheeting for isolating sponge individual from the surrounding benthos. The inlet through which the chamber was aerated formed a ring around the base of the chamber.

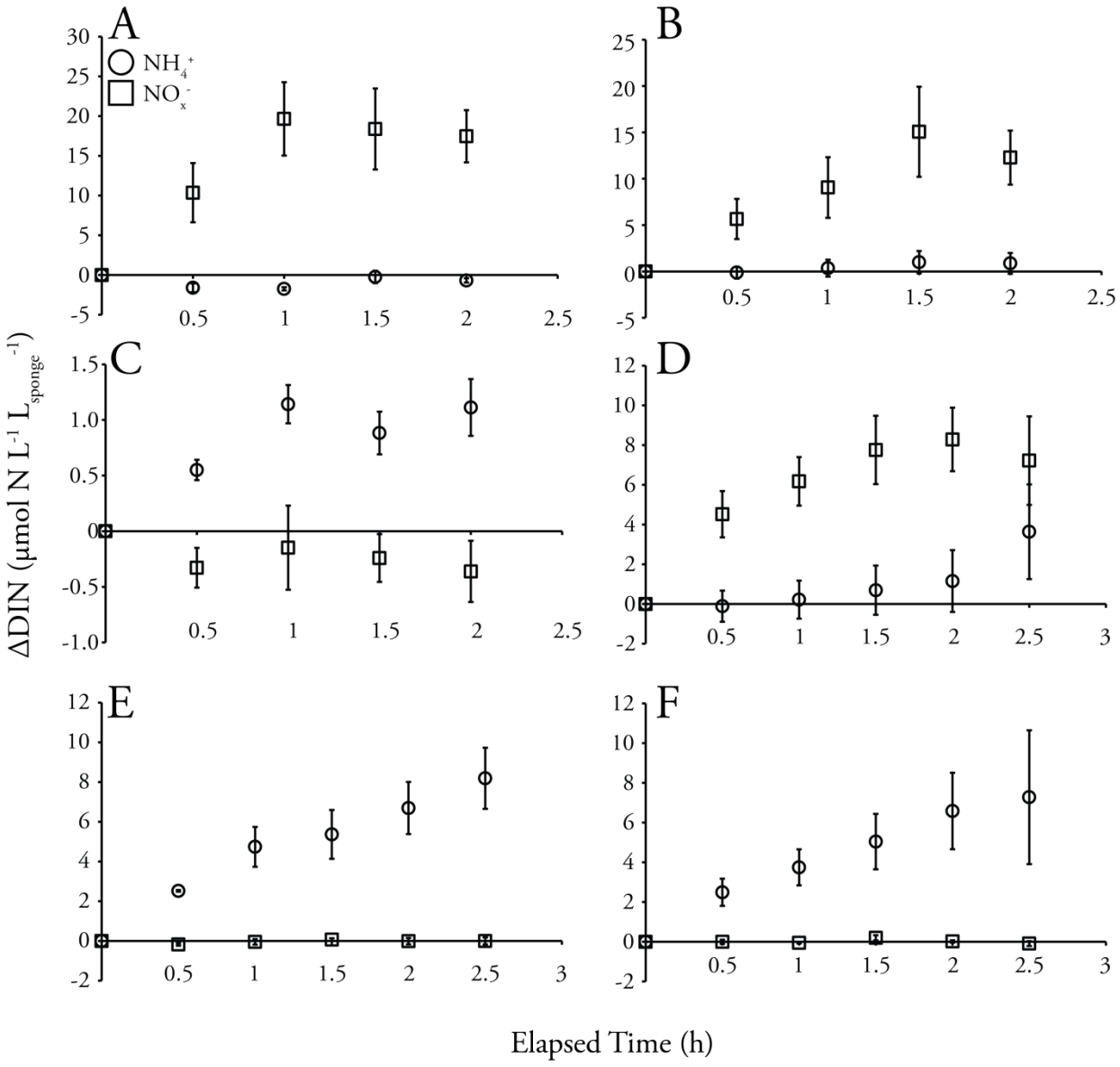

Figure 4.3: NH_4^+ and NO_x^- concentrations during chamber incubations. Values represent the change in concentration over chambered time, normalized to sponge volume (μmol L^{-1} L_{sponge}^{-1}). A. *C. nucula*; B. *G. gibberosa*; C. *S. vesparium*; D. *S. barbara*; E. *Haliclona* sp.; F. *H. melanodocia*.

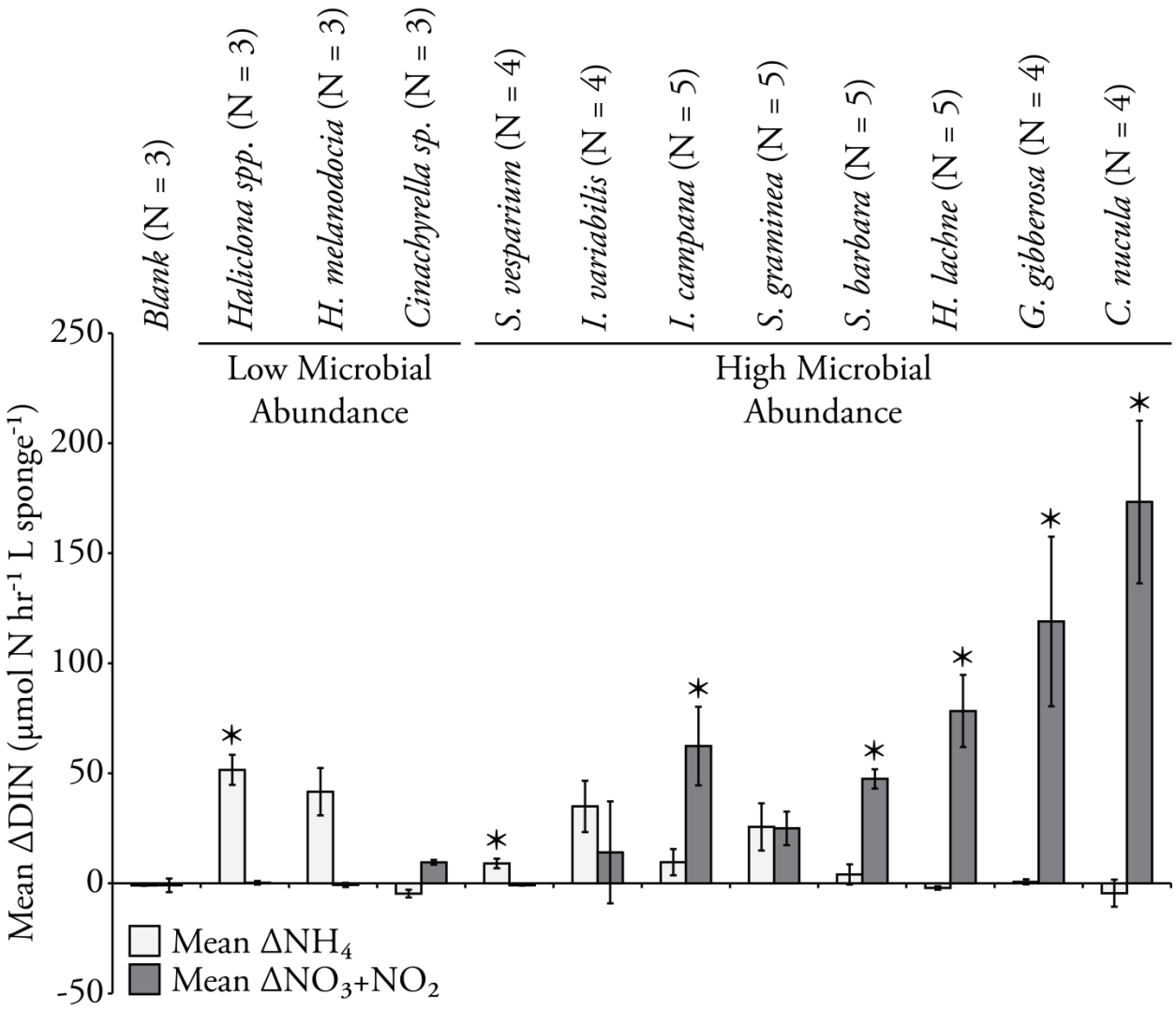

Figure 4.4: Mean volume-normalized rates of DIN production for the 11 tested species in Florida Bay. Error bars represent 1 SE and asterisks (*) indicate significance for both the linear regression of concentration versus incubation time (ANOVA; $p < 0.05$) as well as for the average release rate of replicate individuals (one-sample t-test versus 0; $p < 0.05$).

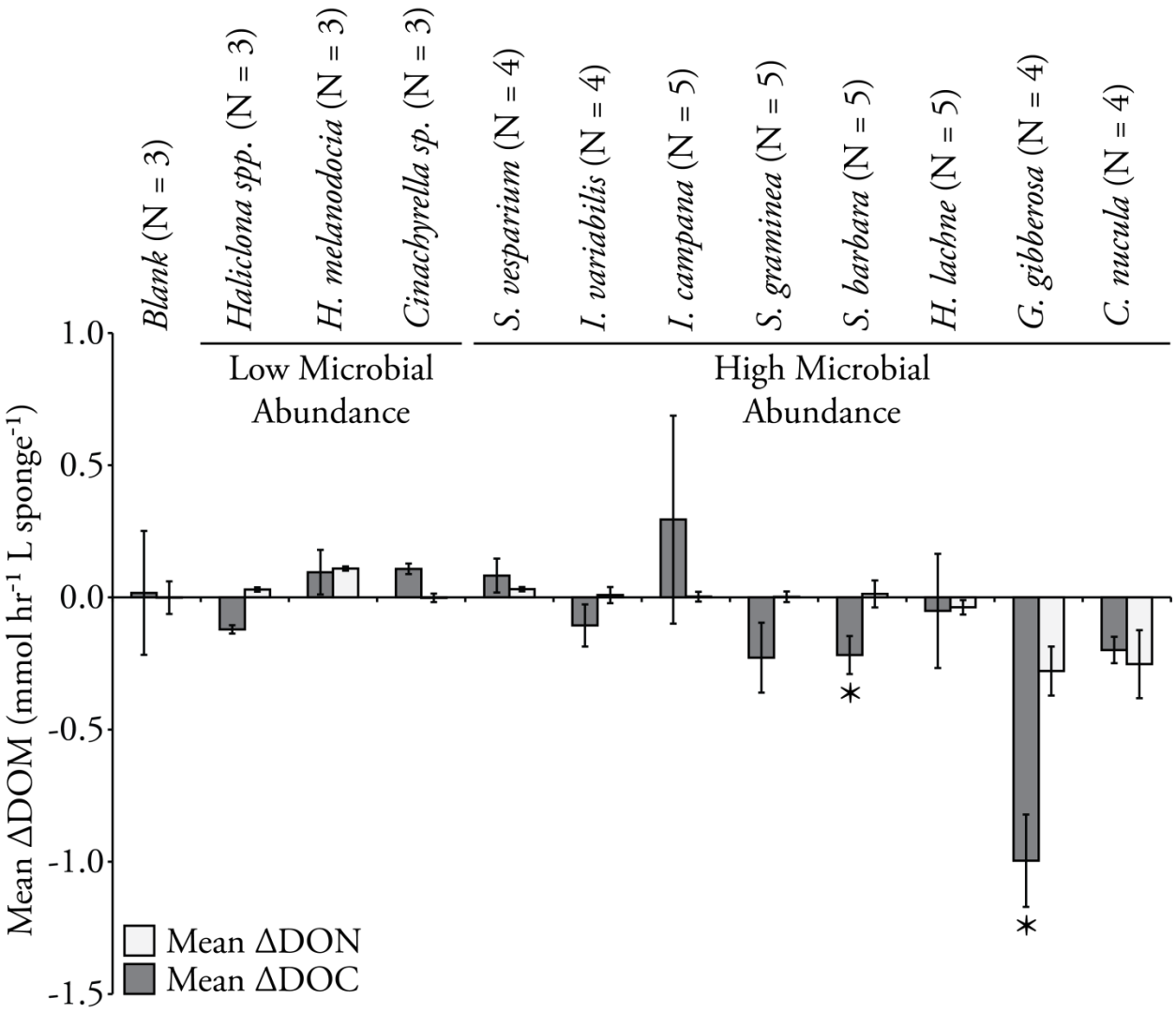

Figure 4.5: Mean volume-normalized rates of DOC and DON production or consumption for the 11 tested species in Florida Bay. Error bars represent 1 SE and asterisks (*) indicate significance for both the linear regression of concentration versus incubation time (ANOVA; $p < 0.05$) as well as for the average release rate of replicate individuals (one-sample t-test versus 0; $p < 0.05$).

CHAPTER 5:

Sponges Represent a Major Source of Recycled Nitrogen in Florida Bay

Introduction

Florida Bay is an estuarine ecosystem bounded on the north by the Everglades wetlands, the south and east by the Florida Keys, and open to the Gulf of Mexico along the western boundary. The bay is uniformly shallow (< 3 m depth) and the water column is clear and oligotrophic (e.g., Boyer et al. 2006). Benthic communities are characterized by diverse sponges (e.g., Peterson et al. 2006), octocorals, small hard corals, seagrasses (primarily *Thalassia testudinum*, *Halodule wrightii*, and *Syringodium filiforme*; Zieman et al. 1989), and macroalgae (calcareous green algae, including *Halimeda* spp. and *Penicillus* spp., and red drift algae, predominantly *Laurencia* spp.; Stevely et al. 2010). Primary productivity is dominated by extensive seagrass meadows, and shows a trend of increasing phosphorus (P) limitation eastward of the broadly marine conditions in western Florida Bay where nitrogen (N) can be limiting (Fourqurean et al. 1993, Lavrentyev et al. 1998).

Bay-wide nutrient budgets constructed for this system (e.g., Rudnick et al. 1999, Boyer and Keller 2007) have shown that most N and P in the water column is in organic forms (Boyer et al. 1997, Boyer et al. 2006), and sources of these nutrients are often similarly dominated by organic matter (as much as 90% of influent N from the Everglades is as DON; Boyer et al. 1999, Childers et al. 2006). Consequently, local recycling processes have been found to regulate the supply of dissolved inorganic nitrogen (DIN) in many locations (Rudnick et al. 2005, Boyer and

Keller 2007, Boyer et al. 2009). Furthermore, carbonate mud banks subdivide the bay into discrete basins which restrict physical exchange and leads to an enhanced role of local processes in chemical cycles (e.g. Fourqurean et al. 1993, Yarbro and Carlson 2008, Zhang and Fischer 2014). The extensive seagrass beds throughout Florida Bay have been highlighted as potentially important sites of dissolved organic nitrogen (DON) remineralization (Yarbro and Carlson 2008). Sponges have also been shown to be important sources of recycled N through remineralization of dissolved and particulate organic matter in this and other environments (e.g., Corredor et al. 1988, Southwell et al. 2008b, Hoer 2015, this volume), yet the role of these organisms in Florida Bay N budgets has been largely unaddressed.

Extensive biomass surveys conducted throughout Florida Bay showed sponge populations at 70% of 207 sites, with densities up to 22 individuals m^{-2} (Peterson et al. 2006). The communities of these organisms are distributed heterogeneously throughout the bay and elevated sponge biomass and population densities were observed in the hardbottom habitats of the southern-central and western regions (Peterson et al. 2006). These organisms have the potential to represent an important source of recycled N to Florida Bay, particularly considering the DIN release rates shown in 11 species common to this system (Hoer 2015, this volume) and results from locally important species in other environments (*C. nucula* and *Ircinia* sp.; Corredor et al. 1988, Diaz and Ward 1997, Southwell et al. 2008b). Furthermore, using sponge biomass data from the surveys conducted by Peterson et al. (2006), Hoer and co-workers (2015) showed the potential for a recycled N flux of 15.3 ± 3.3 mmol N m^{-2} day^{-1} at a *C. nucula* dominated site in the northeastern corner of the bay. Despite the magnitude of this source of recycled N, it may serve little ecological function for local primary producers in the northeastern portion of the bay as it is often severely P limited (Fourqurean et al. 1993, Lavrentyev et al. 1998) and this excess

DIN supply may serve to further exacerbate this limitation. Consequently, we chose to test the hypothesized role of the sponge community in Florida Bay N cycling in a sponge-rich site in west central Florida Bay where the contributed DIN may buffer N limitation from influent marine conditions.

An offshore basin was selected which was thought to be analogous to important sponge-rich sites throughout the bay as a whole. Similar to other basins in the bay, the restricted water exchange at the selected site was hypothesized to allow local processes to dominate nutrient cycling and, coupled with abundant sponge biomass, should improve the capability to quantify the importance of recycled N from these organisms in the overall nutrient budget. We tested the role of sponges by calculating the potential DIN contribution from these organisms through biomass surveys and species-specific DIN release rates (Hoer 2015, this volume), and compared its magnitude to other sources and sinks of N in this system. We hypothesized that the efflux of DIN associated with the sponge community in this system would be a dominant source of recycled N and would be of critical importance to meet the N demand from predominantly seagrass primary productivity. Additionally, we hypothesized that the magnitude and relative importance of sponge efflux and other sources and sinks would vary spatially as a result of varying sponge biomass and sponge community composition (i.e. relative abundance of particular species), and these would contribute to locally visible changes in the quantity and speciation of water column DIN.

Methods

Study area

The role of sponges in the N budget of Florida Bay was tested in a shallow basin (Site ID: Mystery Basin; 24° 56’ 36.6” N, 80° 49’ 32.8” W) located within the boundaries of the

Everglades National park, approximately 13 km north of Long Key, Florida. It is in the west, central portion of Florida Bay (Boyer et al. 1997, Gibson et al. 2008) which tends towards marine conditions due to a dominant role of influent water from the Gulf of Mexico and more minor contribution from Everglades discharge (Fourqurean et al. 1993, Boyer et al. 1997, Rudnick et al. 1999). The tested site is within ~10 km of 5 long-term (1991-2008) monitoring sites within the Southeast Environmental Research Center (SERC) water quality monitoring network (SERC-FIU WQMN Program) which provided historical nutrient data for the surrounding geographic area. The central basin is characterized by a thin veneer (< 5 cm) of carbonate sediment overlying the Pleistocene limestone hardground and is populated by a population of sponges, octocorals, small hard corals, seagrasses, and macroalgae common to the Florida Bay ecosystem. Based on preliminary site visits, the sponge community was roughly analogous to other sponge-rich sites throughout Florida Bay (Peterson et al. 2006); abundant sponge biomass was heterogeneously distributed on the benthos and composed of a variety of different sizes and species. Mystery Basin is approximately elliptical (major axis: ~3 km, minor axis: ~2 km, Figure 5.1) and has a < 2 m water column that is almost completely isolated from surrounding basins by seagrass-covered carbonate mud banks that shoal during low tides. The bank-attenuated water exchange leads to a basin residence time between 4 and 7 days (Martens et al. unpublished), which is approximately similar to the residence time of Rabbit Key Basin located immediately to the north (Cosby et al. 2005).

Water column DIN and TN

Water samples were taken at a total of 34 sites in and around Mystery Basin (Figure 5.1) in August 2012 and May 2013 by divers on SCUBA. At each site, a water sample was collected in a 60 mL polypropylene syringe and filtered in-line at the point of collection. The filter

(Whatman GF/F; ~0.7 μm nominal pore size) and 10 cm of small-diameter, high-density polyethylene tubing were attached to one arm of a polycarbonate 3-way stopcock which was fitted directly to the syringe; the stopcock allowed isolation of collected water or discharge through the open third arm during rinsing. The length of attached tubing helped minimize contamination by allowing the collecting diver to be positioned down-current from the sampled water. A new pre-combusted, 25 mm GF/F was used for the filtration of each water sample. GF/Fs were selected due to their suitability for pre-combustion and use in prior studies of nutrient concentrations in Florida Bay (e.g. Boyer et al. 1997, Boyer et al. 2006, Gibson et al. 2008). During sample collection, the syringe, filter, and tubing were rinsed 3x with filtered target water and the rinsing volume was discharged. The fourth volume was slowly drawn into the syringe (< 2 mL sec^{-1}) to ensure the collection was representative of the desired water mass, and the attached stopcock was closed to prevent accidental sample loss. The sample was returned the surface and stored in a dark ice bath until transport to shore for subsampling and preservation (less than 8 hours from collection to processing or analysis). Samples were immediately divided for DIN (NH_4^+ and NO_2^-+NO_3^- (henceforth NO_x^-)) as well as total dissolved nitrogen (TN) analyses upon return to the laboratory. TN samples (20 mL volume) were put in sample-rinsed borosilicate glass scintillation vials; 100 μL of 50% H_3PO_4 was added, and the vials were stored at 4°C until subsequent analysis; DON was determined as the TN content less DIN. Nitrate and nitrite (NO_x^-) samples (20 mL volume) were placed into sample-rinsed, borosilicate glass scintillation vials and immediately frozen and stored at -20°C until analysis. Ammonium (NH_4^+) samples (20 mL volume) were placed into sample-rinsed amber HDPE bottles. Ammonium samples were analyzed immediately to reduce the potential impact of

degradation on sample integrity. For each location, the sampled parameters were collected in triplicate for quality assurance and control.

Another site inside Mystery Basin (Site ID: CTR; 24° 56' 30.84" N, 80° 49' 58.80" W; Figure 5.1) was intensively sampled over a 3 day period (May 18-20, 2013). During this time, a boat remained anchored at CTR, discrete water collections were performed using peristaltic pumps, and water column NO_x^- was analyzed *in situ*, in real-time via two deployed Spectrophotometric Elemental Analysis System (SEASII-NO_x) autoanalyzers (Steimle et al. 2002; Adornato et al. 2005, Adornato et al. 2007 for detailed descriptions of similarly deployed instrumentation). The SEASII instruments obtained time-series NO_x^- concentrations at different depths, 0.1 and 1.0 m above bottom (mab), in order to compare concentrations near the benthos with those near the surface. During analysis, each instrument sampled water from its respective depth and measured the combined concentrations of NO_3^- and NO_2^-, or NO_x^- ; both NO_2^- in the water column and NO_2^- produced from cadmium reduction of NO_3^- were determined spectrophotometrically utilizing the Griess method (Adronato et al. 2007). The instruments used 15 cm optical pathlengths which increased sensitivity and reduced the method detection limit to 25 nmol NO_x^- L^{-1} (determined by repeated analysis of standard solutions). Both SEASII-NO_x autoanalyzers were calibrated prior to and following deployment and the accuracy of each instrument was checked daily with standards introduced *in situ* by divers on SCUBA. Buffer solution and pre-mixed sulfanilamide/N(1-napthyl)ethylenediamine dihydrochloride reagents were attached to the instruments in darkened compounding bags (VitalMix 9316 and 9318) and these were prepared and replaced daily.

In addition to *in situ* time-series data, peristaltic pumps were set up during the May 2013 sampling mission to collect ambient water from two locations near the boat; one sample inlet

was positioned near the bottommost SEASII-NO_x instrument which allowed discrete confirmation of *in situ* data. Inlets were approximately 15 cm above bottom and target water was pumped through black HDPE tubing for shipboard collection. Sampling was performed four times daily, 08:00, 12:00, 17:00, and 22:00, in order to quantify any diurnal variability in water quality parameters. The pumps ran constantly to prevent stagnation in the tubing and flow rates were set to 20 mL min^{-1} by adjusting pump speeds at the beginning of each sampled time to ensure accuracy in the delivered volumes. Samples were filtered using in-line, 47mm Whatman GF/Fs that were replaced and sample-rinsed prior to each collection. Samples for DIN and TN were collected and stored in a dark ice bath until transport to shore for analysis or further preservation (less than 24 hours from collection to shore-based processing).

Plastics which were used in sample collection and processing (peristaltic pump tubing, HDPE sample tubing, syringes, stopcocks, filter holders, and collection vial lids) were all were soaked in a 0.1 mol L^{-1} HCl bath for >12 hours and triple rinsed with 18.2 MΩ type I water prior to use and between each sampling. Scintillation vials used for sample collection were acid washed and combusted at 450°C for >6 hours to remove any residual organic matter. Combusted glassware was stored wrapped in foil and bagged to minimize contamination prior to use. Filters were combusted at 450°C for >6 hrs and stored in combusted foil. Amber HDPE sample bottles used for ammonium samples were acid washed and rinsed following the aforementioned protocol. Following the wash procedure, small aliquots of o-phthaladehyde working reagent were added to the bottles and allowed to react for 24 hours so as to ensure removal of any residual ammonium from the container. Prior to their use, the pre-treatment solution was rinsed away by triple rinsing with 18.2 MΩ type I water to ensure no residual reagent remained prior to sample or standard addition.

Sample Analysis

Ammonium analyses were performed using the method of Holmes et al. (1999). Sampled volumes were reacted with 5mL of o-phthaladehyde working reagent in amber, HDPE sample bottles and allowed to develop at room temperature for 2.5 hours. After the incubation period, samples were analyzed using a Turner Designs TD-700 laboratory fluorometer equipped with an ammonium optical kit (Turner Designs 10-303). The method detection limit was determined to be 10 nmol L^{-1} by repeated standard measurements. Standards were prepared daily at the point of use by serial dilution of a purchased stock solution (Ricca Chemical Company 693-16), and analyzed with the prepared samples. Nitrate plus nitrite (NO_x^-) discrete samples were analyzed using SEASII-NOx autoanalyzers configured for bench-top use. As with *in situ* analyses, NO_x^- was measured using 15 cm pathlengths and cadmium reduction of NO_3^- to NO_2^- followed by detection based on the Griess reaction. Standards for benchtop and *in situ* NO_x^- measurements were prepared by dilution of a purchased stock (SPEX Certiprep AS-NO39-2Y and ASNO29-2Y), and analyzed daily with collected samples. TN samples were analyzed with a Shimadzu TOC-L/TNM-L organic carbon and total nitrogen analyzer, which employs high temperature catalytic oxidation (HTCO) for analysis of aqueous organic matter. Calibration curves were prepared from lab prepared stock solutions and were closely monitored during analysis. Standards were remade and rerun if the correlation coefficient was found to be less than 0.995, and standards were interspersed within samples to provide additional quality control.

Survey methodology

Sponge habitat in Mystery Basin was divided into two types, hardbottom and seagrass, and the areal extent of both was identified and quantified using satellite images from ArcMap (ESRI; Figure 5.1). Brief field surveys of points along established boundaries were subsequently

conducted to confirm their accuracy. Following field confirmation, a geo-referenced computer generated grid of approximately 400, 100m x 100m squares was overlaid on the image of Mystery Basin and 59 sites were selected, randomly stratified by habitat type (39 hardbottom, 20 seagrass). The geographic coordinates of each site were defined as the centroid of the selected square. The surveys were completed from July 13, 2012 through August 20, 2013. Due to the observed spatial heterogeneity and variable organism size, a combination of sampling methods (belt transects and quadrats) was used to efficiently and accurately quantify the benthic community. At each chosen site, three 25m x 2m non-overlapping transects were established randomly by divers using SCUBA. For each transect divers identified, counted, and measured the dimensions (length (L), width (W), and height (H)) of all sponge biomass that was greater than 10 cm in its largest dimension. Sponges smaller than 10 cm were identified, counted, and measured within four 1 m^2 quadrats equidistantly spaced along the length of the transect. Sponge identifications were performed to the lowest taxonomic level possible and those which could not be identified in the field were photographed and collected for later identification in the lab. In each quadrat, seagrass and macroalgal distribution was also quantified using the Braun-Blanquet cover assessment method (Braun-Blanquet 1972). A score (0-6) was assigned to each species occurring within the quadrat based on its spatial coverage (Braun-Blanquet 1972, Fourqurean et al. 2001).

Species-specific, volumetric biomass was first estimated using morphometric measurements collected during field surveys and roughly approximating their geometry as a rectangular prism (V = L x W x H). This estimate was subsequently converted to a more accurate, displacement-based volume using direct measurements; a subset of the surveyed taxa was chosen for volume determination based on abundance, hypothesized ecological importance,

or geometric complexity. Harvested individuals representative of the chosen taxa were placed in a water-filled container (19 L or 95 L, depending on sponge dimensions) and displacement was measured to the nearest milliliter. This process was replicated for 10 to 30 individuals to ensure a robust quantification and to characterize the spectrum of organism sizes observed in Mystery Basin. A regression was fit to the data using calculated volume (L x W x H) as the independent variable and measured displacement as the dependent variable. This process allowed more accurate species-specific biomass quantifications to be determined with the morphometric measurements obtained from field surveys. Regressions were typically linear, yet non-linear fits were also tested for improvement in the modeled trend. Those species that were not harvested for displacement were approximated using the most morphologically similar taxa for which a regression was derived.

N sourcing to Mystery Basin

The N contribution from the sponge community was calculated using surveyed sponge biomass and species-specific estimates of DIN release rate from 11 sponge species from Florida Bay (Hoer 2015, this volume). The areal flux of recycled N from each species (j_{sponge}; mmol N m^{-2} day^{-1}) was calculated as:

$$j_{sponge} = \frac{(V_{sponge} \times N_{sponge})}{A}$$

Where V_{sponge} is the total surveyed volume of a sponge species in liters (L_{sponge}), N_{sponge} is the calculated DIN release rate for that species (µmol N day^{-1} L_{sponge}^{-1}; Hoer 2015, this volume), and A is the total area of Mystery Basin (both habitat types; m^2). The sum of the fluxes from all species in the sponge population was expanded to A to determine the total N flux to Mystery Basin (mol N day^{-1}). Quantitative estimates of other important fluxes of N to and from the

system (sediment-water exchange, rainfall, Everglades discharge, groundwater flux, N_2 fixation, and denitrification) were obtained through a review of published information on this region.

N demand from primary productivity

The demand from seagrasses represents the majority of photosynthetic N uptake in Florida Bay (Zieman et al. 1989, Fourqurean and Robblee 1999). *Thalassia testudinum* dominated seagreass populations on the western margin of the bay, representing approximately 90% of total biomass, with *Halodule wrightii* and *Syringodium filiforme* representing the remaining 10% (Zieman et al. 1989, Fourqurean and Robblee 1999). Thick seagrass meadows in this region drive primary productivity at a rate of approximately 2.3 g dry weight m^{-2} day^{-1} for *Thalassia* alone (Zieman et al. 1989), and this value was used to approximate benthic productivity for this species along the seagrass-covered shoals of Mystery Basin. With this rate assigned to *Thalassia,* the contributions of each of the other important species (*Halodule* and *Syringodium*) to overall seagrass biomass were obtained from the Braun-Blanquet rapid assessments performed during the sponge biomass surveys and these proportions were used to calculate primary productivity relative to *Thalassia*. Species-specific C:N ratios (Fourqurean et al. 1992, Sprigger Bank, FCE LTER Data, J. Fourqurean 2011) were used in conjunction with the calculated productivity to obtain a photosynthetic N demand that was appropriately weighted for the local seagrass community.

Florida Bay is widely considered an oligotrophic system (Fourqurean et al. 1993, Boyer et al. 2006) and phytoplankton primary productivity in this environment is approximately equivalent to that found in the open ocean (J. Boyer, personal communication). Therefore, net primary productivity values (mg C m^{-3} day^{-1}) from the surface (1 to 5 m depth) and 20 m stations of the Bermuda Atlantic Time-Series were averaged from 1989 to 2011 (bats_production.dat;

batsftp.bios.edu). BATS was selected as it provided a long time record of primary productivity data in the surface ocean that agreed with modeled and experimental results offshore of the Florida Keys (Yoder et al. 1983, Hofmann and Ambler 1988, Fiechter and Mooers 2007). The resultant average productivity was multiplied by the mean water depth in Mystery Basin (1.5 m; Martens et al. unpublished data), and then converted to N demand using Redfield stoichiometry (C/N ~ 6.6; Redfield 1958).

In order to analyze the local importance of various fluxes in the N budget of this basin, a simple model was constructed and evaluated at each surveyed site:

$$N_{flux} = j_{sponge} + j_{new} + j_{sed} + (j_{sg} \times BBCA) - (j_{wcpp} + j_{denitrification} + (j_{sgpp} \times BBCA)$$

where the flux at a given location (N_{flux}; mmol N m^{-2} day^{-1}) is equal to the sum of the sources of N (sponges (j_{sponge}), "new" N (e.g. rainfall, Everglades, N_2 fixation; j_{new}), sediment-water exchange (j_{sed}), and flux from seagrass sediments (j_{sg})) minus the N demand from removal processes (water column primary productivity (j_{wcpp}), denitrification ($j_{denitrification}$), and seagrass primary productivity (j_{sgpp})). Both seagrass related fluxes (j_{sg} and j_{sgpp}) were scaled to local cover in order to reflect an enhanced importance of these processes in more seagrass-rich habitats. This calculation was performed with a factor derived from the Braun-Blanquet density (BBCA) that was proportional to surveyed seagrass cover; densities (D_i; Fourqurean et al. 2001) between 0 and 0.1 were assigned a BBCA value of 0 (~0% cover), $0.1 < D_i \leq 1$ was assigned a value of 0.05 (~5% cover), $1 < D_i \leq 2$ was assigned a value of 0.25 (~25% cover), $2 < D_i \leq 3$ was assigned a value of 0.5 (~50% cover), $3 < D_i \leq 4$ was assigned a value of 0.75 (~75% cover), and $D_i > 4$ was assigned a value of 1 (~100% cover).

Results

Water column DIN and TN

The water quality survey of Mystery Basin yielded a range of DIN values across varying benthic environments (Figure 5.1; Table 5.1). Total DIN (NH_4^+ + NO_x^-) concentrations outside the boundaries of Mystery Basin (HF8-14; 2.0 ± 0.9 μmol N L^{-1}; mean ± 1SD; N = 7 sites) were on average the same as concentrations inside the basin for all collected sites and times (2.0 ± 1.3 μmol N L^{-1}; mean ± 1SD; N = 28 sites), yet the collected samples exhibited a high degree of spatial and temporal variability. Samples collected at hardbottom sites had higher concentrations of all DIN species (NH_4^+, NO_x^-, and total DIN) than those collected over seagrasses (unpaired t-test; $p < 0.02$), and the observed DIN concentrations were significantly elevated in May 2013 relative to observations in August 2012 (3.6 ± 1.5 and 1.5 ± 0.5 μmol N L^{-1} for 2013 and 2012, respectively; mean ± 1SD; unpaired t-test; $p < 0.01$). NH_4^+ was the dominant species of DIN found at all sampled locations, both inside and outside Mystery Basin (Inside: N = 28 paired collections; Wilcoxon signed rank test; $p < 0.001$; Outside: N = 7 paired collections; Wilcoxon signed rank test; $p < 0.01$).

The SEASII-NOx instruments deployed at CTR yielded a 24 hour time-series of NO_x^- from May 19 to 20 that comprised a total of ~13000 individual concentration measurements showing high NO_x^- concentrations inside the basin (Figure 5.2). Inconsistent power delivery corrupted the first 24 hours of the deployment but these problems were solved by the morning of May 19 and the instruments ran virtually uninterrupted until extraction on May 20. The average concentration at 0.1 mab was 3.8 ± 0.7 μmol NO_x^- L^{-1} and 3.0 ± 0.5 μmol NO_x^- L^{-1} at 1.0 mab (mean ± 1SD). These time-series measurements were in agreement with discrete water

collections performed during the same time period (Figure 5.2) and the overall average from discrete NO_x^- collections at CTR (2.6 ± 0.9 μmol NO_x^- L^{-1}; mean ± 1SD; N = 16; Table 5.1).

The water quality survey in May 2013 generated a profile across the center of the basin connecting the presumed influent and effluent points (water mass flow in this area moves roughly N to S; Cosby et al. 2005, Martens et al. unpublished data). The transect, from HF10 to HF7 (Figure 5.3), showed low DIN water at HF10 and HF14 near the entry into Mystery Basin from Rabbit Key Basin to the north-northwest (Total DIN: 0.93 ± 0.04 and 1.8 ± 0.2 μmol N L^{-1} for HF10 and HF14, respectively), DIN content increased upon entering Mystery Basin at HF5 (2.5 ± 0.1 μmol N L^{-1}), further increasing DIN content in central Mystery Basin near HF1 and CTR (5.5 ± 0.1 and 5.0 ± 1.7 μmol N L^{-1} for HF1 and CTR, respectively), and low DIN water at HF4 and HF7 on the south-southeast shoal (1.7 ± 0.2 and 2.2 ± 0.2 μmol N L^{-1} for HF4 and HF7, respectively; Figure 5.3). Site HF10 was located inside Rabbit Key Basin and its water column DIN concentration was not significantly different than that observed in Rabbit Key Basin over the historical duration of SERC sampling in this location (SERC Site ID: 18; unpaired t-test; $p > 0.5$). Conversely, the average water column DIN concentration observed inside Mystery Basin (all sites inside the basin; August 2012 and May 2013 collections) was significantly higher than the average DIN concentration observed in Rabbit Key Basin (unpaired t-test; $p < 0.01$) as well as 2 other nearby SERC sites over their sampled duration (Site IDs: 27 and 28; Sprigger Bank and Old Dan Bank; unpaired t-test; $p < 0.05$).

Total nitrogen concentrations were only measured during the May 2013 sample collections and ranged from 56 ± 10 μmol N L^{-1} inside Mystery Basin (HF1-6 and CTR) to 45 ± 2 μmol N L^{-1} outside (HF8-14; mean ± 1SD), excluding two sites that were determined to be outliers (HF7 and HF10; 250 ± 80 and 210 ± 60 μmol N L^{-1}, respectively; mean ± 1SE). DON

represented the majority of available N in all sampled locations, but the calculated DON concentration did not differ between sites inside and sites outside the basin (unpaired t-test; $p > 0.05$). Inorganic N represented a larger proportion of TN inside the basin as compared to outside (8.3 ± 2.2% inside and 5.0 ± 1.8% outside; mean ± 1SD; N = 21 and 6; unpaired t-test; $p < 0.005$).

Sponge survey

Sponges were found at 57 of the 60 surveyed sites across Mystery Basin and appeared to be the dominant heterotrophic biomass among the surveyed benthos. Population densities ranged from 0.08 to 21 individuals m^{-2} with biomass (volumetric displacement; L_{sponge}) averaging 0.70 ± 0.13 L_{sponge} m^{-2}, and as high as 4.4 L_{sponge} m^{-2} (Figure 5.4, Table 5.2). The observed sponge density at the surveyed sites agreed with previous bay-wide quantifications (Peterson et al. 2006) and provided confirmation of the assumption that the tested site is representative of sponge-rich sites throughout the bay. The total sponge biomass for each survey technique (transects and quadrats) was determined for each species in Mystery Basin by taking the average of the surveyed displacement (L_{sponge} m^{-2}) across both surveyed strata and assessing that value across the areal extent both habitats ($A_{hardbottom} + A_{seagrass}$; m^2). The total biomass for each species was represented by weighted mean of the surveyed results from quadrats and transects which were evaluated separately (e.g., *S. vesparium* biomass (10^6 L_{sponge}) was found to be 2.0 ± 0.42, 0.034 ± 0.007, and 1.9 ± 0.38 for the quadrats, transects, and the weighted mean, respectively; mean ± 1SE; Table 5.2). When calculating the weighted mean, the biomass from each survey technique was weighted based upon the area surveyed using that methodology (12 m^2 for quadrats and 150 m^2 for transects at each assessed location); the error of the weighted mean was calculated by the method of Baker and Nissim (1963). The error in all measures

(transects, quadrats, and weighted mean) was extrapolated to the scale of the basin using the method of C. Krebs (1999). The transect data was not included in the weighted mean for small species which never satisfied the criteria for detection via this methodology (>10 cm in the largest dimension; *Cinachyrella* sp., *C. nucula*, *A. viridis*, *Hyritios* sp., and *Clione* sp.). Conversely, the weighted mean calculated for the species detected in belt transects reflects both utilized survey methodologies (Table 5.2); at some point during their lifetime these organisms would be small enough to be counted in quadrats and therefore using this method did not preclude their detection and these were included in the corresponding weighted mean. The sponge population was diverse and included contributions from 23 identifiable species; approximately 1% of the surveyed biomass was not readily identifiable during field surveys. The observed sponge community was primarily composed of 6 species: *Sphesiospongia vesparium, Ircinia variabilis*, *Geodia gibberosa, Cinachyrella* sp., *Haliclona magnifica*, and *Halichondria melanodocia* (Table 5.2). These represented roughly 97% of surveyed biomass, with *S. vesparium*, *I. variabilis*, and *G. gibberosa* making up approximately 94% of the total (Table 5.2). Sponges were found to be heterogeneously distributed across the tested site; hardbottom habitats, particularly those in the eastern half of Mystery Basin exhibited the largest sponge biomass, and minimal sponge contributions were observed from seagrass sites (Figure 5.4).

The results of the Braun-Blanquet cover assessment (BBCA) were used to calculate species density (D) for surveyed seagrass biomass following the method of Fourqurean and co-workers (2001). Seagrass was found at 55 of the 59 surveyed sites ($D \geq 0.1$; Fourqurean et al. 2001); *Thalassia testudinum* was encountered at all sites where seagrass was found. The other two observed species, *Halodule wrightii* and *Syringodium filiforme*, were found at 66% and 14%

of the surveyed sites, respectively, and typically exhibited much lower densities than *Thalassia*. A fourth species, *Halophila engelmanni*, was observed in a single quadrat, yet exhibited a density less than the threshold assigned for determining the presence/absence of seagrass ($D \geq 0.1$; Fourqurean et al. 2001). The density of *Thalassia* encountered during surveys was greater than that of both *Halodule* and *Syringodium* combined, with the exception of a single site on the southwest shoal where *Halodule* was dominant. At the 20 sites in defined seagrass habitats cumulative density was 75-100% cover ($D > 4$); *Thalassia* composed approximately 81% of this cumulative cover and *Halodule* and *Syringodium* contributed 17% and 2%, respectively. These relative abundances were used to calculate seagrass N demand.

Macroalgae was also found to be abundant throughout Mystery Basin, particularly in hardbottom sites where it often comprised the majority of surveyed macrophyte biomass. The observed macroalgae was dominated by red drift algae, predominantly *Laurencia* sp., with more minor contributions from calcareous green algae (*Halimeda* sp. and *Penicillus* sp.). Densities (D_i) were calculated for macroalgae in order to compare with seagrasses. At 27 of the 59 surveyed sites, the sum of macroalgal species densities ($\Sigma D_{macroalgae}$) was greater than the sum of seagrass densities ($\Sigma D_{seagrass}$), and all of these sites were located in hardbottom habitats. Of these 27, 23 were dominated by red drift algae and the remaining 4 by calcareous green algae. Further, at most of these sites (23 of 27), the dominant type of algae (red drift, calcareous green) represented more than 50% of the total observed algae; at all sites, the dominant organism represented >40% of the total. Sites dominated by red drift algae demonstrated greater areal coverage (25-75% of quadrat area) than sites dominated by calcareous green algae ($\leq$ 5%, one site showed ~25% coverage), yet due to the ephemeral biomass contribution resultant from the mobility of red drift algae, these quantifications are subject to considerable uncertainty.

N budget for Mystery Basin

The available, species-specific DIN release rates represented approximately 97% of the surveyed biomass in Mystery Basin (Hoer 2015, this volume); those species without quantifications of DIN release were excluded from the N budget. The areal flux of N from the sponge community was 0.59 ± 0.28 mmol N m^{-2} day^{-1} (mean ± 1SE; Section I, Table 5.3; Figure 5.5), which was assessed over the area of all habitat types (A) yielding a total N contribution from the sponge community of 2900 ± 1400 mol N day^{-1} (mean ± 1SE; Section I, Table 5.4). The magnitude and speciation of the recycled N flux from the sponge community was spatially heterogeneous and largely dependent upon the quantity of sponge biomass and community composition (Figure 5.6).

The N flux from sediment-water exchange in seagrass beds (0.36 ± 0.27 mmol N m^{-2} day^{-1}; Table 5.3; Yarbro and Carlson 2008) was applied exclusively to the seagrass environments, and the flux from "bare" sediments (0.04 ± 0.01 mmol N m^{-2} day^{-1}; Table 5.3; Capone et al. 1992) was applied to the hardbottom habitats (Section I, Table 5.4). The remaining quantified N sources (Section II, Table 5.3) were applied to the full areal extent of Mystery Basin to obtain the estimated N loading for each of these processes (summarized in Section II, Table 5.4). Benthic denitrification was thought to roughly balance with N_2 fixation in Florida Bay (Kemp and Cornwell 2001), and this assumption was applied to our tested site and presented budget. This generalized pattern was not directly quantified for Mystery Basin and is subject to considerable spatial and temporal variability (Kemp and Cornwell 2001; Boyer and Keller 2007), and therefore represents a slight uncertainty in the budget.

The areal estimates of N demand from seagrasses was expanded to the area of seagrass habitats whereas N demand from water column primary productivity was expanded to the full

area of Mystery Basin (Section III, Table 5.4). The total calculated N demand from primary productivity is approximately 4.2 ± 0.4 mmol N m^{-2} day^{-1} (Section III, Table 5.3); most of the N demand associated with primary productivity is from seagrasses (4.1 ± 0.4 mmol N m^{-2} day^{-1}) and a minor contribution from phytoplankton (0.1 ± 0.07 mmol N m^{-2} day^{-1}; Section III, Table 5.3). The approximate N demand for primary productivity agrees with remote sensing measurements in the dense seagrasses on the Grand Bahama Banks (3.5 ± 1.3 mmol N m^{-2} day^{-1}; mean ± 1 SE; Dierssen et al. 2010) and macrophyte-dominated systems (5 ± 2 mmol N m^{-2} day^{-1}; mean ± 1 SE; reviewed by Gattuso et al. 1998). The surveyed ecosystems of Gattuso et al. (1998) span a broad geographic range from the Chesapeake Bay (USA) to tropical reefs in the Pacific Ocean, and due to its more panoptic view of N demand from benthic primary productivity it may not be as representative of the conditions in Florida Bay. Estimates of N demand from primary productivity do not include a contribution from the surveyed macroalgae, and therefore represent an underestimation of this N uptake, particularly in hardbottom habitats.

The spatial variability in the constructed N budget (Figure 5.7) shows the local importance of N demand along the shoaling, seagrass beds and dominant DIN sourcing throughout the central hardbottom habitat. The modeled N fluxes ranged from net uptake of 3.4 ± 0.4 mmol m^{-2} day^{-1} (mean ± 1SD) in dense seagrass with minimal sponge coverage to net sources of 3.0 ± 1.0 mmol m^{-2} day^{-1} (mean ± 1SD) in sponge-rich hardbottom sites where demand from photosynthesis was minimal.

Discussion

Our results showed that the collective efflux from the sponge community is the largest of the estimated sources of N in Mystery Basin (Tables 5.3 and 5.4). Sponge recycled N represented 45 ± 24 % of the N sources to the tested site or roughly half of the calculated

demand from primary productivity (Table 5.4). Localized, site-specific quantifications of sponge community flux were the largest source of N at 60% of the surveyed locations within the basin. The calculated, average flux from the sponge community in Mystery Basin (0.59 ± 0.28 mmol N m^{-2} day^{-1}; Table 5.3) should be considered a minimum estimate; N release rates were not known for 3% of the surveyed population, and the sponge communities living on the shallow shoals bordering the tested site were potentially underestimated during biomass surveys. These seagrass-covered banks frequently harbored large populations of *C. nucula*, a species that is often found growing attached to seagrass blades (e.g. Corredor et al. 1988, Diaz and Ward 1997, Stevely et al. 2010). Despite direct observations of these populations, the shoaling habitats were exceedingly difficult to survey quantitatively as there is only a thin layer of water overlying the top of the grasses (<0.2 m water depth). The inability to accurately quantify this *C. nucula* population likely led to an underestimation of community DIN flux given the large nitrification rate observed in this species (Corredor et al. 1988, Diaz and Ward 1997, Hoer 2015, this volume).

Nevertheless, these results support the conclusions of previous work in other environments which showed the potential for the sponge community to supply a large proportion of water column DIN, particularly near the benthos (e.g., Corredor et al. 1988, Southwell et al. 2008; Keesing et al. 2013). The calculated flux is of similar magnitude as that observed on the western coast of Australia (0.35 to 0.63 mmol m^{-2} day^{-1}; Keesing et al. 2013), but is lower than the average flux from other selected communities in the Caribbean and Mediterranean (e.g. Corredor et al. 1988, Jiménez and Ribes 2007, Southwell et al. 2008). The differences between the average areal fluxes observed for this and other systems are most likely due to varying sponge community density and its species composition. With the exception of a single site, all

sites surveyed in Mystery Basin exhibited smaller sponge biomass than that found during a survey of Conch Reef in the Florida Keys performed by Southwell and co-workers (2008), and the average benthic sponge cover in Mystery Basin was much lower than the 7-20% areal coverage observed in other environments (Corredor et al. 1988, Jiménez and Ribes 2007). However, Mystery Basin represents a larger area than previously tested sites, and the average sponge community flux does not accurately represent the range of values found, rather the calculated flux was heterogeneously distributed throughout our tested site. Localized peaks in DIN efflux (~3.5 ± 0.9 mmol N m^{-2} day^{-1}; Figure 5.6) were of the same magnitude as values calculated from lower biomass densities found at sites in the Caribbean and Mediterranean (0.12 to 1.5 mmol N m^{-2} day^{-1}, Diaz and Ward 1997; ~2.5 mmol N m^{-2} day^{-1}, Jiménez and Ribes 2007).

Furthermore, the observed peaks in sponge community DIN efflux in this environment often did not collocate with sponge biomass maxima (Figures 5.4 and 5.6) due to variable community composition and the correspondingly variable rates of DIN production dependent on the constituent species. Locally elevated sponge biomass in Mystery Basin was often characterized by dominant populations of *S. vesparium*, which contributed very little DIN per unit of sponge biomass, and peaks in DIN flux were associated with large *G. gibberosa* populations, which exhibit a high rate of DIN production as NO_x^- (Hoer 2015, this volume). Similarly, the sponge communities tested in previous work were dominated by large populations of species that produced large quantities of DIN per unit biomass (*Xestospongia muta*, Southwell et al. 2008; *C. nucula*, Corredor et al. 1988, Diaz and Ward 1997). Assuming *G. gibberosa* populations in Mystery Basin approached the density of *X. muta* on Conch Reef (2.3 L_{sponge} m^{-2}; Southwell et al. 2008), the N flux from this species alone (6.6 ± 2.2 mmol N m^{-2} day^{-1}) would

represent nearly double the maximum N sourced from native sponge assemblages observed in our tested site and would approach the magnitude of estimates by Diaz and Ward (1997) and Jiménez and Ribes (2007). While the constituent species in Florida Bay have the capacity to generate benthic fluxes on the scale of that in other environments, the role of these organisms in the broader Florida Bay ecosystem will be locally variable based upon community size as well as species composition. Quantifying their impact on the N cycle will necessitate high spatial resolution in sponge biomass quantifications.

We hypothesized that sponge recycled N led to locally elevated DIN concentrations which were observed during water quality surveys within Mystery Basin, and we contend that the impact of sponge N recycling was amplified as a result of the shallow water column and bank-attenuated physical exchange with surrounding water masses. The hypothesized local impacts of the sponge population were particularly pronounced in May 2013 samples transecting Mystery Basin along the approximate trajectory of advective transport from HF10 to HF7 (Figure 5.3). These transecting points also reflect the predicted local importance of modeled N sources and sinks to overall water column DIN (Figure 5.7). Specifically, the largest quantified flux of N to the system is from sponge-mediated organic matter remineralization which is greatest in the central basin (Figure 5.7); corresponding samples exhibited elevated DIN concentrations (HF1, HF2, HF3, CTR; Figure 5.3). By contrast, locations where N demand from primary productivity is expected to be great (shoaling seagrass habitats; Figure 5.7) showed low water column DIN concentrations (HF10, HF14, HF4, HF4; Figure 5.3). When physical mixing and exchange with surrounding basins is expected to be at a minimum, it is possible that the nutrient demand at these shoaling locations may be partially satisfied by pulsed input of DIN-

enriched water from seiching of the basin water volume in response to tidal or wind-driven mixing.

Significant seasonality was observed in water column DIN at the tested site. Sites sampled in August 2012 had markedly lower concentrations than similar sites sampled in May 2013. Temporal variability in water column DIN was previously observed at a broader scale in Florida Bay (e.g., Boyer et al. 1999) with DIN concentration peaks occurring during the winter dry season in the western and central regions. The May samples represent the end of the dry season (November through May) and August nears the middle of the rainy season (June through October). Conditions during the dry season would be expected to increase water retention within the basin environments of Florida Bay due to reduced advective mixing, whereas the rainy season would be expected to accelerate flushing (Cosby et al. 2005, Shank et al. 2011). Furthermore, the August 2012 rainy season may have created anomalously high flow due to the recent completion and testing of the C-111 spreader (completed Spring 2012; US Army Corps of Engineers; UNESCO 2013). The C-111 spreader is part of the broader Everglades restoration plan and is designed to increase freshwater delivery to Taylor Slough. It is possible that the restored flow to Taylor Slough and elevated precipitation during the rainy season further enhanced freshwater delivery to the Everglades as compared to that prior to the completed project, which may elevate the wet/dry seasonality of processes in Florida Bay. Further seasonality could be resultant from seasonal shifts in wind direction and speed (Phlips et al. 1999), where windier periods would contribute to enhanced water column mixing or could introduce water from surrounding environments. The wind speed and direction near Mystery Basin (NOAA National Data Buoy Center; Site ID: LONF1) during May 2013 and August 2012

sample collections were not significantly different, and that may indicate a reduced role of wind in generating the observed seasonality.

The shoal-damped exchange in this system yielded moderate water retention relative to other basins in Florida Bay (Cosby et al. 2005); modeled advective flux based on May 2013 data yielded a residence time ($\tau_{advection}$) between 4 and 7 days for this site (C. Martens et al. unpublished data). Conversely, species-specific sponge pumping rates conservatively produced a system filtration time (the time for the water volume to be filtered by the sponge community; τ_{sponge}) of 8 hrs (N. Lindquist et al. unpublished data). With these respective residence times, the same modeled parcel of water would be expected to be filtered over a dozen times by the sponge population while within Mystery Basin. This filtering and the associated heterotrophic processes mediated by the sponge would be minimally expected to exert significant grazing pressure on overlying water (Peterson et al. 2006). We contend that this rapid water column overturn by sponges in conjunction with a slowly exchanging water column with surrounding environments would produce locally detectable changes in water quality based on quantified N sourcing resultant from sponge-mediated chemical transformations. If water residence time in Mystery Basin was elevated due to the dry season conditions, the locally elevated concentrations observed in May 2013 lend support the hypothesis that elevated water residence time can lead to enhanced DIN contribution from the sponge community. Conversely, the lower DIN concentrations observed during the rainy season (August 2012) could be illustrative of decreased water retention in the basin as a consequence of more rapid flushing or enhanced mixing with surrounding water masses. Enhanced flushing could simultaneously lower water residence time in Mystery Basin as well as increase physical delivery of water to seagrass-covered shoals, which dominate local N demand, thereby further depleting water column DIN. The impact of this posited, enhanced

delivery of water to the shoals would be amplified during this time as seagrass primary productivity in Florida Bay is at a maximum in July and August (Fourqurean et al. 2001), and corresponding N demand would be expected to peak contemporaneously. Therefore, we hypothesize that May 2013 represents conditions that approximate the peak DIN concentrations in the basin, and therefore enhanced the visibility of the impact from local processes, whereas samples from August 2012 represent a local concentration minima reflecting enhanced mixing and diminished visibility of local impacts.

The speciation of water column DIN did not directly correlate with that predicted for effluent N from the sponge community at the points of collection (e.g., Figure 5.7), yet an increasing contribution from NO_x^- to total DIN was observed at sites within Mystery Basin (Figure 5.3). Reduced N has been shown to characterize the majority of water column DIN in the wider Florida Bay (e.g., Lavrentyev et al. 1998, Boyer et al. 1999, Gardner et al. 2009), and continuation of this trend at sites in Mystery Basin is not unexpected given that the quantified N sources to this environment were presumed to be largely as NH_4^+ (roughly 60% of total N input is as NH_4^+). Yet by contrast, 60% of DIN from the sponge community is thought to be exuded as NO_x^- (Figure 5.5) and this enhanced delivery of oxidized N may contribute to the observed difference between the tested basin and the surrounding water masses. Nevertheless, the hypothesized local impact of sponge efflux on the speciation of water column DIN was not directly observed. None of the collected samples directly co-located with a presumed peak in sponge NO_x^- or NH_4^+ efflux, and substantial dilution or chemical transformations likely occurred between the point of release from the organism and sample collection. Furthermore, it is possible that the observed spatial variability in sponge community flux may contribute to local hotspots for other N transformations in the water column and surrounding sediments, particularly those

which may be accelerated in the presence of elevated DIN concentrations (nitrification, dissimilatory nitrate reduction to ammonium, denitrification). These additional chemical processes would further complicate efforts to attribute water column DIN speciation to the sponge community at a given location.

The recycled N from the sponge community represents an ecologically relevant source of DIN in this environment, given that it is an important N recycling pathway for the conversion of organic nutrients to more bioavailable inorganic forms. Most of the N in and around Mystery Basin is in organic form, similar to the wider Florida Bay ecosystem (Boyer et al. 1997), yet within the tested basin, DIN comprises a larger fraction of water column TN (8.3% inside as compared to 5.0% outside) potentially due to sponge remineralization processes. Further, this input of recycled N from sponges may be of particular importance at sites along the western margin of Florida Bay which experience levels of N limitation as compared to the majority of the bay where primary productivity is P limited (Fourqurean et al. 1993, Lavrentyev et al. 1998). The presence of a semi-diurnal tidal signal in Mystery Basin (approximately 0.2 m range; Martens et al. unpublished data) which occurred in phase with a nearby coastal gauge (NOAA tide gauge Vaca Key, FL; ID: 8723970) provided an indication of oceanic influence and potential P input; influx along the margin with the Gulf of Mexico is presumed to be among the largest sources of P to Florida Bay (Rudnick et al. 1999, Fourqurean and Robblee 1999 and citations within). Yet, without direct assessments of P in Mystery Basin, the role of DIN from the sponge community in altering the local stoichiometric ratios of nutrient elements remains purely conjectural.

The calculated budget represents best estimates of a variety of contributing processes occurring simultaneously, and provides a means to comparatively analyze the potential

importance of a newly quantified flux of recycled N. With the addition of sponge recycled N, the calculated budget shows approximate balance within the uncertainty of the quantified sources and sinks to Mystery Basin (Table 5.4), but this balance should be viewed cautiously due to unknown local applicability of the various estimates of nutrient input. The flux of N from groundwater discharge utilized in the budget (Table 5.3) was based upon an average seepage rates for basins in the middle of FL Bay and interstitial DIN concentrations from eastern sites near the Florida Keys (Corbett et al. 1999). We consider this rate of seepage plausible for Mystery Basin as similar rates have been observed directly north in Rabbit Key Basin (Corbett et al. 2000), but the N content of the seeped groundwater in this area is unknown and potentially lower than is found nearer to the Florida Keys island chain. Nevertheless, the recycled N input from sponge flux conservatively generates twice the DIN input that is associated with the theoretical maximum "new" input from groundwater discharge (Tables 5.3 and 5.4). Additionally, the local flux of N from the Everglades is subject to similar uncertainty, as it was obtained from a bay-wide estimate of TN discharge assessed evenly across the spatial extent of FL Bay (2220 km^2; Rudnick et al. 1999). This value most likely represents an overestimation of the N contributed from this source to the tested site because the magnitude and chemical composition would be expected to change significantly due to dilution and chemical transformations occurring as it is transported from the point of introduction. The TN contribution from the Everglades is mostly as organic compounds (Rudnick et al. 1999), which is hypothesized to be an important feedstock for sponge-mediated remineralization processes (Hoer 2015, this volume).

Furthermore, the exclusion of macroalgal primary productivity represents a considerable source of uncertainty in the quantified uptake flux at sites where primary productivity of these

organisms outweighed that of seagrasses (27 of 59 surveyed sites). At these locations the macroalgal biomass was dominated by red drift algae (primarily *Laurencia* sp.), and this biomass is notably ephemeral due to its susceptibility to physical transport, which contributes to significant uncertainties in the quantified biomass (Madden et al. 2009). Conversely, the contribution of the most abundant attached species (calcareous green algae) at these sites was significantly lower and represented typically ≤ 5% coverage, even at locations where its populations dominated. As a result of the uncertainty areal contribution of the dominant macroalgal taxa, the contribution of these organisms to the calculated N demand is excluded. Improved quantifications of these drifting organisms would greatly improve the accuracy of the N uptake from photosynthesis.

The N contribution from the sponge community is expected to exhibit significant temporal variability. Similar to the other N fluxes from the literature, the rate of DIN release from the sponge population exhibits short-term temporal variability on the scale of days to weeks (Southwell et al. 2008, Gibson 2011, Hoer 2015, this volume) which is not directly accounted for in the presented budget. The utilized species-specific N release rates were collected over many months, and therefore may represent a degree of this temporally dynamic behavior (Hoer 2015, this volume), yet the nature of this variability for the tested species is unknown. Furthermore, Florida Bay ecosystems are also subject to dramatic long-term variability as a result of near-total eradication of the sponge population during phytoplankton blooms (Butler et al. 1995, Peterson et al. 2006, Stevely et al. 2010) including a 2013-14 event that occurred in Mystery Basin and the surrounding areas. These blooms, which have become a recurring phenomenon in Florida Bay (Fourqurean and Robblee 1999), have the potential to quickly decimate dominant sponge populations which, based upon our estimates of their N contribution, should significantly alter

the nutrient budget of surrounding waters, particularly basins with limited exchange across shallow shoals. Nevertheless, our results further indicate a potentially dominant role of sponge populations in the N budget of shallow coastal ecosystems, and the spatially heterogeneous sponge biomass within Florida Bay may contribute to some of the variability observed in water column DIN at the ecosystem scale. Additional comparative assessments with other environments within the bay with differing sponge densities and community compositions could provide evidence as to the importance of recycled DIN to the overall N budgets in this ecosystem.

Table 5.1: Summarized DIN determinations from various sites within and surrounding Mystery Basin (Figure 5.1). N species concentrations (NO_x^-, NH_4^+, total DIN, and TN) are in µmol N L^{-1} and represent the mean ± 1SD.

Date	**Collections (n)**	**NO_x^-**	**NH_4^+**	**DIN**	**TN**	**Hardbottom/Seagrass**
August 2012	16	0.5 ± 0.2	1.1 ± 0.3	1.6 ± 0.5		Hardbottom
August 2012	5	0.4 ± 0.1	0.5 ± 0.4	1.0 ± 0.5		Seagrass
May 2013	CTR (SEASII; n ≈ 7300)	3.0 ± 0.5				Hardbottom (1.0 mab)
May 2013	CTR (SEASII; n ≈ 6100)	3.8 ± 0.7				Hardbottom (0.1 mab)
May 2013	CTR (n = 16)	2.6 ± 0.9	2.2 ± 0.9	4.8 ± 1.3	54 ± 8	Hardbottom
May 2013	3	1.7 ± 0.3	2.9 ± 0.7	4.5 ± 0.9	60 ± 12	Hardbottom
May 2013	3	0.6 ± 0.3	1.6 ± 0.7	2.1 ± 0.4	65 ± 22	Seagrass
May 2013	3	0.8 ± 0.5	2.1 ± 0.5	2.9 ± 0.8	44 ± 2	Hardbottom; Outside MB*
May 2013	4	0.4 ± 0.1	1.1 ± 0.3	1.4 ± 0.4	45 ± 2	Seagrass; Outside MB*

*MB = Mystery Basin

Table 5.2: Total sponge biomass across both hardbottom and seagrass habitats in Mystery Basin (10^3 L_{sponge}). Values are shown for each utilized survey methodology (belt transect and 1 m^2 quadrat) and represent the mean ± 1SE in the calculated displacement. † indicate species which have measured DIN production rates and ‡ indicate species which were too small to be measured in transects and these data were excluded from the corresponding weighted mean.

Species	**Transect Biomass (10^3 L_{sponge})**	**Quadrat Biomass (10^3 L_{sponge})**	**Weighted Mean (10^3 L_{sponge})**
S. vesparium †	2000 ± 410	34 ± 7.1	1900 ± 380
I. variabilis †	1000 ± 220	78 ± 18	930 ± 210
G. gibberosa †	510 ± 123	130 ± 10	480 ± 120
Cinachyrella sp.†‡	0	58 ± 5.4	58 ± 0.40
Haliclona magnifica	34 ± 8.5	25 ± 3.7	33 ± 7.8
Halichondria melanodocia †	24 ± 8.0	94 ± 5.4	29 ± 7.4
Unidentified sponges	27 ± 13	42 ± 11	28 ± 12
Spongia sp. †	12 ± 3.7	9.3 ± 4.2	12 ± 3.4
Dysidea etheria	11 ± 3.2	19 ± 3.7	12 ± 3.0
Tedania ignis	9.9 ± 2.6	8.1 ± 1.9	9.7 ± 2.4
Hippospongia lachne †	7.7 ± 2.5	1.9 ± 1.9	7.2 ± 2.3
Lissodendoryx stigmata	5.7 ± 1.5	3.6 ± 1.3	5.5 ± 1.4
C. nucula †‡	0	5.1 ± 1.3	5.1 ± 0.1
Amphimedon viridis‡	0	4.3 ± 2.8	4.3 ± 0.21
Haliclona sp. †	2.4 ± 0.98	27 ± 2.4	4.2 ± 0.92
Hyritios sp. ‡	0	2.2 ± 0.54	2.2 ± 0.04
I. strobilina	1.6 ± 0.82	0	1.5 ± 0.76
Tectitethya crypta	1.4 ± 0.70	2.3 ± 1.4	1.4 ± 0.66
Callyspongia sp.	0.49 ± 0.37	5.0 ± 0.77	0.83 ± 0.34
I. campana†	0.70 ± 0.70	0	0.65 ± 0.65
Aaptos lithophaga	0.61 ± 0.45	0	0.56 ± 0.42
Ircinia sp.	0	5.8 ± 4.5	0.43 ± 0.33
Niphates erecta	0.19 ± 0.12	1.0 ± 0.57	0.25 ± 0.12
Clione sp. ‡	0	0.24 ± 0.24	0.24 ± 0.02
Total biomass	**3600 ± 500**	**560 ± 27**	**3500 ± 450**

Table 5.3: Nitrogen fluxes (mmol m^{-2} day^{-1}) for Florida Bay. Sponge recycled N was determined from Mystery Basin biomass surveys and species-specific N release rates (Hoer 2015, this volume). The remaining fluxes were calculated based upon published quantifications from Florida Bay or analogous environments ("Bare" Sediment; Great Barrier Reef, Australia). Reported values represent the mean ± 1SE.

I. Recycled N Sources in FL Bay	**mmol N m^{-2} day^{-1}**	**References**
Sponge DIN Flux (Mystery Basin Biomass)	0.59 ± 0.28	This Study
Sediment-Water Flux (Diffusive; Seagrass Beds)	0.36 ± 0.27	Yarbro and Carlson (2008)
Sediment-Water Flux (Diffusive; "Bare" Sediment)	0.04 ± 0.01	Capone et al. (1992)
Total Recycled N Inputs	**0.99 ± 0.39**	
II. New N Inputs to FL Bay		
Rainfall	~ 0.06	Prospero et al. (1996)
Everglades	~ 0.12	Boyer and Keller (2007); Rudnick et al. (1999)
Groundwater Discharge	< 0.3 ± 0.05	Boyer and Keller (2007); Corbett et al. (1999)
N_2 Fixation (Benthic Algae)	~ 0.1	Boyer and Keller (2007)
Total New N Inputs	**0.57 ± 0.06**	
III. N Demand – FL Bay Net Primary Productivity		
Seagrasses (90% *Thalassia* sp.)	4.1 ± 0.4	Zieman et al. (1989); Fourqurean et al. (2002); FCE LTER, Fourqurean J. (2011)
Phytoplankton	0.1 ± 0.07	BATS NPP (0-20m Water Depth, Averaged from 1989-2011); Boyer personal communication
Minimum Total N Demand	**4.2 ± 0.4**	
IV. Nitrogen Loss from FL Bay		
Benthic Denitrification	~ 0.1	Kemp and Cornwell (2001)

Table 5.4: The nitrogen budget for Mystery Basin calculated using the quantifications from Table 5.3. The areal nutrient fluxes were assessed across the appropriate habitat types (see Figure 5.1). Reported values represent the mean ± 1SE.

I. Recycled N Sources in Mystery Basin	**mol N day^{-1}**	**References**
Sponge DIN Flux (Mystery Basin Biomass)	2900 ± 1400	This Study
Sediment-Water Flux (Diffusive; Seagrass Beds)	680 ± 510	Yarbro and Carlson (2008)
Sediment-Water Flux (Diffusive; "Bare" Sediment)	130 ± 30	Capone et al. (1992)
Total Recycled N Inputs	**3700 ± 1500**	
II. New N Inputs to Mystery Basin		
Rainfall	~ 300	Prospero et al. (1996)
Everglades	~ 400	Boyer and Keller (2007); Rudnick et al. (1999)
Groundwater Discharge	< 1500 ± 250	Boyer and Keller (2007); Corbett et al. (1999)
N_2 Fixation (Benthic Algae)	~ 500	Boyer and Keller (2007)
Total New N Inputs	**2700 ± 250**	
III. N Demand – Mystery Basin Net Primary Productivity		
Seagrasses (90% *Thalassia* sp.)	8000 ± 800	Zieman et al. (1989); Fourqurean et al. (2002); FCE LTER, Fourqurean J. (2011)
Phytoplankton	510 ± 370	BATS NPP (0-20m Water Depth, Averaged from 1989-2011); Boyer personal communication
Minimum Total N Demand	**8510 ± 880**	
IV. Nitrogen Loss from Mystery Basin		
Benthic Denitrification	~ 500	Kemp and Cornwell (2001)

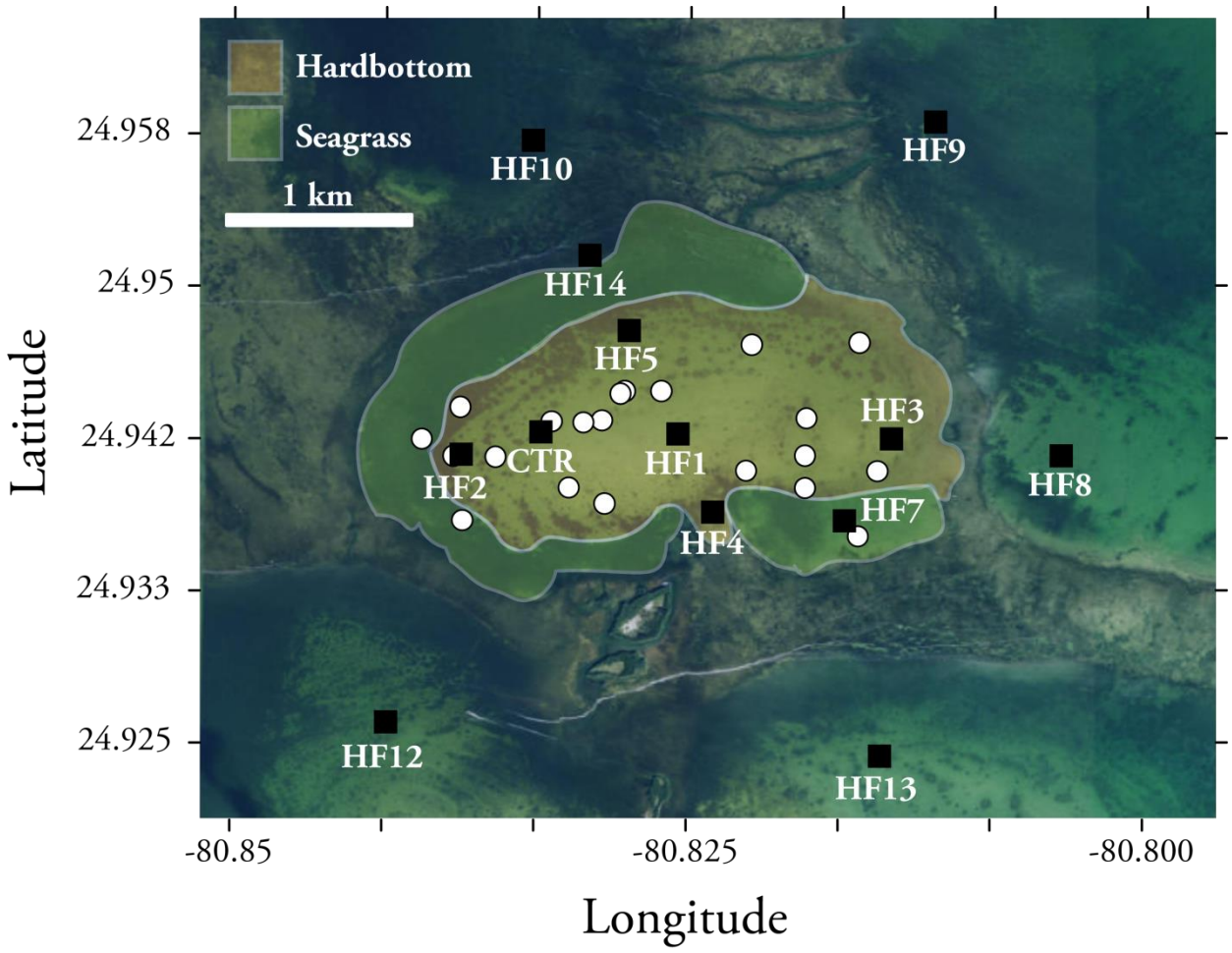

Figure 5.1: ArcMap image of the study area showing the location of water quality collection sites as well as the areal extent of seagrass and hardbottom habitat types. White circles indicate collections performed during August 2012 and labeled black squares were sampled in May 2013. SEASII-NO_x instrumentation was deployed at CTR during May 2013.

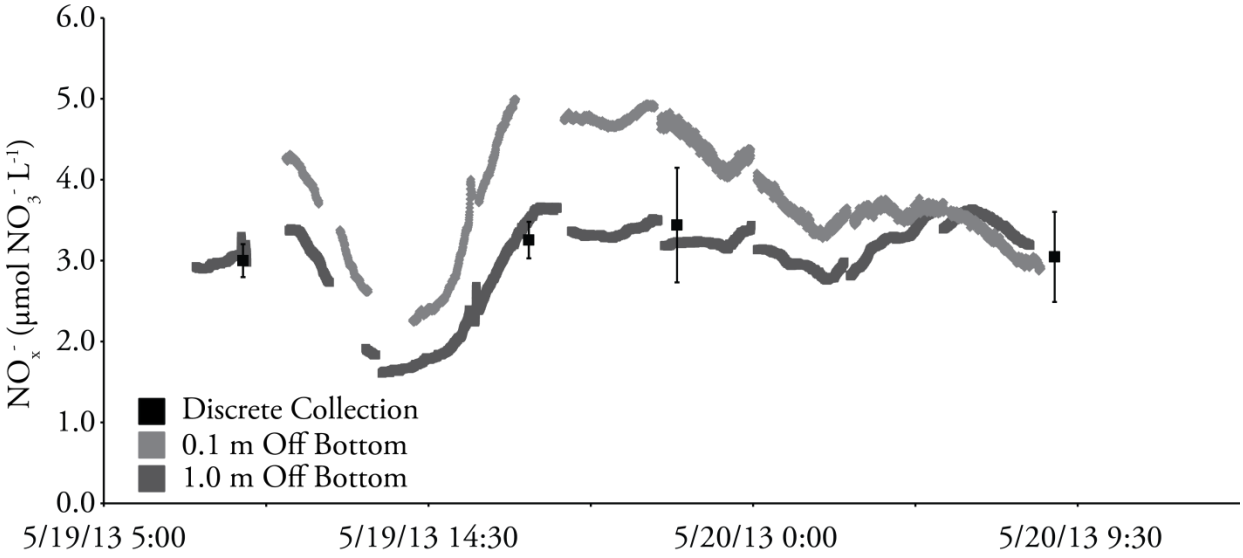

Figure 5.2: Time-series data collected at CTR with discrete collections performed by peristaltic pump. Discrete collections represent the mean ± 1SE. Breaks in the data record are indicative of gaps where the instrument is programmed to obtain a new optical reference or instrument maintenance.

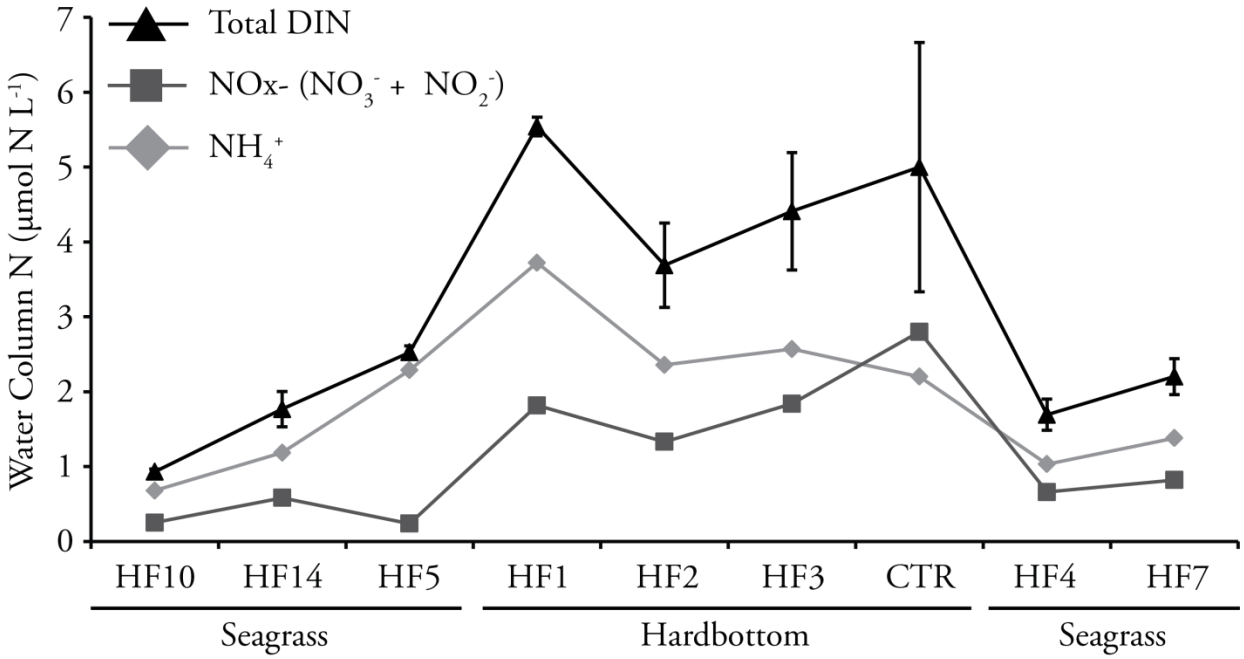

Figure 5.3: Water column samples transecting Mystery Basin along the approximate trajectory of water transport into and out of the basin. Proximate site characterization is listed below the site IDs and each point represents the mean of the sampled parameter. Error bars indicate ± 1 SE.

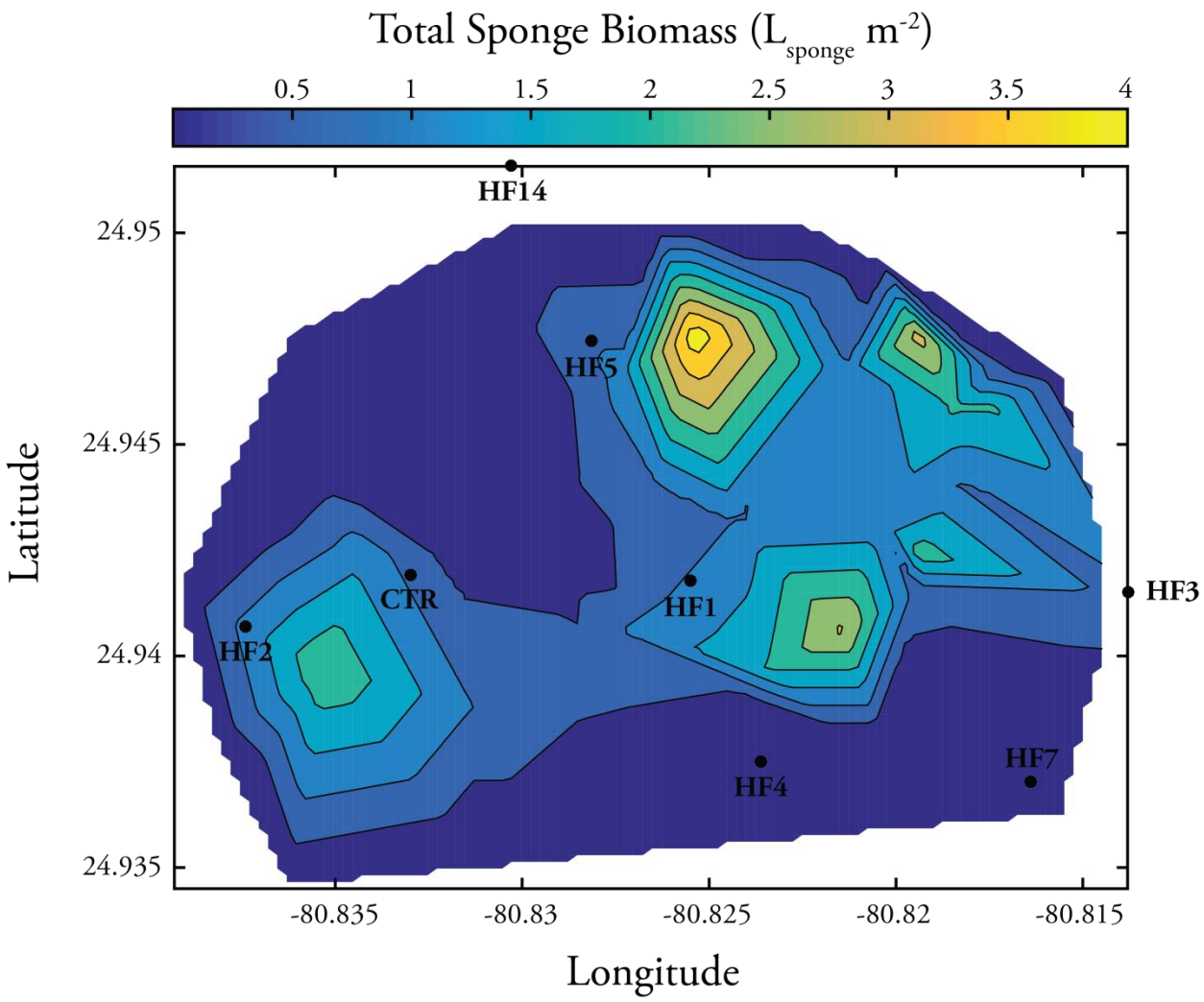

Figure 5.4: Contour plot of surveyed sponge biomass in Mystery Basin with 8 site IDs which were sampled during the water quality survey.

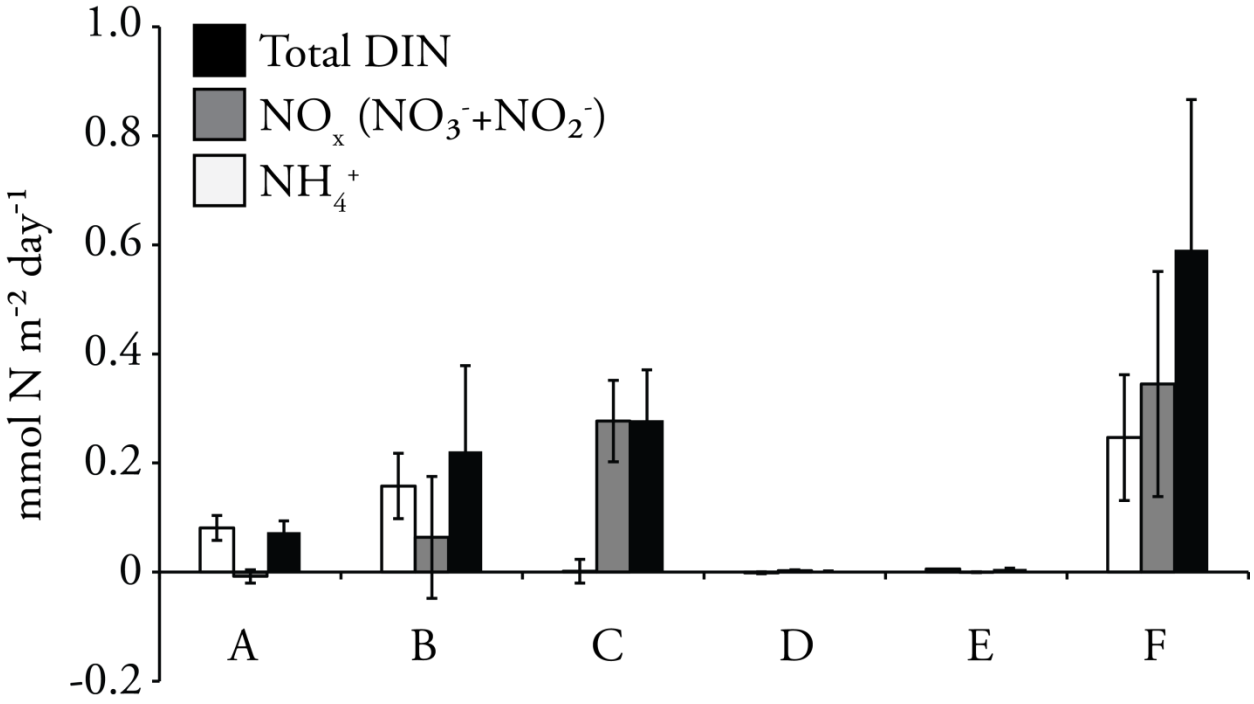

Figure 5.5: Average NH_4^+, NO_x^-, and total DIN contributions from the sponge community in Mystery Basin. DIN fluxes are separated by species and arranged in descending order of biomass contribution (largest to smallest; from left to right): A. *S. vesparium*; B. *I. variabilis*; C. *G. gibberosa*; D. *Cinachyrella* sp.; E. *H. melanodocia*; F. All surveyed species. Error bars are ± 1 SE.

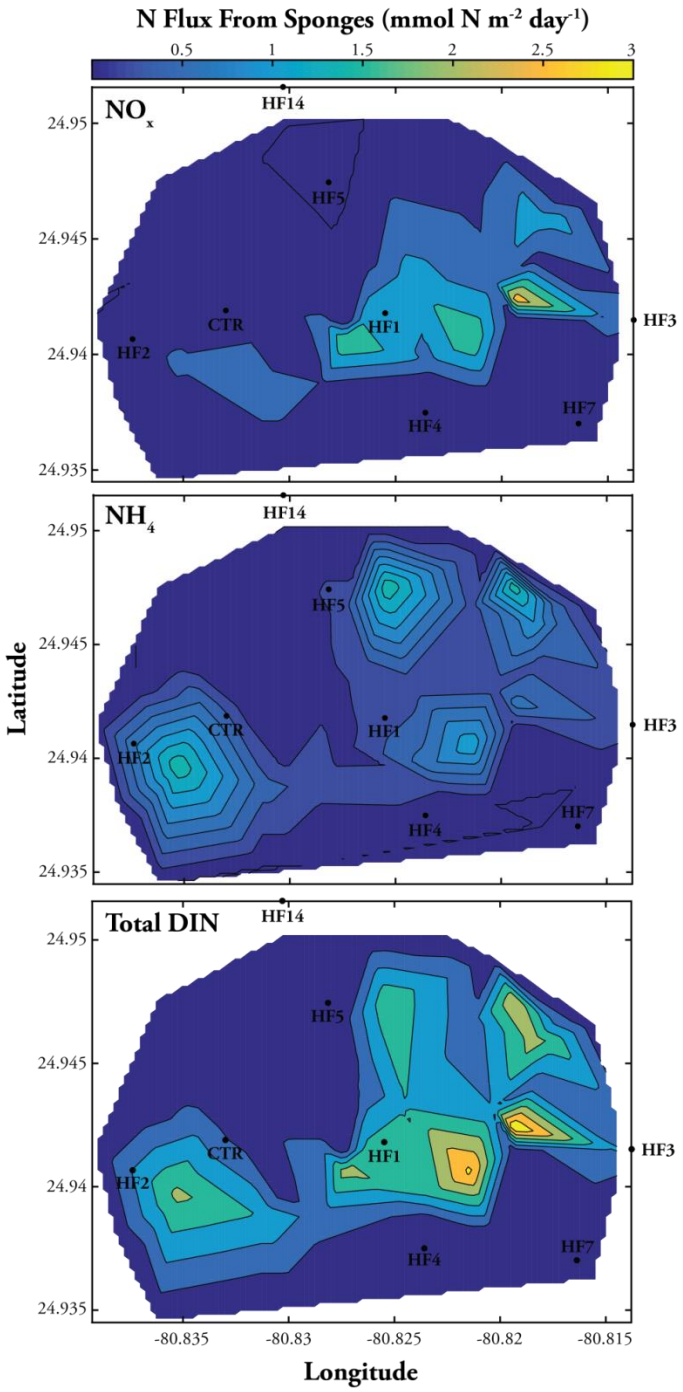

Figure 5.6: Contour plots of NH_4^+, NO_x^-, and total DIN contributions from the sponge community in Mystery Basin. Site IDs and locations are shown for 8 points sampled during the water quality survey.

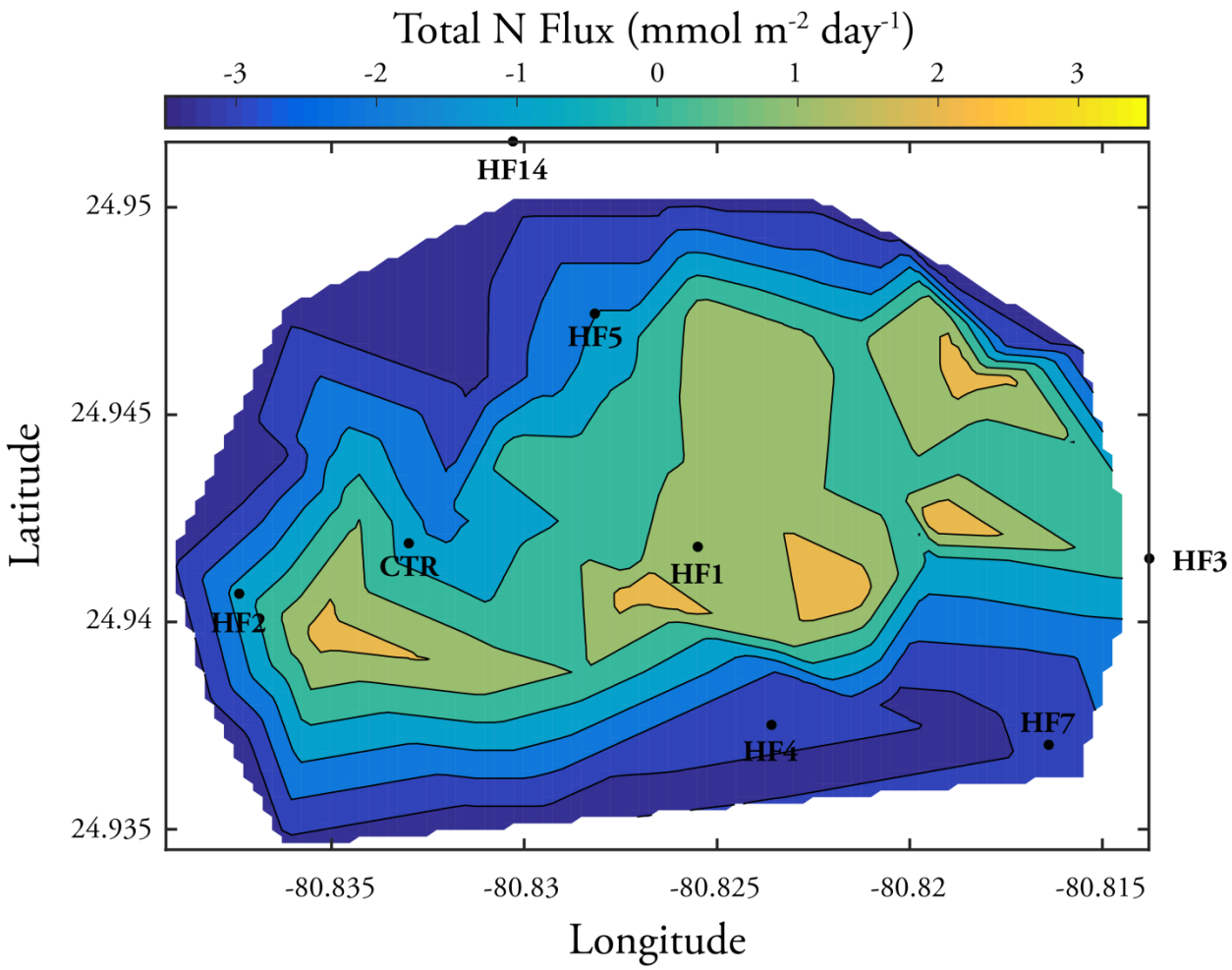

Figure 5.7: Contour plot of the calculated N flux model used to determine local importance of N sources and sinks throughout Mystery Basin.

CHAPTER 6:

Impacts of a Cyanobacterial Bloom on the Sponge Population and Chemical Structure of an Offshore Basin in Florida Bay

Introduction

Florida Bay is an estuarine lagoon between peninsular Florida and the Florida Keys archipelagic island chain. The shallow water column (< 3 m water depth) is generally oligotrophic (e.g., Boyer et al. 2006) and is compartmentalized into multiple small basins by a series of shoaling carbonate mud banks that reduce physical exchange between neighboring environments (e.g., Phlips et al. 1995, Boyer et al. 1997, Fourqurean and Robblee 1999). Shoal-attenuated water exchange between these basins generates highly variable water residence times throughout the bay, thus augmenting the influence of local processes in chemical cycles (e.g., Fourqurean et al. 1993, Rudnick et al. 2005, Zhang and Fischer 2014). The benthic community is characterized by a variety of macrofauna (sponges, octocorals, and small hard corals) and macrophytes (seagrasses and macroalgae). Seagrasses (primarily *Thalassia testudinum*, *Halodule wrightii*, and *Syringodium filiforme*) usually dominate primary productivity throughout the Florida Bay ecosystem (Zieman et al. 1989) and thereby drive nutrient demand. Dense seagrass meadows in the eastern portion of the bay are phosphorus (P) limited however, there is evidence for nitrogen (N) limitation in the western portion of the bay, which is characterized by marine conditions through exposure to the open Gulf of Mexico (Fourqurean et al. 1993, Lavrentyev et al. 1998). Sponges dominated by *Spheciospongia vesparium*, *Ircinia* sp., and *Chondrilla nucula* (Peterson et al. 2006; Hoer 2015, this volume) are also a common feature

throughout the bay (Chiappone and Sullivan 1994, Butler et al. 1995). Sponges can serve a variety of functions, and where biomass is high, provide important habitat for juvenile organisms (Butler et al. 1995), exert large grazing pressure on water column productivity (Officer et al. 1982, Peterson et al. 2006), and contribute a large quantity of recycled, dissolved inorganic nitrogen (DIN) to the overlying water (Corredor et al. 1988, Southwell et al. 2008, Hoer 2015, this volume). The recycled N from these and other dominant organisms (e.g., seagrasses; Yarbro and Carlson 2008) may be particularly important source of DIN to the water column as local recycling processes have been found to regulate the supply of bioavailable N in many locations (Rudnick et al. 2005, Boyer et al. 2009, Hoer 2015, this volume).

Florida Bay has experienced a series of ecological disturbances that intensified in the late 1980s (Fourqurean and Robblee 1999) and have continued into the present (Berry et al. 2015). These changes have the potential to profoundly shift the trophic structure of the bay as a whole towards water column productivity and away from the currently dominant role of seagrasses (Chasar et al. 2006). Widespread *Thalassia* mortality was observed throughout the ecosystem in the late 1980s and early 1990s with pronounced impacts in the western and north-central regions (Robblee et al. 1991). The losses of seagrass biomass, including the root structure, led to sediment destabilization and a consequent increase in turbidity (Hall et al. 1999), while the decay of dead tissue and resuspended sediments likely enhanced local nutrient loading (Fourqurean et al. 1993, Boyer et al. 1999, Peterson et al. 2002). Contemporaneously, intense phytoplankton blooms were observed throughout the bay, exhibited extremely high cell densities ($>10^6$ cells mL^{-1}), and often persisted for many months at a time (Phlips and Badylak 1996, Phlips et al. 1999). Generally, these blooms consisted of rapidly growing populations of cyanobacteria (*Synechococcus*) or diatoms (Phlips et al. 1999), both of which have been shown to satisfy their

photosynthetic N requirements through uptake of nitrate (NO_3^-) and ammonium (NH_4^+) (Mulholland and Lomas 2008). Blooming populations of cyanobacteria have been shown to reduce water column DIN ($NO_x^- + NH_4^+$) to near trace concentrations as a result their large autotrophic N draw-down (Lindell et al. 2005). These blooms have become a recurring feature in Florida Bay and their persistence may exacerbate the loss of seagrasses through bottom-water hypoxia, toxin production, and shading of benthic macrophytes (Phlips and Badylak 1996, Phlips et al. 1999). Furthermore, these dense blooms are hypothesized to have precipitated rapid sponge die-offs (Butler et al. 1995, Wall et al. 2012); in two consecutive bloom events in central Florida Bay (1991-1992 and 1992-1993) locally dominant species (*S. vesparium*, *Iricina* sp., and *Spongia* sp.) experienced mortalities ranging from 40 to 100% (Butler et al. 1995). Yet, despite temporal overlap, a direct, causal link between blooming *Synechococcus* and sponge mortality remains unknown (Butler et al. 1995, Lynch and Phlips 2000, Peterson et al. 2006).

Substantial loss of sponge biomass has the potential to generate cascading ecosystem impacts due to the sudden absence of the ecological and biogeochemical contributions from these organisms (Butler et al. 1995, Peterson et al. 2006, Hoer 2015, this volume). In addition to the lost contribution to ecosystem processes, the decaying biomass from the decimated sponge population may serve as important source of recycled nutrients, potentially generating local eutrophication. Decaying biomass of fish killed during blooms of the toxic dinoflagellate *Karenia brevis* can supply quantities of regenerated nutrients that are capable of providing significant N and P to the blooming organisms, thereby contributing to increased bloom duration (Vargo et al. 2008, Vargo 2009, Killberg-Thoreson et al. 2014). While fish mortality has not been noted during *Synechococcus* blooms in Florida Bay (Butler et al. 1995, Lynch and Phlips 2000), mass sponge mortality has the potential to provide a similar magnitude of regenerated

nutrients, and consequently enhance bloom intensity and longevity by lending nutritive support to blooming cyanobacteria. Yet, the ephemeral nature of intense blooms and their often sudden onset makes observation of such impacts difficult. In early September 2013, during an ongoing investigation of the role of sponges in Florida Bay N cycling, bloom conditions were found in Mystery Basin, a small, offshore basin located just north of the Arsnicker Keys (Site ID: HF1; Figure 6.2). The bloom was first noted by fishermen at locations in Rabbit Key Basin just to the north of Mystery Basin, prior to our observations (W. Sharp, personal communication). Site descriptions by both our group and reports from fishermen to the Florida Fish and Wildlife Conservation Commission indicated that the bloom appeared to be expanding southward from a point of origin north or northeast of Rabbit Key. We had generated significant data characterizing this location prior to the onset of bloom conditions (extensive water quality sampling and surveyed sponge biomass; Hoer 2015, this volume) that enabled a unique examination of the ecosystem response of a sponge-rich basin throughout a cyanobacterial bloom and following its dissipation.

The primary objective of this study was to quantify how the sponge community of Mystery Basin was impacted by bloom conditions and how the basin's water chemistry and the biogeochemical role of sponges may have changed following return to a more typical water column plankton community. We hypothesized that the bloom would result initially in significant sponge mortality as well as a large reduction in ambient DIN concentrations due to the sudden increase of photosynthetic N demand. We predicted that the massive quantity of destroyed sponge biomass would quickly decay and generate a sustained injection of recycled nutrients, potentially contributing to the intensity or longevity of the bloom. We expected dissolved organic matter (dissolved organic carbon and dissolved organic nitrogen) to peak

during bloom maxima as a result of exuded photosynthate from actively expanding cyanobacteria populations, and predicted that these values would quickly return to pre-bloom concentrations due to heterotrophic remineralization and physical export. Additionally, we hypothesized that after cessation of the bloom, water column DIN would stabilize at a concentration significantly lower than pre-bloom conditions as a result of the functional absence of the sponge community. This ecosystem disturbance provided a unique opportunity to observe the *in situ* evolution of bloom conditions in a previously studied location, the timing of impacts on the benthic community and the overall impacts of these changes. The results of these measurements provide information that is critical to understanding and predicting the array of responses of similar sponge-rich environments in Florida Bay to future bloom events.

Methods

Water quality samples

Immediately upon discovery of bloom conditions in Mystery Basin, water quality samples were collected from sites previously sampled during pre-bloom surveys (September 26, 2013; HF1-HF13; Hoer 2015, this volume; Figure 6.1). At each site, triplicate water samples were collected through an in-line filter using 60 mL polypropylene syringes. The in-line filter (Whatman GF/F; ~0.7 μm nominal pore size) and 10 cm of small-diameter, high-density polyethylene tubing were attached to one arm of a polycarbonate 3-way stopcock which was fitted directly to the syringe; the stopcock allowed isolation of collected water or discharge through the open third arm during rinsing. The length of attached tubing helped minimize contamination by allowing the collecting diver to be positioned down-current from the sampled water. A new pre-combusted, GF/F was used for the filtration of each water sample. The diameter of the utilized GF/F (25 or 47 mm) was selected depending upon the bloom conditions

at the sampled site; dense bloom conditions necessitated a larger diameter filter to increase the filterable volume prior to clogging. GF/F material was selected due to its suitability for pre-combustion and similar use in prior studies of nutrient concentrations in Florida Bay (e.g. Boyer et al. 1997, Boyer et al. 2006, Gibson et al. 2008). During sample collection, the syringe, filter, and tubing were rinsed 3x with filtered target water and the rinsing volume was discharged. The fourth volume was slowly drawn into the syringe (< 2 mL sec^{-1}) to ensure the collection was representative of the desired water mass, and the attached stopcock was closed to prevent accidental sample loss. The sample was returned the surface and stored in a dark ice bath until transport to shore for subsampling and preservation (less than 8 hours from collection to processing or analysis). At the shore lab samples were immediately split for DIN (NH_4^+ and NO_2^-+NO_3^- (henceforth NO_x^-)) as well as total dissolved nitrogen (TN) analyses; DON was determined as the TN content less DIN. TN samples (20 mL volume) were put in sample-rinsed borosilicate glass scintillation vials; 100 µL of 50% H_3PO_4 was added, and the vials were stored at 4°C until subsequent analysis. Nitrate plus nitrite (NO_x^-) samples (20 mL volume) were placed into sample-rinsed, borosilicate glass scintillation vials and frozen. Ammonium (NH_4^+) samples (20 mL volume) were placed into sample-rinsed amber HDPE bottles. Ammonium concentrations were determined immediately to reduce the potential impact of degradation on sample integrity. For each location, the sampled parameters were measured in triplicate for quality assurance and control. Used GF/F filters were wrapped in foil and frozen to be analyzed for chlorophyll *a* concentration.

Following this initial collection when the bloom was detected, recurring water quality samples were gathered at four locations across a north/south transect running from a presumed control site on the bay side of Long Key (Site ID: J01, 24° 49' 54.30" N, 80° 48' 44.82" W) to a

site in the center of Mystery Basin (Site ID: HF1, 24° 56' 30.42" N, 80° 49' 31.80" W; Figure 6.2). Two sites were sampled between the northern and southern endpoints: one immediately south of the carbonate mud bank which defines the southern sill of Mystery Basin (Site ID: HF13, 24° 55' 27.00" N, 80° 48' 52.08" W), and the second, which was 2 km due south of this southern edge (Site ID: WP96, 24° 54' 30.36" N, 80° 48' 49.14" W; Figure 6.2). The north-south transect was selected due to the presumed southward expansion of bloom conditions from a presumed point of origin in the north-central region of Florida Bay. Samples were collected weekly from October 7 to November 26, 2013. By November 26th, bloom conditions appeared to have receded and, as a result, sampling continued with collections being performed every third week from November 26, 2013 to March 13, 2014. Logistical limitations prevented sampling from March 13 to July 29, 2014, at which point recurring collections resumed with surface samples being collected every six weeks from July 29, 2014 to March 4, 2015. Sampling was concluded March 4, 2015 because water column nutrient concentrations at impacted sites appeared to be at a steady state. In total, the bloom sampling campaign collected 90 bottles from each of the 4 sites, spanning 17 months.

At each site, unfiltered water samples were collected from the surface and immediately above the bottom in triplicate, 125 mL amber HDPE bottles. Surface water was collected approximately 10 cm below the water surface by reaching over the gunnel of the boat, and water at depth was collected by a diver using SCUBA. Sample bottles were rinsed three times with target water, filled, and capped. Rinses were also performed in triplicate at depth by allowing the bottle to fill and voiding it with air from the collecting diver's secondary air supply. Collected water was immediately frozen shipboard using dry ice and shipped overnight to the University of North Carolina for filtration and analysis. On the day of arrival in North Carolina,

the samples were thawed, vacuum filtered (47mm Whatman GF/F), and split into separate vials for NO_x^-, NH_4^+, DOC, and TN analyses. NO_x^- samples (20 mL volume) were preserved in sample-rinsed, borosilicate glass scintillation vials and frozen at -20°C until analysis. DOC/TN samples (20 mL volume) were placed in sample-rinsed borosilicate glass scintillation vials, 100 µL of 50% H_3PO_4 was added, and the vials were stored at 4°C until analysis. NH_4^+ samples (20 mL volume) were collected in duplicate from each field bottle and placed into sample-rinsed amber HDPE vials. Used 47mm GF/Fs were reserved, wrapped in foil which had been baked at 450°C for >6 hours (henceforth, pre-combusted), and frozen at -80°C for subsequent acetone extraction of chlorophyll *a* and fluorescent measurement (US EPA Method 445.0; Arar and Collins 1997). The collected NH_4^+ samples were analyzed the day of arrival to ensure sample quality, whereas DOC/TN and NO_x^- samples were stored after filtration and analyzed within 48 h.

The large differences observed between pre-bloom (May 2013) and post-bloom water column DIN concentrations lead to concerns regarding the potential for methodological error introduced by transporting samples frozen versus immediate filtration and analysis or preservation in the field. In order to examine this possibility, samples were collected at HF1 over a 4 day period in July 2014 by syringe using in-line filtration *in situ* as in the first bloom collections (September 26, 2013) and pre-bloom water quality surveys (Hoer 2015, this volume). During this time, one set of samples was also collected using 125 mL amber HDPE bottles providing direct comparison between the utilized sampling methods. Samples collected using HDPE bottles were vacuum filtrated in the field, the ammonium was analyzed the day of collection, and NO_x and DOC/TN samples were preserved for analysis upon return to North Carolina. Two weeks following these collections (July 29, 2014), a water sample was obtained

using the outlined technique for recurring collections and shipped frozen to NC which provided additional comparability between sampling methodologies.

All glassware and plastics which were used in sample collection and processing (HDPE bottles, filter holders, filtration tubing, scintillation vial lids, etc.) were soaked in a 0.1 mol L^{-1} HCl bath for >12 hours and triple rinsed with 18.2 MΩ type I water prior to use and between each sampling. The amber HDPE sample bottles used for ammonium samples were treated with small aliquots of o-phthaladehyde working reagent following the acid wash procedure. This added volume of reagent was allowed to react for 24 hours to ensure removal of any residual ammonium from the container, and the pre-treatment solution was rinsed away by triple rinsing with 18.2 MΩ type I water immediately prior to use for standards or samples. Scintillation vials used for sample collection were acid washed by the above procedure and baked at 450°C for >6 hours to remove any residual organic matter. This combusted glassware was stored wrapped in pre-combusted foil and bagged to minimize contamination prior to use. Filters were baked at 450°C for >6 hrs and stored in pre-combusted foil.

Sample analysis

Ammonium analyses were performed using the method of Holmes et al. (1999). Sampled volumes were reacted with 5mL of o-phthaladehyde working reagent in amber, HDPE bottles and allowed to develop at room temperature for 2.5 hours. After the incubation period, samples were analyzed using a Turner Designs TD-700 laboratory fluorometer equipped with an ammonium optical kit (Turner Designs 10-303). The method detection limit was determined to be 10 nmol L^{-1} by repeated standard measurements. Standards were prepared daily at the point of use by serial dilution of a purchased stock solution (Ricca Chemical Company 693-16), and analyzed with the prepared samples. Nitrate plus nitrite (NO_x^-) samples were analyzed using

SEASII-NOx autoanalyzers configured for bench-top use (Steimle et al. 2002, Adornato et al. 2005, Adornato et al. 2007 for detailed descriptions of similarly utilized instrumentation). NO_x^- was measured using a 15 cm pathlength and cadmium reduction of NO_3^- to NO_2^- followed by detection based on the Griess reaction. The method detection limit was calculated as 25 nmol NO_x^- L^{-1}, and was determined by repeated analysis of standard solutions. Standards were prepared daily by dilution of a purchased stock (SPEX Certiprep AS-NO39-2Y and ASNO29-2Y), and analyzed with collected samples. DOC/TN samples were analyzed with a Shimadzu TOC-L/TNM-L organic carbon and total nitrogen analyzer, which employs high temperature catalytic oxidation (HTCO) for analysis of aqueous organic matter. Calibration curves were prepared from lab prepared stock solutions and were closely monitored during analysis. Lab prepared carbon standards were batch checked against commercially produced stock solutions (La-Mar-Ka Chemical Company) to ensure accuracy. Standards were remade and rerun if the correlation coefficient was found to be less than 0.995, and standards were interspersed within samples to provide additional quality control.

Post-bloom sponge survey

An abbreviated sponge biomass survey was conducted in April 2014 to determine the extent of damage to the previously observed sponge community following the dissipation of bloom conditions (Hoer 2015, this volume). Fifteen (10 hardbottom, 5 seagrass) of the 59 previously surveyed sites were randomly selected to be reassessed, and methodologically identical surveys were conducted at the chosen sites. As in the pre-bloom surveys, three 25m x 2m non-overlapping transects were established randomly by divers using SCUBA. For each transect, divers identified, counted, and measured the dimensions (length (L), width (W), and height (H)) of all sponge biomass that was greater than 10 cm in its largest dimension. Sponges

smaller than 10 cm were identified, counted, and measured within four 1 m^2 quadrats equidistantly spaced along the length of the transect. Sponge identifications were performed to the lowest taxonomic level possible and those that could not be identified in the field were photographed and collected for later identification in the lab. The volumetric displacement of the surveyed sponge biomass was determined using previously derived relationships between the morphometric measurements obtained in the field and lab-measured water displacement (Hoer 2015, this volume). In each quadrat, seagrass and macroalgal distribution was also quantified using the Braun-Blanquet cover assessment method (Braun-Blanquet 1972). A score (0-6) was assigned to each species occurring within the quadrat based on its spatial coverage (Braun-Blanquet 1972, Fourqurean et al. 2001).

Post-bloom N sourcing from sponges

The N contribution from the sponge community was recalculated using the updated sponge biomass to test the impact on sponge sourced N as compared to pre-bloom determinations (Hoer 2015, this volume). The areal N flux from the sponge community was calculated using the same pre-bloom method and the sum of the fluxes from contributing species was expanded to the area of Mystery Basin to determine the total N flux from sponges (mol N day^{-1}; Hoer 2015, this volume).

Results

Water quality samples: chlorophyll a

Samples collected on September 26, 2013 showed elevated chlorophyll *a* concentrations at sites north of central Mystery Basin (HF9 and HF10; 21 ± 1 and 19 ± 1 µg L^{-1}, respectively; mean ± 1SE; Figure 6.3) and inside the basin near the eastern shoal (HF3; 19 ± 2 µg L^{-1}; mean ± 1SE). The observed concentrations decreased moving south and west across the basin (Figure

6.3). Chlorophyll *a* concentrations peaked during the first of the recurring collections (October 7, 2013) and the highest concentrations were observed at sites outside Mystery Basin (HF13 and WP96; Figure 6.4). The concentration of chlorophyll *a* during these maxima were similar to those found during previously observed Florida Bay *Synechococcus* blooms (22, 25, 33 µg L^{-1} for HF1, HF13, and WP96, respectively; 20-40 µg L^{-1}, Phlips et al. 1999); flow cytometry implicated *Synechococcus* as the blooming taxon (A. Corcoran et al. unpublished data). The trend observed in *Synechococcus* cell counts was closely correlated with measured chlorophyll *a* concentrations and supported the use of chlorophyll *a* as a proxy for cyanobacterial biomass; water column concentration maxima of *Synechococcus* cells and chlorophyll *a* occurred contemporaneously (October 7, 2013; 22-33 µg chlorophyll *a* L^{-1} and 16-21 x 10^6 cells mL^{-1} at impacted sites; A. Corcoran et al. unpublished data) and exhibited similar declining trajectories as they decreased to background concentrations which corresponded to measured values for both parameters at the controls site J01 (0.2-0.3 µg chlorophyll *a* L^{-1} and 10 x 10^4 cells mL^{-1}; A. Corcoran et al. unpublished data). Chlorophyll *a* was uniformly low at the control site (Site ID: J01), exhibiting concentrations less than 1 µg L^{-1} (Figure 6.4). The concentrations at J01 were likely below the limit of detection for the utilized method due to the low volume of sample filtered (125 mL), so the absolute concentrations measured near background levels should be viewed cautiously. Despite this analytical limitation, the chlorophyll *a* concentrations at the control site are representative of normative conditions in the oligotrophic waters of Florida Bay (e.g, Phlips et al. 1999, Armitage et al. 2011), and are in agreement with a long-term dataset collected at a nearby SERC water quality monitoring site (SERC ID: 28; SERC-FIU WQMN Program). Following peak conditions, concentrations at HF13 and WP96 quickly decreased to the background by late October 2013, whereas elevated chlorophyll *a* concentrations persisted at

HF1 until late November 2013 (Figure 6.4). "Bloom" and "post-bloom" conditions were defined for each site based on the measured chlorophyll *a* concentration; bloom conditions were defined as time during the sampled period where chlorophyll concentrations were elevated relative to the control site, and the bloom was considered to have left a site (i.e., post-bloom) when concentrations of chlorophyll returned to background levels for two consecutive samplings.

Water quality samples: DIN

The first water quality samples taken during the bloom (September 26, 2013) showed a range of initial responses to increased phytoplankton populations; water column DIN decreased at all sampled sites relative to pre-bloom conditions (Hoer 2015, this volume), yet the magnitude of the difference varied considerably. Using chlorophyll *a* concentrations as a proxy for phytoplankton biomass, sites with high concentrations of cyanbacteria (chlorophyll *a* >10 µg L^{-1}; HF3, HF5, HF9, HF10) exhibited water column DIN that ranged from values below the limit of detection (< 0.04 µmol N L^{-1}) to 0.1 µmol N L^{-1}. Interestingly, phytoplankton biomass at HF1 was considerably elevated during the first sampling (8 ± 1 µg L^{-1}; mean ± 1SE), yet the DIN concentration remained near 1 µmol N L^{-1}, the vast majority of which was as NH_4^+ (0.9 µmol NH_4^+ L^{-1}). Even moderately increasing phytoplankton biomass (1 – 6 µg L^{-1}) was observed to be coupled with decreasing DIN concentrations, and the magnitude of this decrease was largely proportional to the degree to which chlorophyll *a* was elevated.

Water column DIN decreased significantly as the bloom progressed, eventually dropping NH_4^+ and NO_x^- concentrations to levels well below 200 nmol L^{-1} at all bloom impacted sites (Figures 6.5, 6.6, and 6.7). Conversely, the control site exhibited no significant change in DIN over the sampled time period (Table 6.1; Figure 6.8). There was no significant difference

between collections performed at the surface and those at depth for any of the sampled dates or sites, and therefore data were averaged to yield single mean concentrations which were taken to be representative of the whole water column at each site. Further, no significant difference was observed between the two methods of collection (syringe versus 125 mL HDPE bottles; July 2014), nor was there a difference between the comparative samples collected in early July and when samples were collected 2 weeks later and shipped to NC on dry ice (Figure 6.9). Total DIN concentrations at impacted sites during bloom conditions were 0.34 ± 0.34, 0.55 ± 0.52, and 0.46 ± 0.11 μmol N L^{-1} for HF1, HF13, and WP96, respectively (mean ± 1SD; Table 6.1). Average bloom DIN concentrations at HF1 and HF13 were elevated by including samples collected at the very beginning of the bloom (September 26, 2013) which were taken when phytoplankton biomass was still expanding at both sites, as evidenced by chlorophyll *a* (Figures 6.5 and 6.6); excluding the collection from that date yields average bloom concentrations of 0.23 ± 0.22 and 0.25 ± 0.11 for HF1 and HF13, respectively (mean ± 1SD).

As the bloom conditions dissipated at impacted locations, total DIN concentrations increased nearly ten-fold (Figures 6.5, 6.6, and 6.7), but concentrations inside Mystery Basin remained slightly lower than those observed during pre-bloom collections (Site ID: HF1; Table 6.1; Figures 6.10, 6.11, and 6.12). The average total DIN concentration in Mystery Basin following the bloom (November 26, 2013 to March 4, 2015) was 1.1 ± 0.5 μmol N L^{-1} (mean ± 1SD; Table 6.1), which was significantly lower than the conditions during May 2013 collections performed at HF1 and nearby CTR (5.5 ± 0.2 and 4.8 ± 1.3 μmol N L^{-1}, respectively; Table 6.1; unpaired t-test, $p < 0.0001$). CTR is included for comparative purposes because pre-bloom sampling at HF1 was limited, the two sites are in close proximity to one another, and are very similar in terms of water depth and benthic cover. In Mystery Basin the average post-bloom

concentrations of total DIN and both measured DIN species (NO_x^-, NH_4^+) were significantly lower than the average of all values measured at sites within Mystery Basin during May 2013 (Table 6.1; Figures 6.10, 6.11, and 6.12; unpaired t-test; $p < 0.001$), and also significantly lower than the average NO_x from the August 2012 water quality survey (0.5 ± 0.2 µmol N L^{-1}; Table 6.1; Figure 6.11; unpaired t-test; $p < 0.05$). Conversely, there was no significant difference between the average post-bloom NH_4^+ and total DIN concentrations and those measured during the August 2012 water quality survey (Table 6.1; Figures 6.10 and 6.11). The substantial decrease in water column NO_x^- concentration inside Mystery Basin following the bloom is evidenced by the large decrease in the ratio of NO_x^-:NH_4^+; prior to the bloom, sites inside the basin had a NO_x^-:NH_4^+ ratio of 0.62 ± 0.34 (mean ± 1SD; n = 28; August 2012 and May 2013 collections) and this ratio fell to 0.17 ± 0.06 at HF1 following the bloom (mean ± 1SD; n = 13).

The total DIN concentrations measured inside Mystery Basin following the bloom were not significantly different than the median concentration observed in Rabbit Key Basin from 1991 to 2008 (SERC Site ID 18; 0.8 ± 0.4 µmol N L^{-1}; median ± 1 MAD; unpaired t-test; $p = 0.7$; SERC-FIU WQMN Program; Figure 6.10), nor did they differ from the observations made during the pre-bloom survey at sites HF10 and HF14 which were presumed to be characteristic of influent water from Rabbit Key Basin (Hoer 2015, this volume).

Water quality samples: DOC, TN, and DON

DOC, TN, and DON (DON = TN – DIN) also showed a marked response to bloom conditions, with peak concentrations occurring simultaneously with peak chlorophyll *a* (Figures 6.13, 6.14, and 6.15) followed by a rapid resumption of pre-bloom conditions following the decline of the bloom (Hoer 2015, this volume). DOC reached >1000 µmol C L^{-1} at impacted sites (Figures 6.13, 6.14, and 6.15) with mean concentrations under bloom conditions averaging

between 750 and 1000 µmol C L^{-1} (Table 6.1). Nitrogenous material (TN and DON) responded similarly, although the increase in TN during the bloom was relatively minor, yet post-bloom TN concentrations were significantly lower than pre-bloom values measured at all sites inside Mystery Basin (unpaired t-test; $p < 0.0001$; Table 6.1). The control site (J01) exhibited little change in DOC and TN over the measured period (Figure 6.16) and remained relatively low as compared to values measured at impacted sites (Table 6.1).

Post-bloom sponge survey

During sample collections shortly following the onset of bloom conditions (September 26, 2013), significant sponge mortality was observed at impacted sites both inside and outside Mystery Basin (N. Lindquist and D. Hoer, personal observations). Similar to pre-bloom quantifications, a weighted mean volumetric displacement was obtained for each site and species by weighting the biomass values measured by each survey technique (transects and quadrats) by the area surveyed using that methodology (12 m^2 for quadrats and 150 m^2 for transects at each assessed location; Hoer 2015, this volume). The average of the resultant weighted mean biomass for each species (L_{sponge} m^{-2}) was then calculated from all 15 surveyed sites and expanded to the full areal extent of Mystery Basin in order to generate an estimate of the surviving sponge community (Table 6.2). The error of the weighted mean was calculated by the method of Baker and Nissim (1963), and extrapolated to the scale of the basin using the method of C. Krebs (1999).

Based on these estimates, we calculated that total sponge biomass in Mystery Basin fell by approximately 99% as compared to pre-bloom populations (Table 6.2). Surveys conducted at 14 of the 15 chosen sites showed a drastic reduction in sponge biomass relative to communities observed prior to the bloom; sites which hosted as much as 3.1 ± 1.9 L_{sponge} m^{-2} (Hoer 2015, this

volume) had only 0.02 ± 0.01 L_{sponge} m^{-2} of surviving biomass following bloom dissipation and the remaining community was dominated by sparsely populated, small individuals. A total of 4 sponges that were large enough to be detected in the belt transects were found across all tested sites following the bloom (>10 cm in the largest dimension), yet at the same 15 sites, 1178 sponges which satisfied this criteria were observed prior to the bloom (Hoer 2015, this volume).

The species which were top contributors to the sponge community before the bloom (*S. vesparium, Ircinia variabilis, Geodia gibberosa, Cinachyrella* sp., and *Haliclona magnifica*) were largely absent from post-bloom surveys, and the remaining organisms were dominated by different species whose populations were only moderately impacted by elevated phytoplankton concentrations (*Cinachyrella* sp., *Chondrilla nucula*¸ *Haliclona* sp., *H. magnifica*, and *Aaptos lithophaga*; Table 6.2). With the exception of *A. lithophaga* that showed no significant change, all species showed a reduction in their calculated biomass, and these reductions ranged from 45 to 100% loss of the pre-bloom population (Table 6.2; Wilcoxon signed-rank test; $p < 0.001$). The dominant 5 surviving species represented almost 99% of the calculated total biomass, and *Cinachyrella* sp. alone represented nearly 87% (Table 6.2). Sites outside Mystery Basin exhibited similar sponge mortality, yet the magnitude of community destruction is unknown due to a lack of pre-bloom quantifications; subjective assessments at these sites were obtained during the bloom by tagging individuals which were presumed to represent dominant taxa at these sites (*S. vesparium* and *I. variabilis*; HF13 and WP96), and subsequently monitoring the tagged individuals during recurring water quality sampling; all tagged individuals died and decayed prior to decline of bloom conditions. Prior to the bloom, sponge taxa presumed to have dense microbial consortia living within their tissues (high microbial abundance or HMA *sensu* Hentschel et al. 2006) represented approximately 95% of the estimated sponge biomass whereas

species with tissue microbes at approximately seawater concentrations (low microbial abundance LMA; Hentschel et al. 2006) and those with unknown microbial density represented 2 and 3% respectively (Table 6.2). Conversely, the sponge community was dominated by LMA species (94% of the estimated biomass) following the bloom, with minor contributions from HMA species and those with unknown microbial density (4 and 2%, respectively; Table 6.2). Approximately 96% of the biomass lost to bloom-mediated sponge mortality was estimated to be HMA species (Table 6.2).

In contrast to the sponge community, macrophyte biomass showed a minimal response to bloom conditions. As with surveys conducted prior to the bloom, post-bloom Braun-Blanquet cover assessment data were used to calculate species density (D_i; Fourqurean et al. 2001) for comparative assessment to pre-bloom values. Seagrasses were found at all 15 surveyed sites ($D_i \geq 0.1$; Fourqurean et al. 2001) with *Thalassia testudinum* dominating discovered biomass at all tested locations. As in pre-bloom surveys, only two other species were found in any abundance (*Halodule wrightii* and *Syringodium filforme*); *Halodule* was found at 66% of the surveyed sites, whereas *Syringodium* was observed in several quadrats, yet had a calculated density which never exceeded the threshold for determining the presence or absence of seagrasses ($D_i \geq 0.1$; Fourqurean et al. 2001). For each of the post-bloom surveyed sites, the cumulative seagrass density was not significantly different than the surveys conducted prior to the bloom (paired t-test; $p > 0.6$). Similarly, macroalagal biomass showed no significant change between pre and post-bloom surveys (paired t-test; $p > 0.5$), and exhibited a continued dominance of calcareous green, primarily *Halimeda* sp. and *Penicillus* sp., and non-calcareous red algae, largely *Laurencia* sp.

Post-bloom N sourcing from sponges

Rates of DIN release were previously quantified for 4 of the 10 sponge taxa found in post-bloom surveys, representing approximately 95% of the surveyed biomass (*Cinachyrella* sp., *Haliclona* sp., *C. nucula*¸ and *H. melanodocia*; Hoer 2015, this volume); the species without quantified DIN release were excluded from the calculation of N sourcing. The surviving sponge population contributed 4 ± 1 µmol N m^{-2} day^{-1} (mean ± 1SE; Figure 6.17), which represents a 99% reduction from the 590 ± 280 µmol N m^{-2} day^{-1} contributed by the pre-bloom population (mean ± 1SE; Hoer 2015, this volume). A minimal change to the calculated N contribution from the surviving sponge population (5 ± 1 µmol N m^{-2} day^{-1}; mean ± 1SE) occurs if we assume the unquantified 5% of surveyed biomass released DIN at a rate equal to the highest quantified rate for a survivor taxon (*Haliclona* sp.; 75 ± 7 µmol N L_{sponge}^{-1} hr^{-1}; Hoer 2015, this volume). Assessing the areal flux from the surviving community (4 ± 1 µmol N m^{-2} day^{-1}) over the calculated area of Mystery Basin yielded 20 ± 6 mol N day^{-1} (mean ± 1SE) as a conservative estimate of the total N contributed by the post-bloom sponge population.

Discussion

Significant sponge biomass and water column chemistry changes were documented during and following a phytoplankton bloom in Mystery Basin. As planktonic biomass increased, water column N content decreased to near trace concentrations at impacted sites and slowly recovered over approximately 4 months following dissipation of the blooming cyanobacteria (Figures 6.5, 6.6, and 6.7). The apparent introduction of bloom conditions from the north-northeast (Figure 6.3) corresponds with previous observations suggesting that blooms in the west central region of Florida Bay are typically sourced from north-central locations and are often coincident with a seasonal shift in the dominant wind direction (Phlips et al. 1999).

Near total mortality of sponge biomass was observed in Mystery Basin and surrounding sites during surveys conducted following the dissipation of bloom conditions (Table 6.2). Mass loss of multiple sponge species has been previously reported following cyanobacterial blooms in Florida Bay (e.g., Butler et al. 1995, Lynch and Phlips 2000, Wall et al. 2012) however, our extensive pre-bloom biomass surveys of the native sponge population created a unique opportunity to directly observe the community transition after the bloom had receded. HMA species appeared to be more severely impacted by elevated cyanobacterial populations than LMA sponges (Table 6.2). Yet, neither the underlying mechanism that led to the observed mass sponge mortality nor the source of the differential response between HMA and LMA species is definitively known. A prevailing hypothesis based on previous studies is that the exceptionally high concentration of cells in the water column during blooms leads to mechanical blockage of sponge aquiferous canals, which in turn quickly contributes to a cessation in pumping and eventually leads to tissue necrosis or organismal death (Butler et al. 1995, Lynch and Phlips 2000, Wall et al. 2012). Observed bloom conditions were characterized by exceedingly dense *Synechococcus* cell populations (16-21 x 10^6 cells mL^{-1} at impacted sites; A. Corcoran et al. unpublished data) which vastly increased particulate loading and potentially mediated clogging and mortality of the sponge population; cell concentrations in Mystery Basin were more than triple those observed during previous blooms (5 x 10^6 cells mL^{-1}; Phlips et al. 1999) and those tested during laboratory experiments (Phlips and Lynch 2000). The hypothesized clogging of pumping structures within the sponge may be supported by observations of a shift in the *Synechococcus* community during cyanobacterial bloom development towards chain-forming organisms whose cells are coated in mucilage (Berry et al. 2015). The extracellular polysaccharide coating increases cellular stickiness, which may increase the ability for these

organisms to adhere to the flagellated choanocyte cells within the organism and consequently slow or halt filtration. Physical obstruction may be enhanced in HMA species as they possess denser, more complex aquiferous structures than their LMA counterparts (Weisz et al. 2008), and this increased surface area to volume ratio of internal structures may raise their susceptibility to clogging. Conversely, no significant change was observed in macrophyte biomass following dissipation of blooming cyanobacteria. The brevity of the bloom (~2 month duration, Figure 6.4) likely reduced the long-term impacts on seagrasses which are generally thought be associated with harmful algal blooms (Phlips and Badylak 1996, Phlips et al. 1999).

In addition to the near complete eradication of sponge biomass, drastic changes in water quality occurred during and following the bloom. Dissolved organic matter (DOC and DON) concentrations showed a marked response to bloom conditions, with peak concentrations for both correlated with the observed chlorophyll a maxima followed by rapid restoration of pre-bloom conditions that followed the reduction in cyanobacterial abundance (Figures 6.13, 6.14, and 6.15). Rapid DOM production is expected from active and abundant phytoplankton populations (e.g., Baines and Pace 1991, Biddanda and Benner 1997, Engel et al. 2011). The organic matter in the collected samples displayed a relatively high C:N ratio (DOC:DON >15) during peak bloom conditions indicative of increased rates of N-poor polysaccharide release as available N became depleted (e.g., Norrman et al. 1995, Biersmith and Benner 1998, Engel et al. 2011). Physical export, phytoplankton utilization, and bacterial degradation most likely dominated the removal of the produced DOM from the sampled region during the bloom and as it waned (Carlson 2002). Assessment of the relative importance of each process was complicated by the fact that the time between collections was typically greater than the water residence time in Mystery Basin. However, on timescales of 4 to 7 days, bacterial

remineralization and direct phytoplankton utilization of the labile fraction of the produced DOM may have been significant during the bloom with physical export dominating following bloom cessation (Norrman et al. 1995, Zubkov et al. 2003, Weinbauer et al. 2011).

Water column DIN at HF1 recovered to the pre-bloom conditions measured in August 2012 approximately 4 months after phytoplankton biomass returned to background concentrations (Figure 6.5). There was a large difference between the post-bloom concentrations of all DIN species and those measured during May 2013 (Figures 6.10, 6.11, and 6.12), but this large N reduction was not observed in comparative assessments with August 2012 values (Table 6.2, Figures 6.10, 6.11, and 6.12). The diminished effect on the overall concentration of DIN was unexpected, given the large contribution to the water column DIN reservoir from the sponge community estimated in an N budget (45 ± 24 %; Hoer 2015, this volume). There was only a small difference in the NO_x^- concentrations from August 2012 and those following the bloom (Table 6.2, Figure 6.11) that we could hypothesize to be due to the loss in sponge biomass. However, we only have limited pre-bloom DIN data with which to compare. The destruction of the pre-bloom sponge community should be associated with a consequent loss of a large source of NO_x^- contributed by these organisms; 60% of the total, pre-bloom DIN contributed by sponges was as NO_x^- while the surviving community contributed less than 1% of this quantity (Figure 6.17; Hoer 2015, this volume). The absence of a strong seasonal N signal in the post-bloom dataset (specifically, a dry-season elevation in water column N loading) may also be indicative of the missing DIN from sponge recycling processes, however, the lack of a long pre-bloom time series of nutrient concentrations precludes this from being asserted definitively. The highly elevated DIN concentrations observed in May 2013 were associated with increased water retention during the dry season, a factor that was thought to enhance the visibility and thus the

spatial heterogeneity of N sources and sinks (Hoer 2015, this volume). Minimally, the reduced sponge presence has significantly reduced the heterotrophic pressure exerted by the pre-bloom community (Peterson et al. 2006) and increased the filtration time for Mystery Basin (time for a modeled parcel of water to be filtered by a member of the sponge community) from 8 hrs to 36 days (N. Lindquist unpublished data). The reduced grazing (filtration) potential of the surviving sponge community may increase the susceptibility of this environment to future cyanobacterial blooms or allow for the presence of an elevated standing crop of phytoplankton biomass (Peterson et al. 2006).

The return to background phytoplankton concentrations in Mystery Basin lagged that observed at other sites which experienced bloom conditions (HF13, WP96; Figure 6.4), and this retained cyanobacterial population also delayed water column DIN recovery to higher normal concentrations at HF1 (Figure 6.4). The restricted physical exchange observed at this site could have been responsible for the retention of bloom conditions within Mystery Basin; reduced rates of water turnover have been proposed as a mechanism for accumulating elevated standing cyanobacterial populations and enhancing bloom susceptibility (Phlips et al. 1999, Cloern 2001).

The high intensity of the retained bloom at HF1 (>8 µg L^{-1}; Figure 6.4) raises questions regarding the sustaining source of N and P concentrations. We propose that the accelerated resupply of nutrient elements derived from the large quantity of decaying sponge material in Mystery Basin served as an additional mechanism that enhanced the retention of high density bloom conditions at HF1 relative to the other tested sites (Figure 6.4). Sponges were destroyed at both sampled sites outside Mystery Basin, yet at HF1 the entirety of the decay process was spatially restricted due to the inability to export the rotting sponge tissue over the shoaling perimeter of the basin. An extreme stench associated with rotting animal tissue was easily

detected during the initial month of the bloom and was noted by FWC researchers that collected water quality samples (W. Sharp, G. Delgado, personal communication). The decay and remineralization of rotting sponge biomass was likely focused within Mystery Basin because other, less enclosed sites lacked the physical isolation and therefore presented reduced barriers to physical export. Based on tagged individuals at the sampling sites, it took less than 3 weeks from initial physiological deterioration to full organismal death and decay to a spicule skeleton (W. Sharp personal communication) therefore we will assume that nutrients sourced from decaying sponge biomass were injected slowly over the entire decay period rather than as a sudden pulse of material. We hypothesize that the moderately elevated ammonium concentrations observed at HF1 shortly after bloom conditions appeared in Mystery Basin (September 26, 2013; approximately 0.9 μmol NH_4^+ L^{-1}) were the result of remineralized nutrients from dead sponge biomass which began to decay prior to the onset of peak bloom conditions. Additionally, a visible peak in DOM observed at HF1 on October 30, 2013 (Figure 6.13) may be due to organic matter efflux from decaying sponge biomass, which fits within the assumed timeline for sponge death and decay as a result of the phytoplankton bloom. This DOM peak occurs in the absence of contemporaneously elevated chlorophyll *a* thus removing increased primary productivity as a potentially causative agent. Similar DOM elevation is observed at HF13 (Figure 6.14) and to a lesser degree at WP96 (Figure 6.15), perhaps resulting from locally important degradation by-products (both sponge and dead *Synechococcus* biomass) or due to DOM export from Mystery Basin (physical transport generally runs north to south; Cosby et al. 2005). The proximity of HF13 to Mystery Basin made physical export a particularly important factor to consider as the DOM signature is significantly different at this location as

compared to WP96 despite the apparent similarity in photosynthetic biomass (Figures 6.14 and 6.15).

In order to determine the potential for nutrient element release through decaying sponge biomass, the N content of the two most abundant species prior to the bloom (*S. vesparium* and *I. variabilis*; Table 6.2) was calculated using species-specific sponge densities as well as tissue N content (4.7 and 7.5% N for *S. vesparium* and *I. variabilis*, respectively; Martens et al. unpublished data). The eradication of these two species represented 82% of the total sponge biomass lost during the die-off (Table 6.2). The decomposition of *S. vesparium* contributed approximately 150 ± 14 µmol N L^{-1} while *I. variabilis* liberated 68 ± 7 µmol N L^{-1} (mean ± 1SE). Assuming this rate of release is conserved for the remaining 18% of biomass lost, the estimated total N introduction from decaying sponge biomass is 260 ± 20 µmol N L^{-1} (mean ± 1SE) distributed across the 3 week decay period (approximately 12 ± 1 µmol N L^{-1} day^{-1} if assumed to be released evenly over time). The decay of sponge tissue may also represent an important source of P to blooming cyanobacteria. Sponges have been observed to retain particularly large quantities of P from filtered water, specifically HMA sponges whose microbial consortia have been shown to sequester P as polyphosphate (Zhang et al. 2015). Similar to N release, potential P liberation through sponge decay was calculated using the proportion of P in HMA sponge tissue (~0.32 ± 0.03% dry weight; Zhang et al. 2015) and the dry weight of destroyed HMA sponge biomass estimated using the densities of representative species (*S. vesparium* and *I. variabilis*; Table 6.2). The decaying HMA tissue was estimated to produce approximately 7.1 ± 0.1 µmol P L^{-1} (mean ± 1SE; 0.34 ± 0.01 µmol P L^{-1} day^{-1}). At locations where the sponge population was most dense (~4 L_{sponge} m^{-2}; Hoer 2015, this volume), these

organisms may be able to provide a similar source of regenerated N and P as that expected from decaying fish biomass during dinoflagellate blooms (Killberg-Threson et al. 2014).

The estimated values of nutrient resupply from remineralized sponge tissue were compared to estimated nutrient demand from the blooming cyanobacterial community. These were approximated using the cellular N and P quota for *Synechococcus* (20-50 fg N $cell^{-1}$ and 0.5 – 3.3 fg P $cell^{-1}$; Bertilsson et al. 2011) and the concentration of these cells in the water column during peak bloom and background conditions (approximately 16 x 10^6 and 10 x 10^4 cells mL^{-1}, respectively; A. Corcoran et al. unpublished data). The estimated N and P required to generate the observed increase in cell concentration during peak bloom conditions (23-57 µmol N L^{-1} and 0.3 – 1.7 µmol P L^{-1}) greatly exceeded the potential supply from measured pre-bloom DIN, yet is approximately equal to the total measured N reservoir during pre-bloom collections (May 2013; Table 6.1). Despite the demonstrated ability of *Synechococcus* to uptake organic nitrogen to satisfy cellular N requirements (Zubkov et al. 2003), it seems unlikely that the entirety of water column organic N in this environment was available to these organisms, given the range of compounds represented by the DOM pool. Limiting conditions were expected for total P availability (e.g., Boyer et al. 2006 and citations therein), but the lack of direct measurements of local P concentrations precluded any definitive statement regarding the pre-bloom availability of this nutrient. Assuming the cyanobacterial population that was retained beyond what was observed at HF13 and WP96 renewed the entirety of its biomass daily, it would require a maximum uptake rate of 0.9 to 13 µmol N L^{-1} day^{-1} and 0.01 to 0.99 µmol P L^{-1} day^{-1}. The nutrient demand associated with these retained populations is well within the quantity of nutrients estimated to be resupplied through sponge biomass decay (12 ± 1 µmol N L^{-1} day^{-1} and 0.34 ± 0.01 µmol P L^{-1} day^{-1}).

We hypothesize that the remineralized nutrients from decaying sponge tissue at HF1 would be rapidly incorporated into blooming biomass as a means for the cyanobacteria to fill the apparent gap between water column availability and their estimated nutrient demand. The presumed time-dependent release of this material would have created a long-term supply of N and P that could have served as a mechanism for maintaining necessary nutrient concentrations to support residual bloom conditions observed within Mystery Basin. However, the available data do not allow us to distinguish the recycled nutrients resulting from sponge decay versus those sourced from internal nutrient recycling mediated by remineralization of dead *Synechococcus* biomass as well as viral lysis of heterotrophic bacteria within the bloom (Weinbauer et al. 2011). While these processes were likely occurring simultaneously throughout the duration of the bloom, the latter two would be expected to occur at sufficiently rapid rates within the water column to be similarly effective agents of nutrient regeneration (in both organic and inorganic forms) in regions of open exchange as in the shoal-attenuated water column of a basin (Norrman et al. 1995, Zubkov et al. 2003, Weinbauer et al. 2011). Conversely, sponge tissue is likely less bioavailable to the bloom-stimulated heterotrophic bacterial community on short timescales, and therefore, nutrient resupply from decomposing sponge biomass would be expected to be drastically enhanced as a result of the shoal-attenuated exchange present in Mystery Basin.

In order to assess the possibility that simple physical restriction can maintain high intensity bloom conditions in this region of Florida Bay, four sites were selected within the SERC water quality monitoring network and examined to determine how rapidly normative chlorophyll *a* concentrations reestablished following a high magnitude bloom event. These events were identified during the 1991 to 2008 data records as times when the measured

chlorophyll *a* concentration was greater than 8 µg L^{-1}, which represents the concentration that was measured during times of active bloom in Mystery Basin (Figure 6.4). SERC stations 18, 19, 20, and 28 (Rabbit Key Basin, Twin Key Basin, Peterson Keys, and Old Dan Bank, respectively; SERC-FIU WQMN Program) were selected because these had previously-modeled water residence times (τ) which was approximately similar to that measured for Mystery Basin (median $\tau < 10$ days; Cosby et al. 2005) and were all within a region of Florida Bay which had approximately similar environmental conditions for planktonic biomass (south-central; Phlips et al. 1995). Restricting the assessed sites to a zone of similar influence was important in order to analyze sites where phytoplankton biomass was subject to approximately similar environmental conditions as those that were experienced during the bloom in Mystery Basin (Phlips et al. 1999). In order to reflect bloom conditions with nutrient demand roughly equivalent to those observed at the tested site, bloom duration at each location was determined as the interval between the time when chlorophyll *a* first rose above 8 µg L^{-1} until it fell below the threshold value; the start and end dates for the noted blooms are subject to uncertainty given the monthly sample collection interval at these sites (SERC-FIU WQMN Program). There are abundant examples of long-term blooms in Florida Bay (>6 month prior to resumption of oligotrophic conditions; e.g., Butler et al. 1995, Phlips et al. 1999), however, we chose to focus on the most intense bloom events in this region as these would create the highest demand for water column nutrients and may not be sustainable by internal nutrient recycling alone. Neither the Peterson Keys nor Old Dan Bank locations (Site IDs: 20 and 28, respectively) exhibited any bloom events which exceeded the threshold value while both Rabbit Key Basin and Twin Key Basin (Site IDs 18 and 19, respectively; directly north and east of Mystery Basin) had two events which exceeded the 8 µg L^{-1} threshold and lasted between 20 and 38 days (SERC-FIU WQMN

Program). Calculated identically to those from the SERC sites, the bloom durations at HF1, HF13, and WP96 were 50, 23, and 14 days, respectively (Figure 6.4). Therefore, historical analysis for basins near Mystery Basin supports the hypothesis that simply increasing site residence time relative to a freely exchanged water column may increase planktonic standing stock and susceptibility to blooms (Phlips et al. 1999), but it is insufficient to produce blooms which match the intensity or duration of that within Mystery Basin.

A comparison of the analyzed SERC sites to those surveyed by Peterson and co-workers (2006) during a bay-wide assessment of sponge biomass allowed the relative sponge cover to be determined for each water quality monitoring location from the nearest sites with a surveyed sponge community. When determining the nearest quantified sites, care was taken to select those within the same basin as the water quality station (i.e., Rabbit Key Basin and Twin Key Basin). The sites within Rabbit Key Basin had relatively low sponge biomass (5 surveyed sites; median: 5 g m^{-2}; maximum: 41 g m^{-2}), and sites in twin Key Basin had somewhat higher sponge cover (8 surveyed sites; median: 25 g m^{-2}; maximum: 88 g m^{-2}). The approximate sponge biomass at these sites was much lower than what was observed in Mystery Basin (assuming density of the dominant species *S. vesparium*; 59 surveyed sites; median: 61 g m^{-2}; maximum: 740 g m^{-2}). Sponge mortality at either of these sites would be expected to yield significantly lower quantities of reminieralized nutrients due to reduced sponge cover, and may have had led to the observed difference in retention times for high-intensity blooms. Undoubtedly the success of the observed *Synechococcus* bloom in Mystery Basin was due to a plurality of factors. However, we hypothesize that the sustained intensity of the bloom was due in part to the nutrient amendment supplied by decaying sponge biomass, and that this source of remineralized nutrients

may have an important role in controlling or enhancing phytoplankton blooms in sponge-rich environments.

The changes observed in the Mystery Basin and surrounding waters provided important information about how sponge-rich areas within Florida Bay may respond to and possibly modulate recurring bloom phenomena. Significant impacts were observed in both water column DIN and the sponge population of Mystery Basin during and following the observed bloom conditions, but there was minimal discernable evidence for a reduced source of DIN from the sponge community. However, we believe that the stored nutrients released from the tissues of the decaying sponges contributed significantly to the intensity and longevity of the 2013-14 bloom. Sponge mortality may not only increase the susceptibility of an impacted environment to future blooms through lost top-down pressure, but also contribute to the intensity or longevity of the bloom that occurs simultaneously with the die-off.

Table 6.1: Summarized DIN concentrations from various sites within and surrounding Mystery Basin (Figure 6.1) prior to, during, and following the bloom. Both C and N species (NO_x^-, NH_4^+, total DIN, and TN) are in µmol L^{-1} and represent the mean ± 1SD. † indicates data from Hoer (2015) and ‡ indicates data from Hoer (unpublished).

Date	**Sites (n)**	**NO_x^-**	**NH_4^+**	**DIN**	**DOC**	**TN**	**Pre/During/Post Bloom**
August 2012	21	0.5 ± 0.2†	1.0 ± 0.4†	1.5 ± 0.5†			Pre-Bloom
May 2013	CTR (SEASII; n ≈ 7300)	3.0 ± 0.5†					Pre-Bloom
May 2013	CTR (SEASII; n ≈ 6100)	3.8 ± 0.7†					Pre-Bloom
May 2013	CTR (n = 16)	2.6 ± 0.9†	2.2 ± 0.9†	4.8 ± 1.3†	540 ± 50‡	54 ± 8†	Pre-Bloom; Inside MB*
May 2013	7	1.1 ± 0.7†	2.2 ± 1.0†	3.3 ± 1.5†	610 ± 70‡	62 ± 14†	Pre-Bloom; Inside MB*
May 2013	7	0.6 ± 0.4†	1.5 ± 0.6†	2.0 ± 0.9†	570 ± 60‡	45 ± 2†	Pre-Bloom; Outside MB*
Nov. 2013 – Mar. 2015	13	0.17 ± 0.09	0.96 ± 0.39	1.1 ± 0.5	470 ± 140	34 ± 9	Post-Bloom; HF1
Oct. 2013 – Mar. 2015	17	0.17 ± 0.09	1.0 ± 0.4	1.2 ± 0.5	410 ± 170	31 ± 11	Post-Bloom; HF13
Oct. 2013 – Mar. 2015	17	0.33 ± 0.20	0.95 ± 0.31	1.3 ± 0.4	360 ± 190	29 ± 12	Post-Bloom; WP96
Oct. 2013 – Mar. 2015	19	0.28 ± 0.23	0.58 ± 0.46	1.2 ± 0.48	190 ± 130	15 ± 10	Control; J01
Sep. 2013 – Nov. 2013	7	0.09 ± 0.04	0.24 ± 0.33	0.34 ± 0.34	750 ± 380	47 ± 19	Bloom; HF1
Sep. 2013 – Oct. 2013	3	0.13 ± 0.04	0.42 ± 0.53	0.55 ± 0.52	890 ± 240	59 ± 5	Bloom; HF13
Oct. 2013	2	0.28 ± 0.06	0.18 ± 0.09	0.46 ± 0.11	1000 ± 220	59 ± 10	Bloom; WP96

*MB = Mystery Basin

Table 6.2: Pre and post-bloom weighted mean sponge biomass across both hardbottom and seagrass habitats in Mystery Basin (10^3 L_{sponge}) with their associated microbial abundance (HMA versus LMA, *sensu* Henstschel et al. 2006). Pre-bloom values are from Hoer (2015, this volume). † indicates species which have calculated N flux (Hoer 2015, this volume) and ‡ indicate species which were too small to be measured in transects and these data were excluded from their weighted mean. Italicized entries for microbial abundance indicate a genus level distinction and the superscripted letter indicates the literature source. [a] Gloeckner et al. 2014; [b] Hoffmann et al. 2009; [c] Sipkema et al. 2009; [d] Weisz et al. 2008; [e] Ereskovsky et al. 2005; [f] Ereskovsky et al. 2004; [g] Hill et al. 2006; [h] Reiswig 1974.

Species	**Microbial Abundance**	**Weighted Mean Biomass (Pre-Bloom; 10^3 L_{sponge})**	**Weighted Mean Biomass (Post-Bloom; 10^3 L_{sponge})**	**Percent Decline**
S. vesparium †	HMA[a]	1900 ± 380	0	100%
I. variabilis †	*HMA*[a]	930 ± 210	0	100%
G. gibberosa †	*HMA*[b]	480 ± 120	0	100%
Cinachyrella sp. †‡	*LMA*[a]	58 ± 0.40	32 ± 1.0	45%
Haliclona magnifica	*LMA*[c]	33 ± 7.8	0.88 ± 0.39	97%
Halichondria melanodocia †	LMA[d]	29 ± 7.4	0.22 ± 0.20	99%
Unidentified sponges	Unknown	28 ± 12	0.21 ± 0.13	99%
Spongia sp. †	*HMA*[e]	12 ± 3.4	0	100%
Dysidea etheria	*LMA*[a]	12 ± 3.0	0.03 ± 0.03	99%
Tedania ignis	LMA[a]	9.7 ± 2.4	0	100%
Hippospongia lachne †	HMA[f]	7.2 ± 2.3	0	100%
Lissodendoryx stigmata	Unknown	5.5 ± 1.4	0.03 ± 0.03	99%
C. nucula †‡	HMA[g]	5.1 ± 0.1	1.52 ± 0.07	70%
Amphimedon viridis‡	*LMA*[a]	4.3 ± 0.21	0	100%
Haliclona sp. †	*LMA*[c]	4.2 ± 0.92	1.45 ± 0.57	65%
Hyritios sp. ‡	Unknown	2.2 ± 0.04	0	100%
I. strobilina	*HMA*[a]	1.5 ± 0.76	0	100%
Tectitethya crypta	LMA[h]	1.4 ± 0.66	0	100%

Species	**Microbial Abundance**	**Weighted Mean Biomass (Pre-Bloom; 10^3 L_{sponge})**	**Weighted Mean Biomass (Post-Bloom; 10^3 L_{sponge})**	**Percent Decline**
Callyspongia sp.	*LMA*[a]	0.83 ± 0.34	0.04 ± 0.02	95%
I. campana†	*HMA*[a]	0.65 ± 0.65	0	100%
Aaptos lithophaga	Unknown	0.56 ± 0.42	*0.59 ± 0.59*	*+5%*
Ircinia sp.	*HMA*[a]	0.43 ± 0.33	0	100%
Total biomass		**3500 ± 450**	**37 ± 1.4**	**99%**

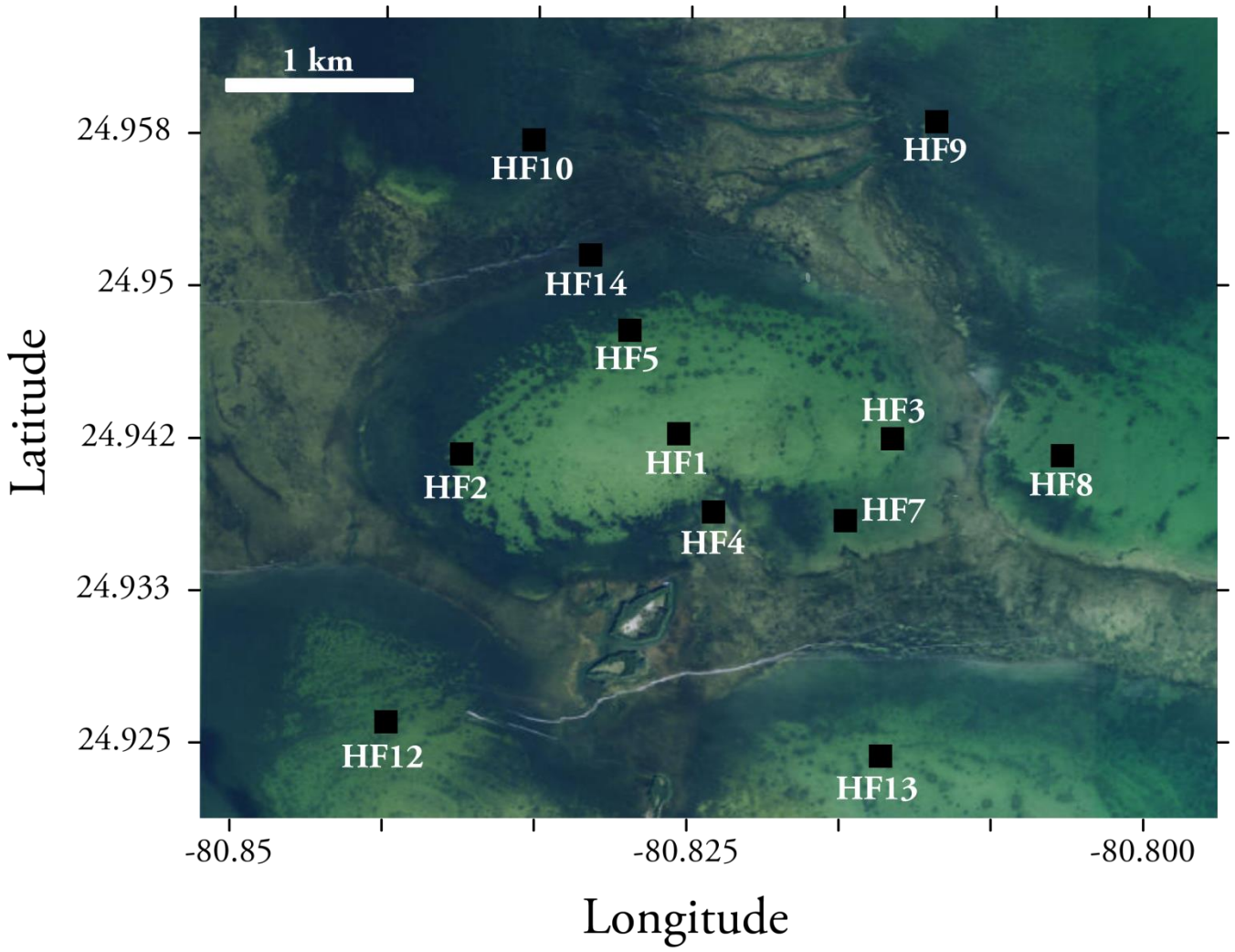

Figure 6.1: ArcMap image of Mystery Basin showing the location of water quality collections that were performed immediately following the discovery of bloom conditions at this location (September 26, 2013).

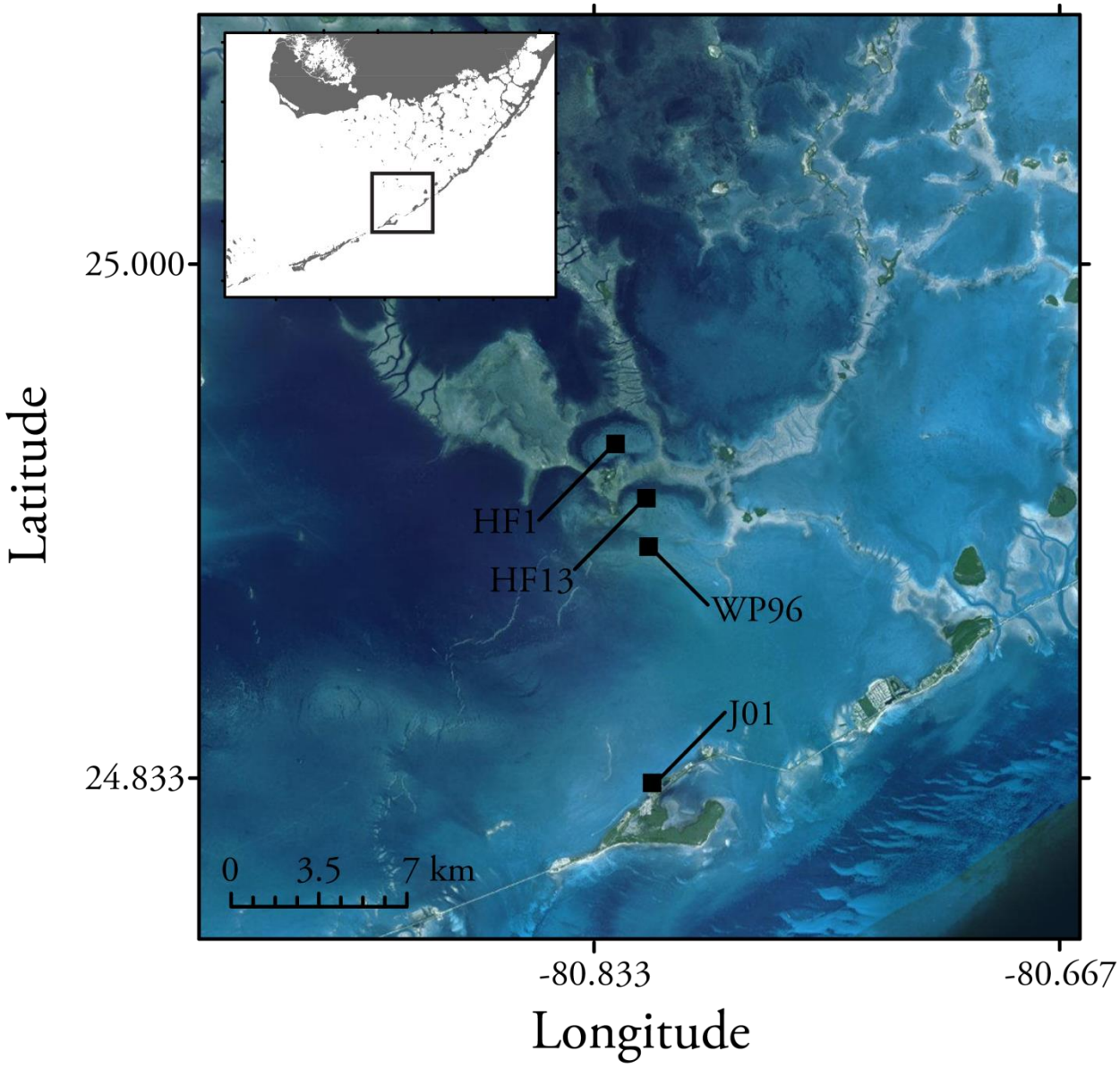

Figure 6.2: ArcMap of recurring water quality samples. HF1 represents the center of the target basin and J01 represented the control site just north of Long Key, Florida. The inset shows the position of the target area relative to broader Florida Bay.

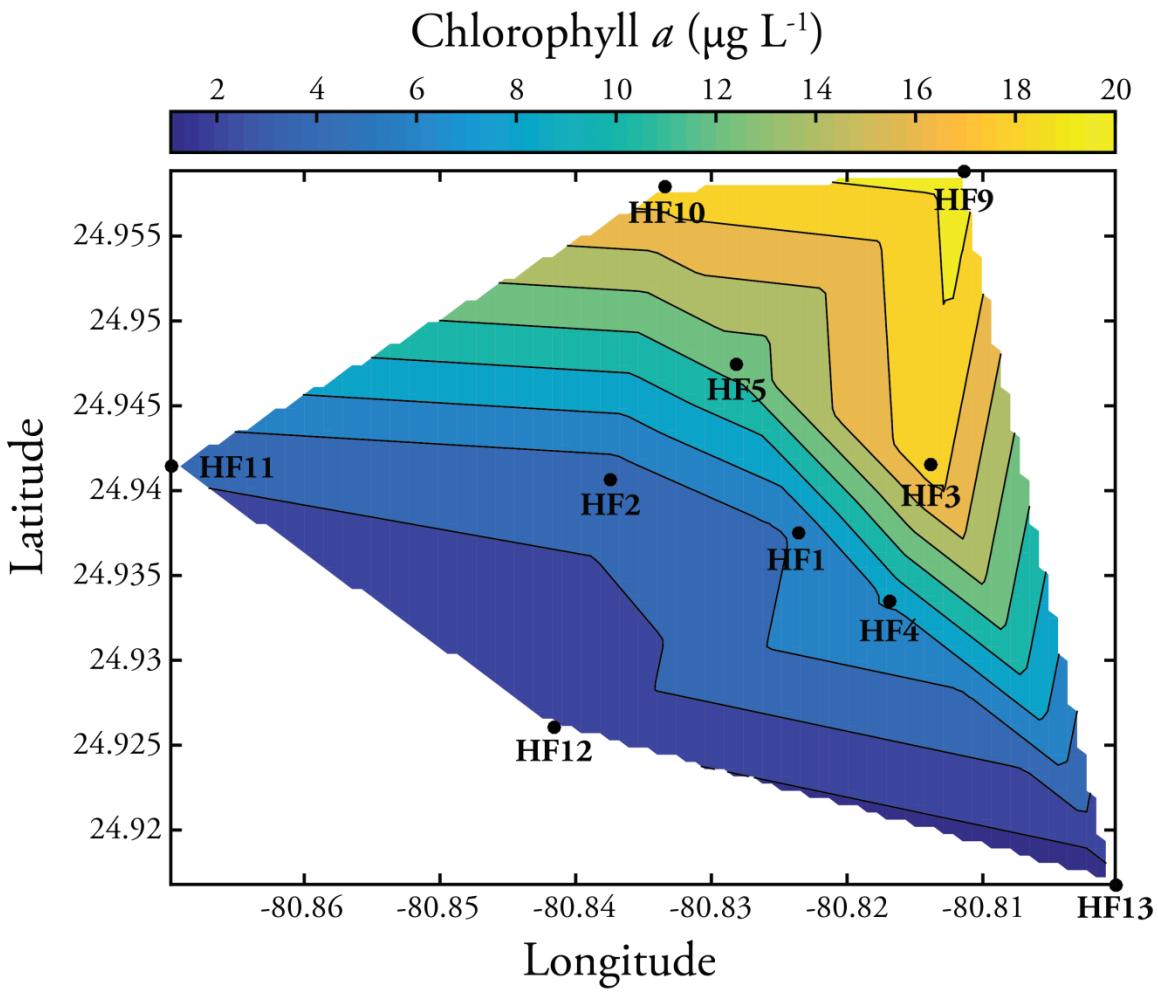

Figure 6.3: Contour plot of September 26, 2013 chlorophyll *a* concentration at points inside and outside of Mystery Basin. Sites HF1, HF2, HF3, HF4, and HF5 are all within the boundaries of Mystery Basin.

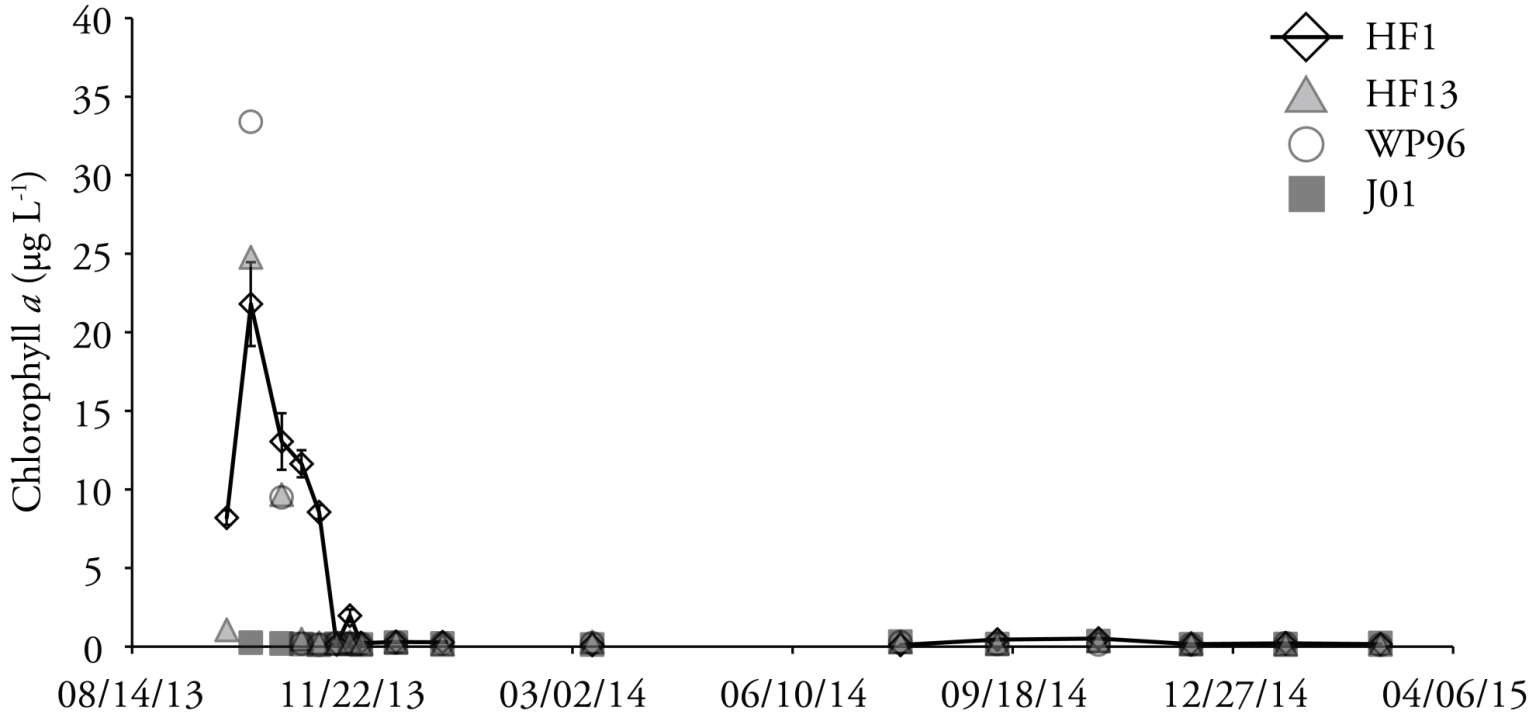

Figure 6.4: Mean chlorophyll *a* concentrations at all sampled sites from September 26, 2013 to March 4, 2015 (see Figure 6.2 for relative site locations). Error bars indicate ± 1SE.

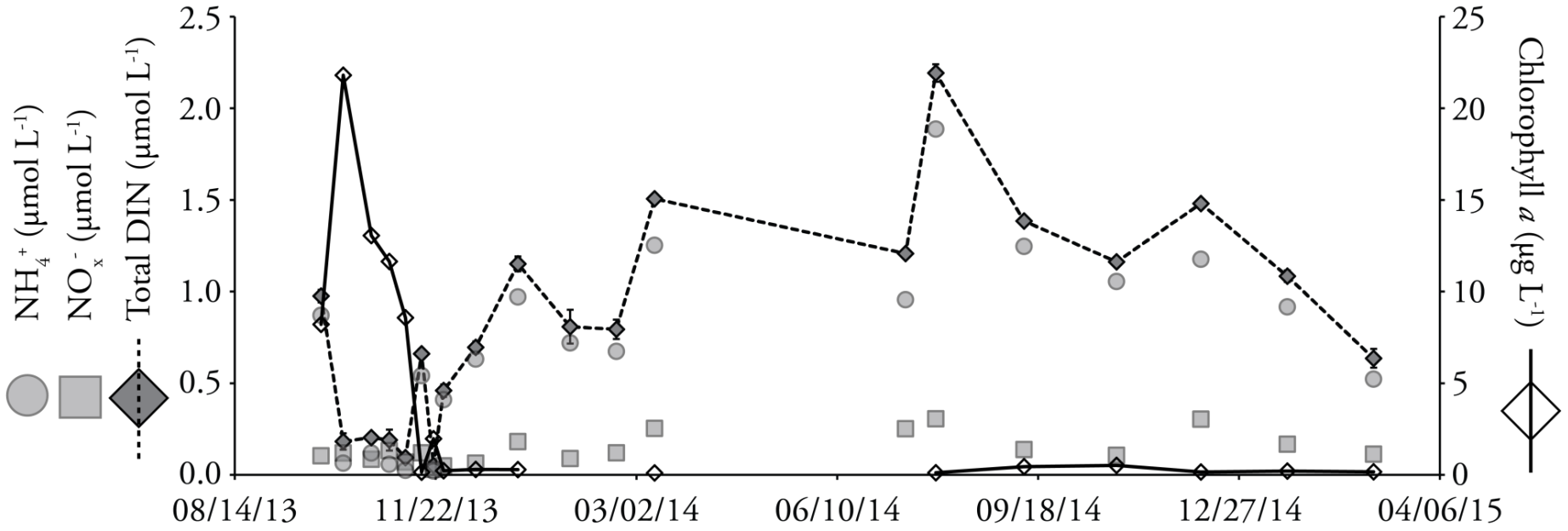

Figure 6.5: Mean DIN (NO_x^- + NH_4^+), NO_x^-, NH_4^+, and chlorophyll *a* concentrations for HF1 from September 26, 2013 to March 4, 2015 (see Figure 6.2 for relative site locations). Error bars are ± 1 SE.

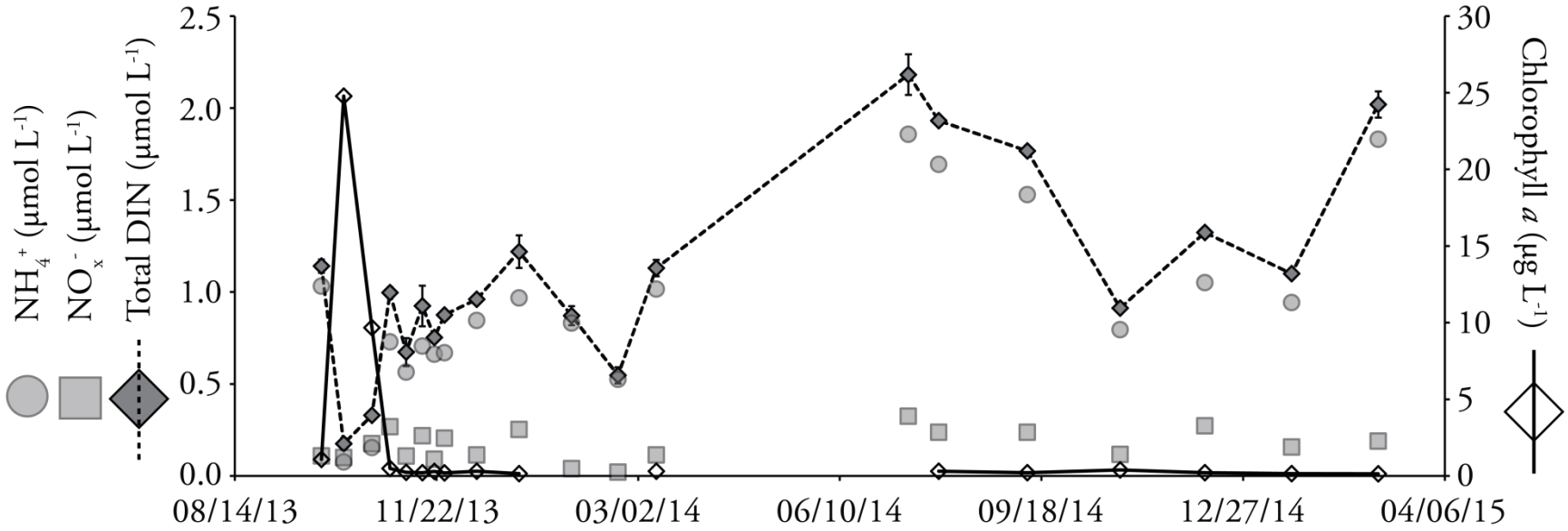

Figure 6.6: Mean DIN (NO_x^- + NH_4^+), NO_x^-, NH_4^+, and chlorophyll *a* concentrations for HF13 from September 26, 2013 to March 4, 2015 (see Figure 6.2 for relative site locations). Error bars are ± 1 SE.

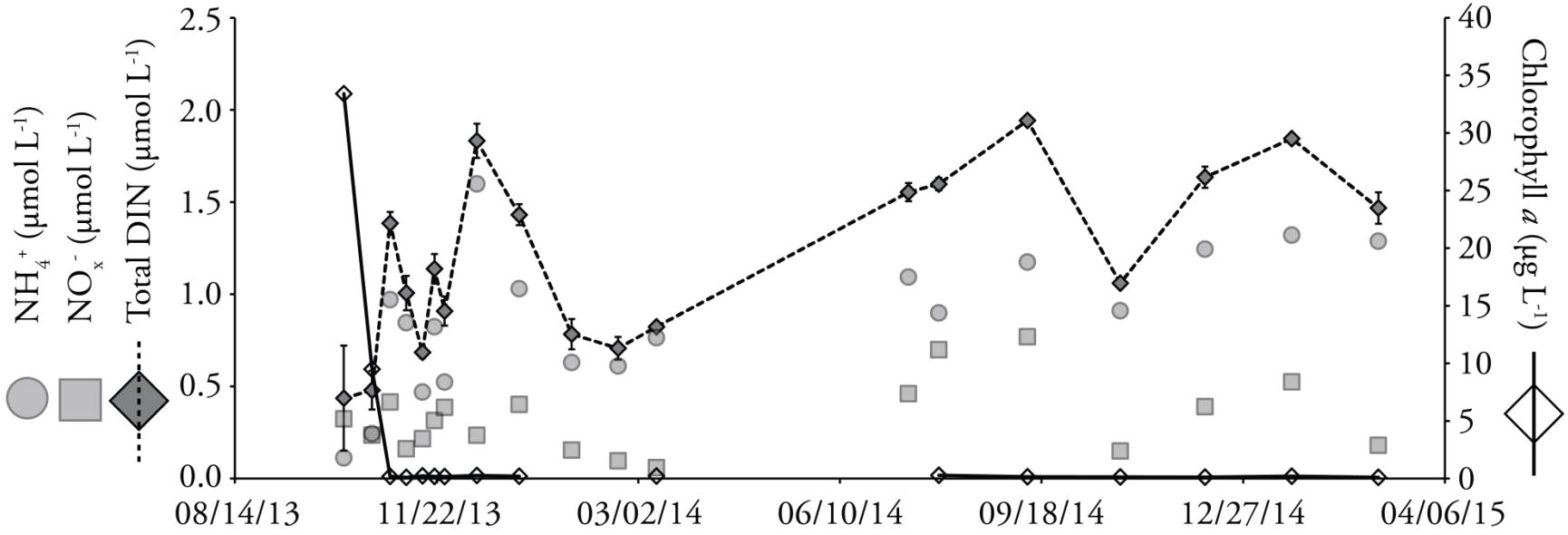

Figure 6.7: Mean DIN (NO_x^- + NH_4^+), NO_x^-, NH_4^+, and chlorophyll *a* concentrations for WP96 from September 26, 2013 to March 4, 2015 (see Figure 6.2 for relative site locations). Error bars are ± 1 SE.

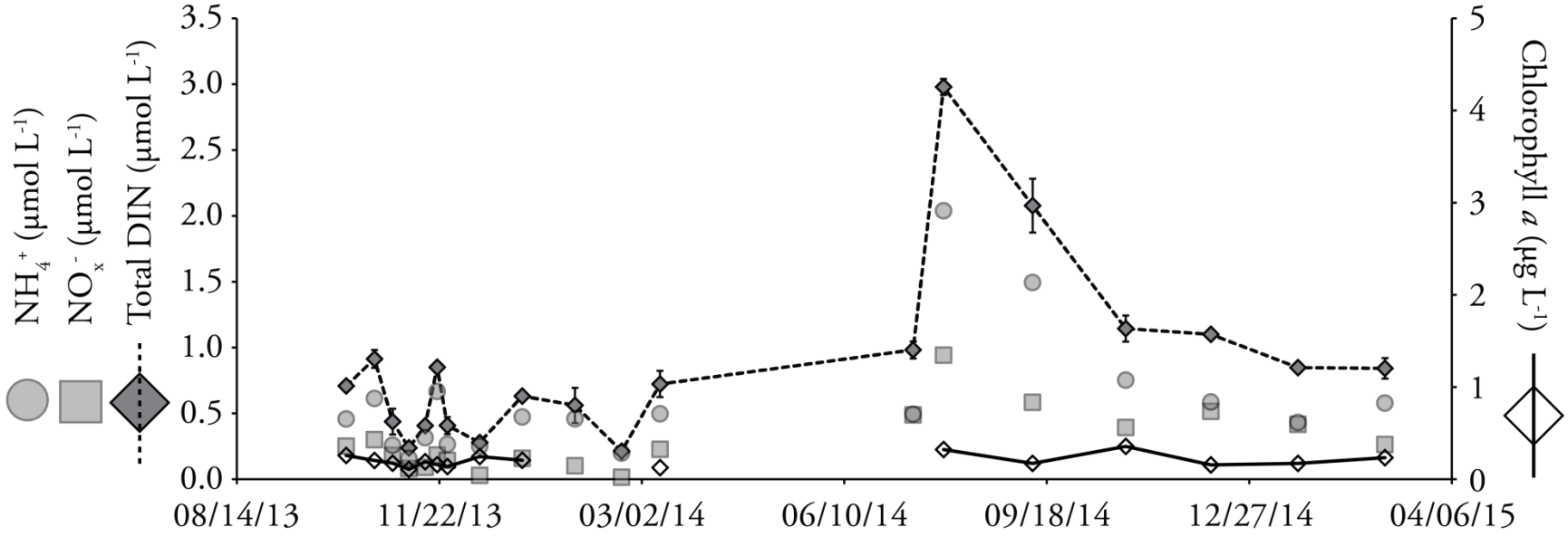

Figure 6.8: Mean DIN (NO_x^- + NH_4^+), NO_x^-, NH_4^+, and chlorophyll *a* concentrations for J01 from September 26, 2013 to March 4, 2015 (see Figure 6.2 for relative site locations). Error bars are ± 1 SE.

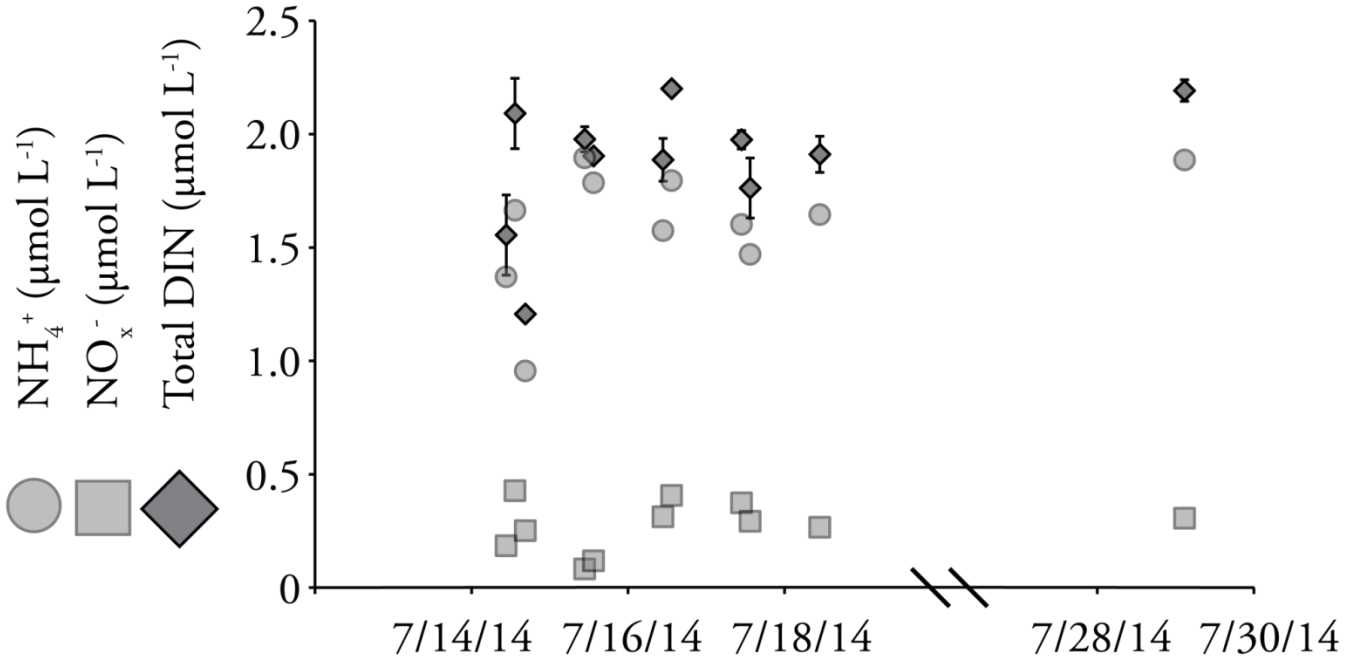

Figure 6.9: Methodological comparison for water quality parameters (mean surface DIN (NO_x^- + NH_4^+), NO_x^-, and NH_4^+) at HF1. This illustrates data from conventionally collected samples (frozen and shipped on dry ice) and syringe collected samples which were filtered and processed in the field. Data at the far right (July 29, 2014) represents a conventional collection whereas the preceding samples (July 14 to 18, 2014) represent field processed collections. Error bars are ± 1 SE.

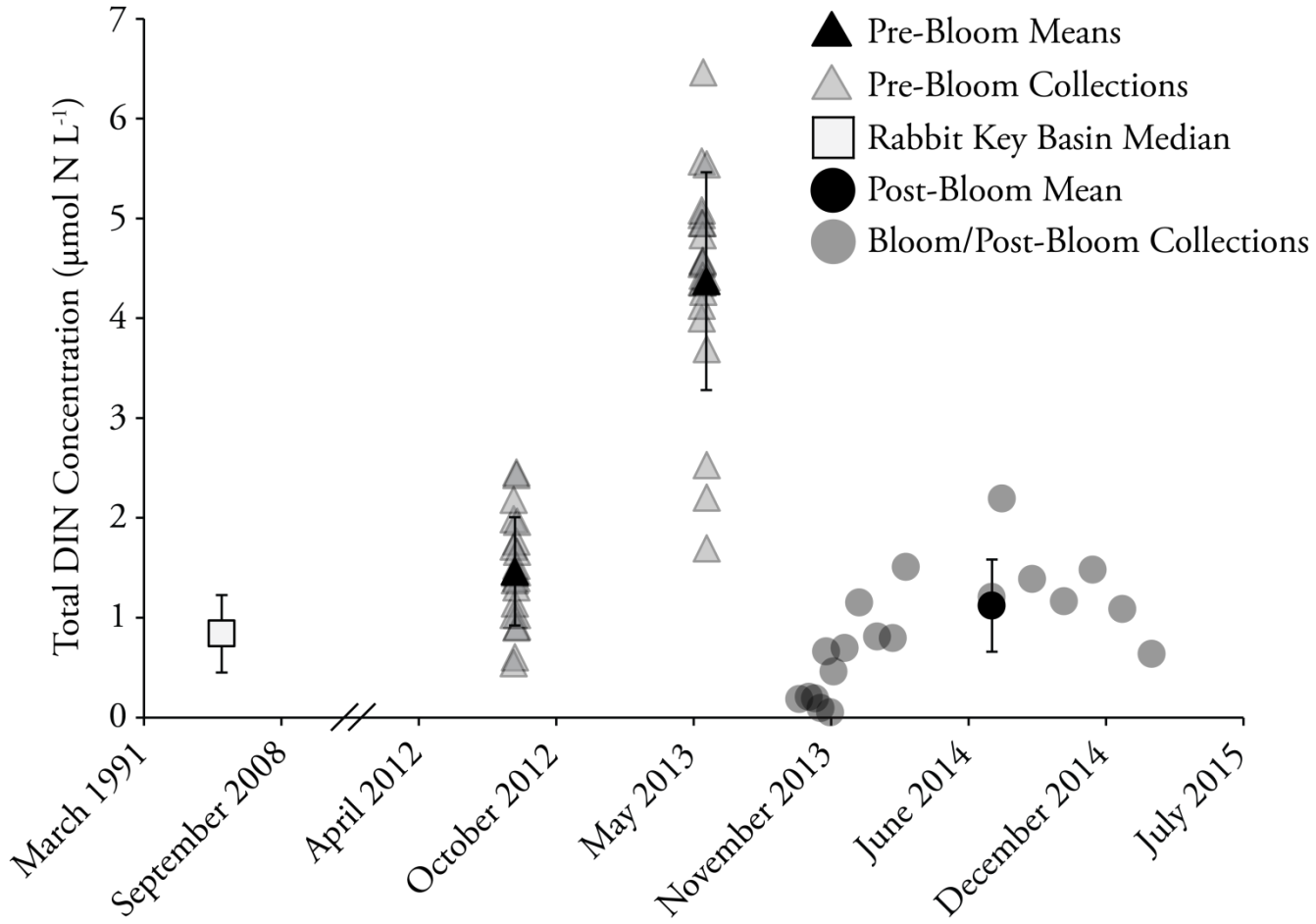

Figure 6.10: Comparison DIN concentrations at HF1 during and following the bloom with those measured prior to its onset (May 2013 and August 2012) as well as the median from Rabbit Key Basin (SERC Site ID: 18). Error bars represent ± 1SE and ± 1MAD for Mystery Basin and Rabbit Key Basin values, respectively.

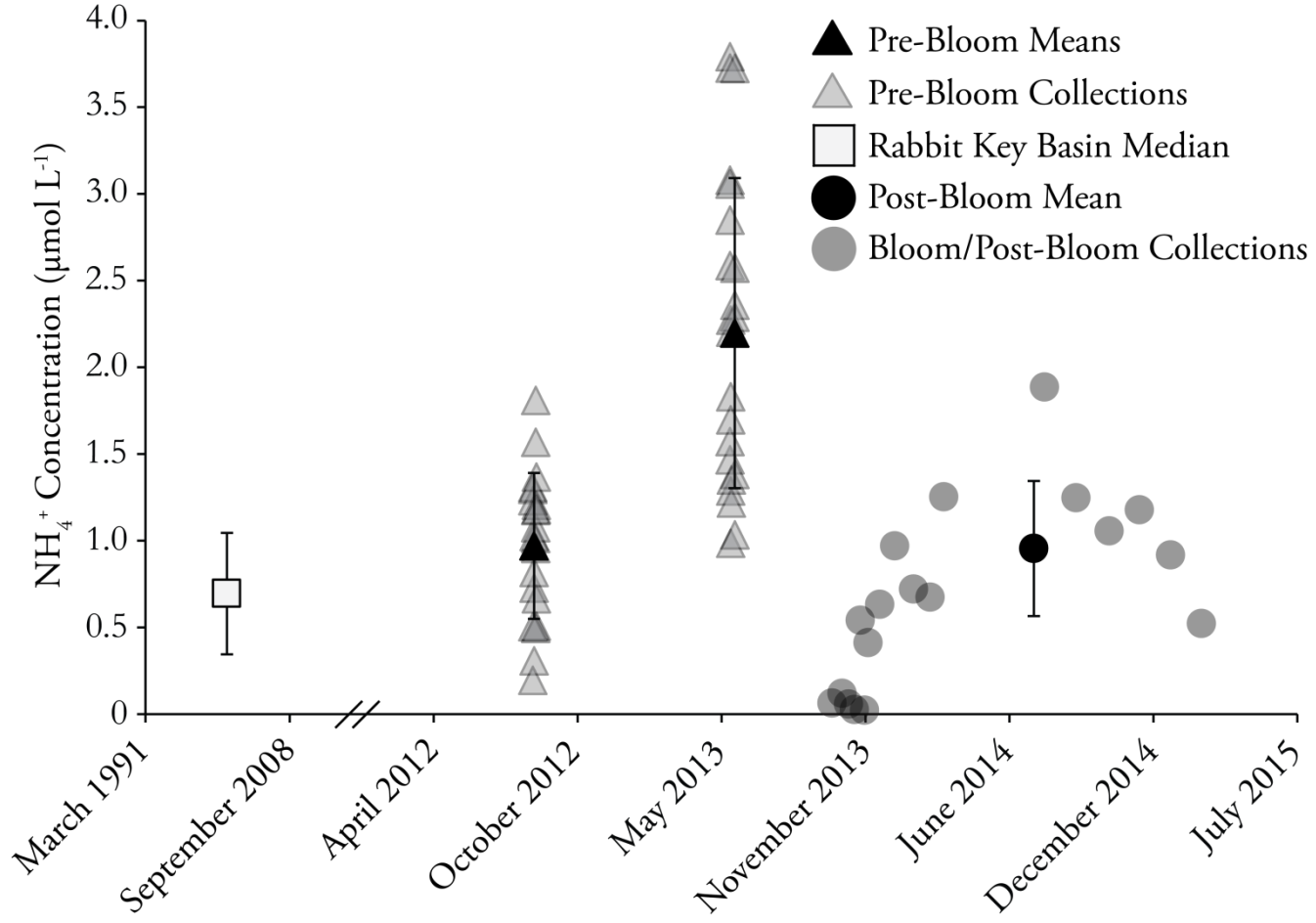

Figure 6.11: Comparison NH_4^+ concentrations at HF1 during and following the bloom with those measured prior to its onset (May 2013 and August 2012) as well as the median from Rabbit Key Basin (SERC Site ID: 18). Error bars represent ± 1SE and ± 1MAD for Mystery Basin and Rabbit Key Basin values, respectively.

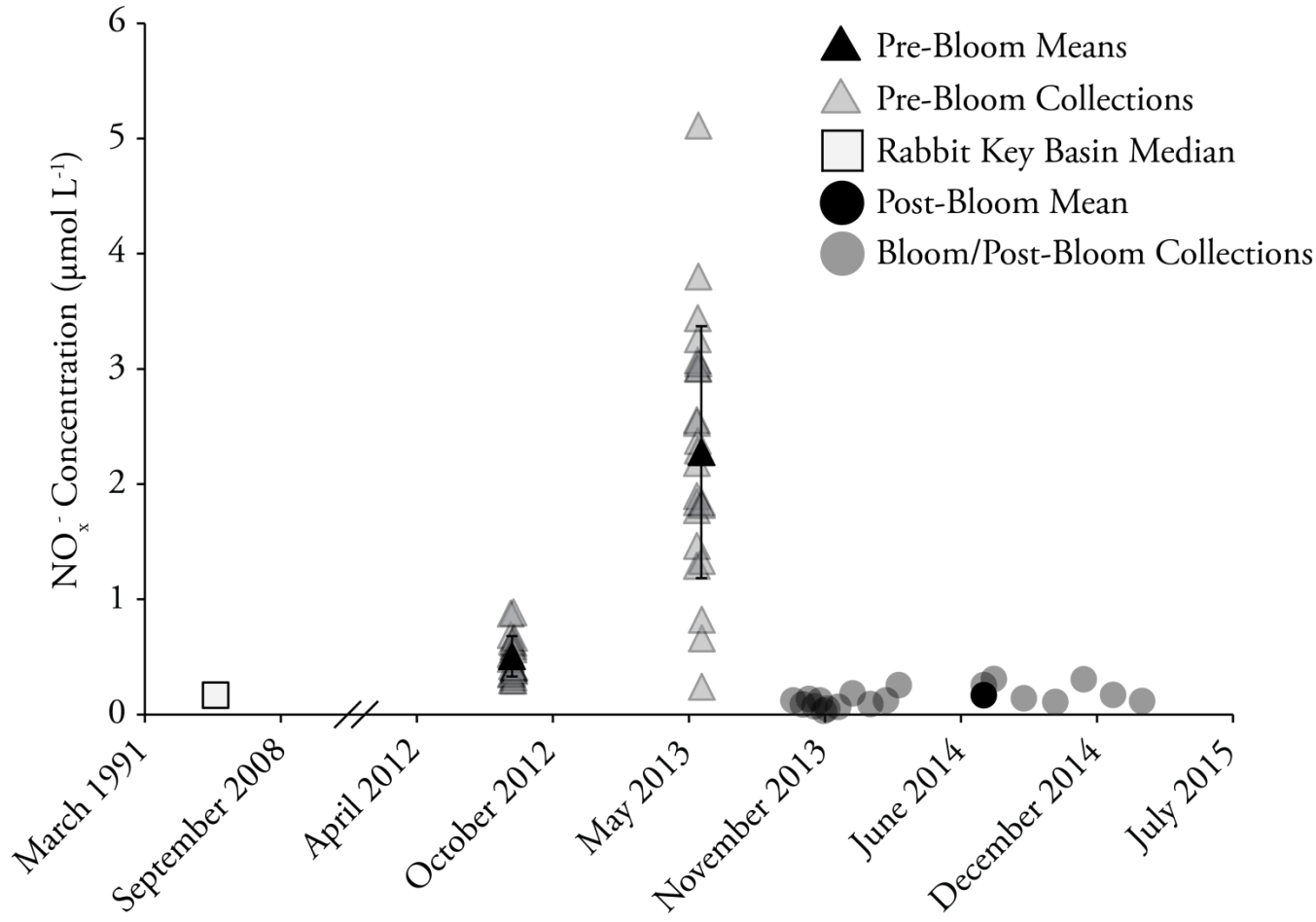

Figure 6.12: Comparison NO_x^{-1} concentrations at HF1 during and following the bloom with those measured prior to its onset (May 2013 and August 2012) as well as the median from Rabbit Key Basin (SERC Site ID: 18). Error bars represent ± 1SE and ± 1MAD for Mystery Basin and Rabbit Key Basin values, respectively.

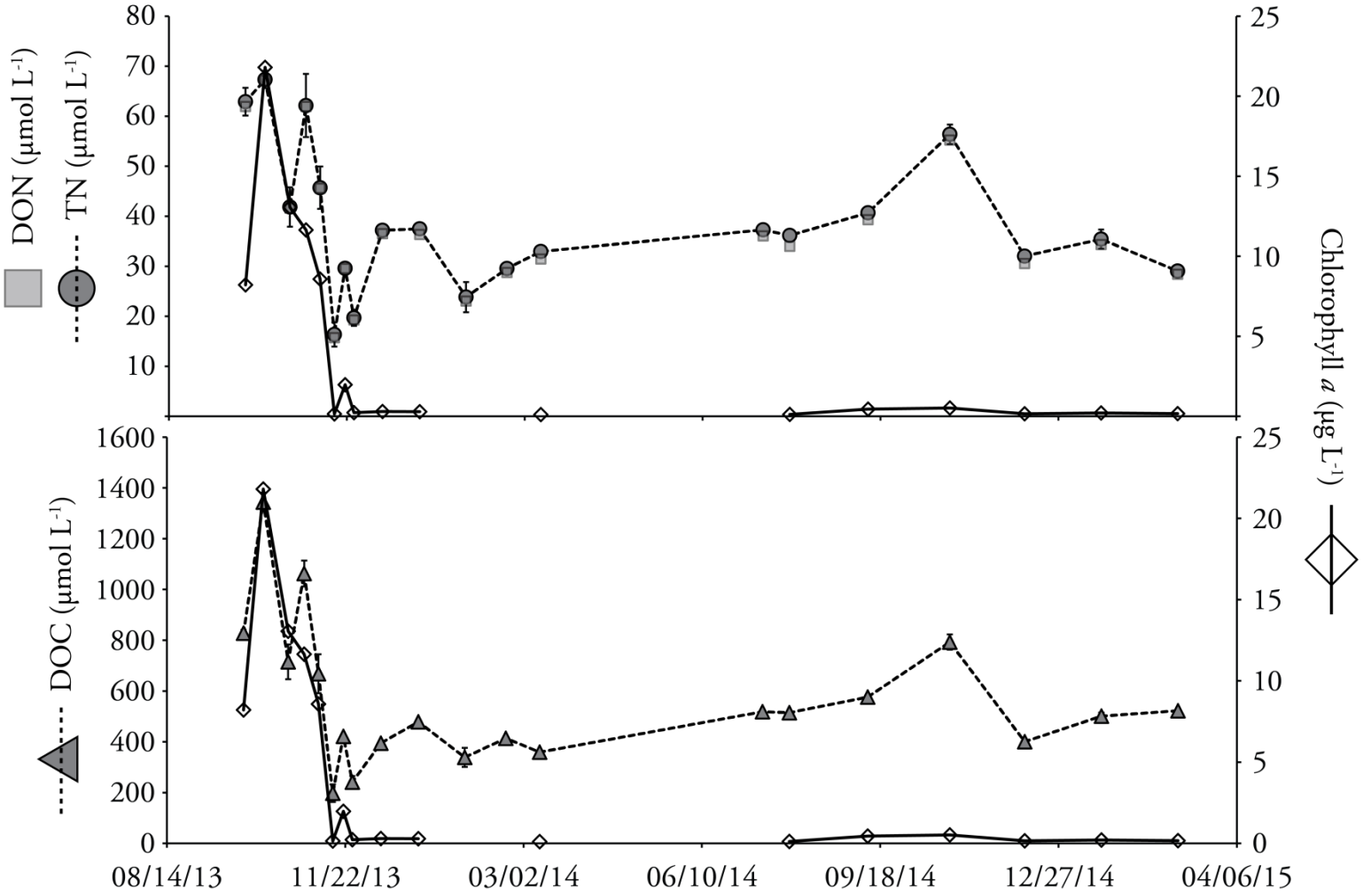

Figure 6.13: Mean DOC, TN, DON, and chlorophyll *a* concentrations for HF1 from September 26, 2013 to March 4, 2015 (see Figure 6.2 for relative site locations). Error bars are ± 1 SE.

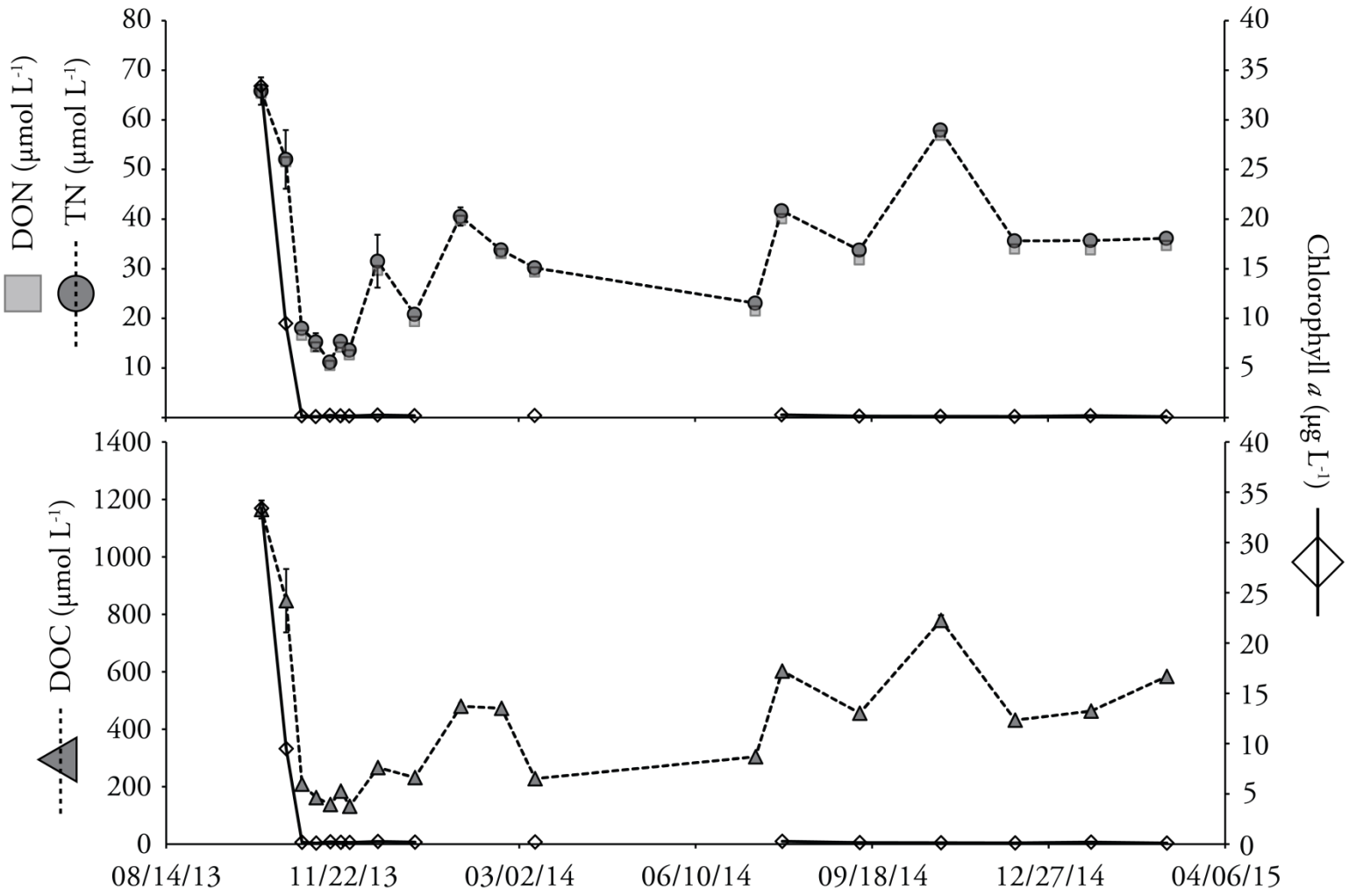

Figure 6.14: Mean DOC, TN, DON, and chlorophyll *a* concentrations for HF13 from September 26, 2013 to March 4, 2015 (see Figure 6.2 for relative site locations). Error bars are ± 1 SE.

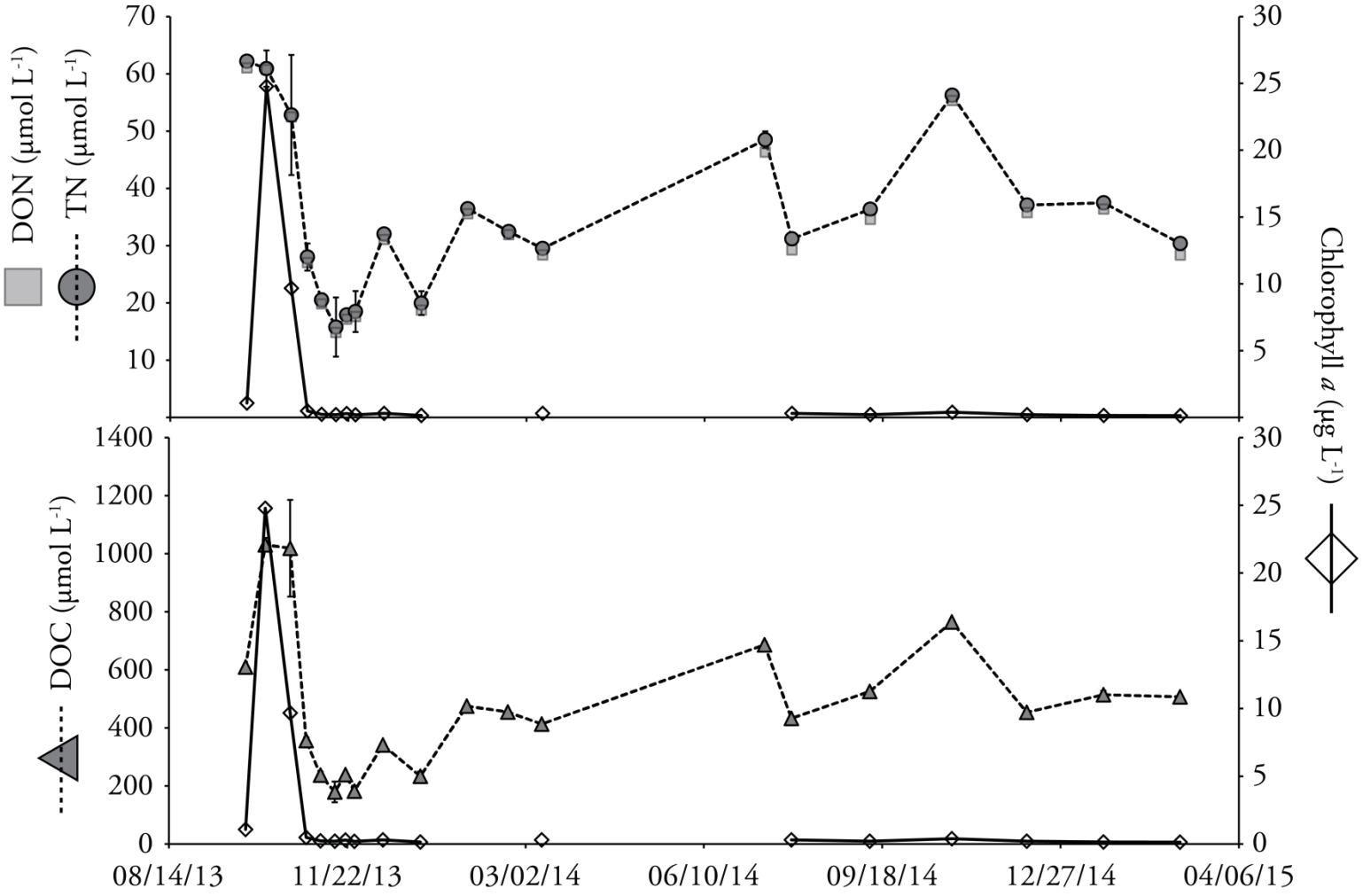

Figure 6.15: Mean DOC, TN, DON, and chlorophyll *a* concentrations for WP96 from September 26, 2013 to March 4, 2015 (see Figure 6.2 for relative site locations). Error bars are ± 1 SE.

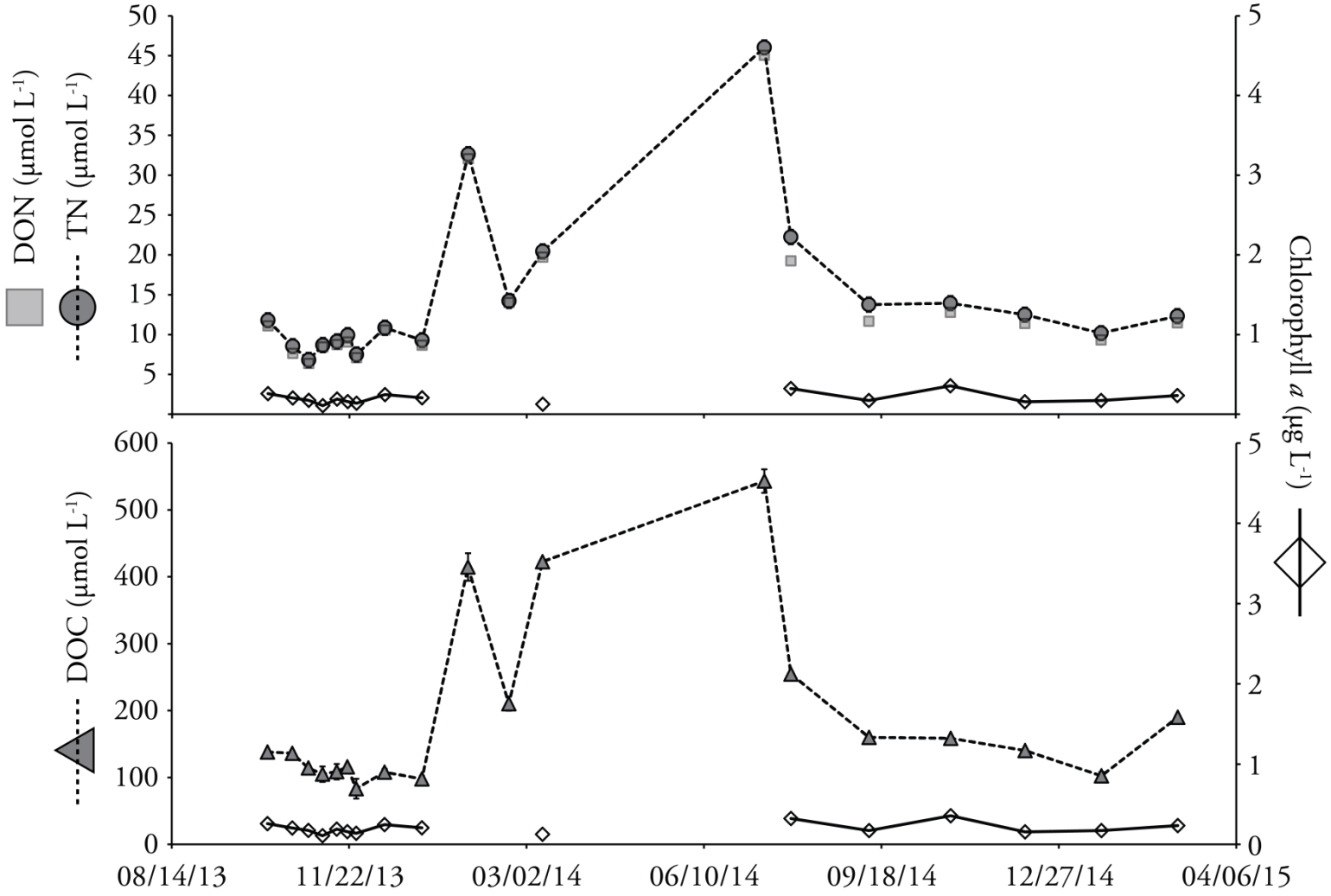

Figure 6.16: Mean DOC, TN, DON, and chlorophyll *a* concentrations for J01 from September 26, 2013 to March 4, 2015 (see Figure 6.2 for relative site locations). Error bars are ± 1 SE.

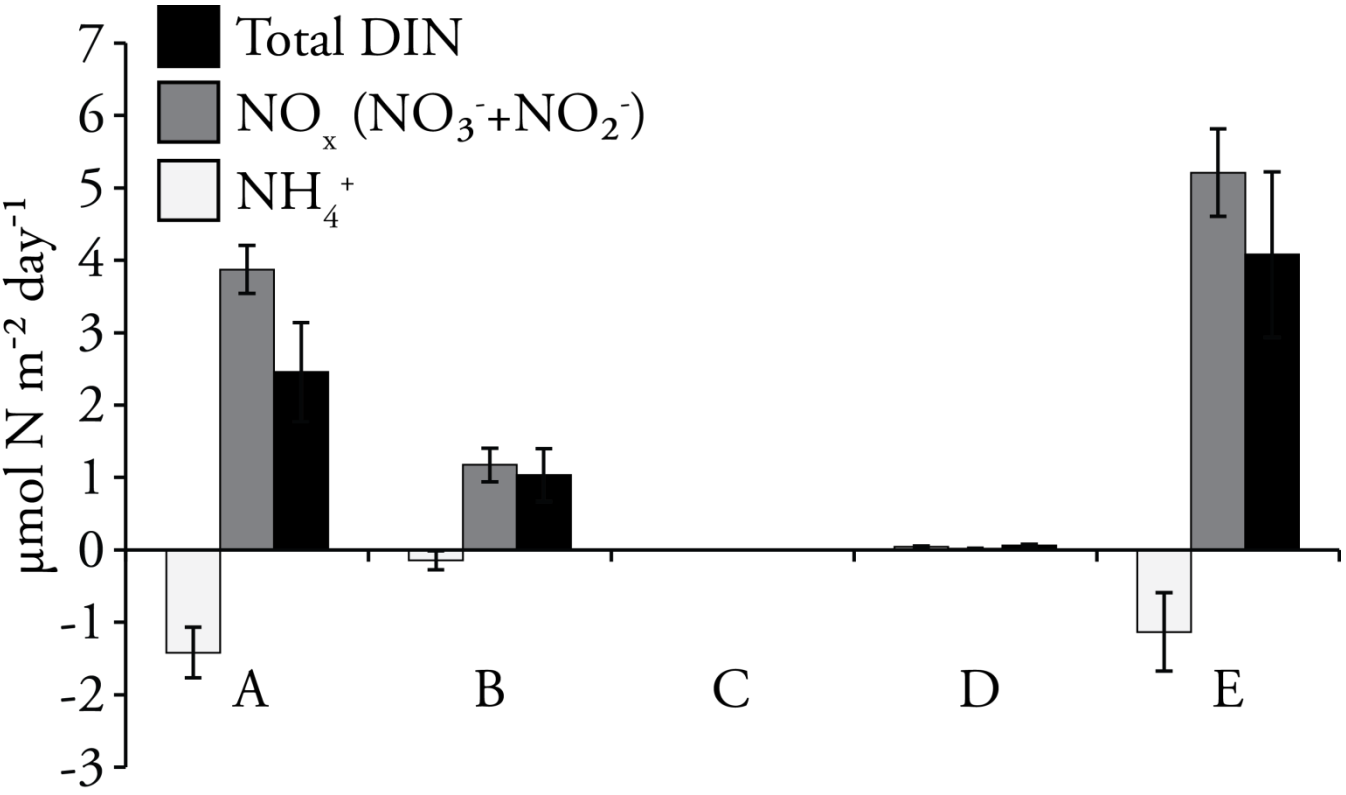

Figure 6.17: Average NH_4^+, NO_x^-, and total DIN contributions from the sponge community in Mystery Basin. DIN fluxes are separated by species and arranged in descending order of biomass contribution (largest to smallest; from left to right): A. *Cinachyrella* sp.; B. *C. nucula*; C. *Haliclona* sp.; D. *H. melanodocia*; E. All surveyed species. Error bars are ± 1 SE.

CHAPTER 7:

Summary of Findings, Conclusion, and Future Directions

This dissertation investigated the role of sponges in the cycling of carbon (C) and nitrogen (N) in carefully selected reef and Florida Bay environments and found that these fascinating organisms are capable of dominating the transformations and resupply of these elements to surrounding waters. The research focused on collecting data needed to test hypotheses largely through *in situ* measurements with undisturbed organisms so as to avoid any physiological changes resultant from sponge manipulation.

The giant barrel sponge *X. muta* was observed to satisfy the majority of its metabolic C demand from dissolved organic carbon (DOC). The quantity of DOC removed by this species and the size of its population on Conch Reef suggest that it has a large role in the C cycling in this environment. This organism retained a considerable proportion of absorbed DOC in excess of apparent respiration demand, which we hypothesize to be allocated to cellular maintenance and repair. However, we observed no export of particulate or mucosal organic matter from the organism to confirm its participation in the putative "sponge loop" (de Goeij et al. 2013). Additionally, the exhalent water from this species contains high concentrations of dissolved inorganic nitrogen (DIN) as a result of the observed respiration processes as well as chemical transformations mediated by the dense microbial consortia hosted in its tissues. The rate of DIN efflux observed in the Florida Keys was found to be very similar to that observed on the oligotrophic reefs of the Bahamas, indicating that this process may be conserved within the

species. Furthermore, the estimated contribution of respired organic N appears to fill the N gap observed between absorbed and exported DIN. This rapid remineralization of dissolved organic matter may represent an ecologically relevant source of DIN on nutrient-poor reefs by rapidly recycling nutrients bound in organic compounds into more bioavailable inorganic forms (e.g., Diaz and Ward 1997, Ribes et al. 2005, de Goeij et al. 2013).

Results of investigations of DIN release from *X. muta* and evidence from a variety of sponge species in the Caribbean and other environments (e.g., Corredor et al. 1988, Jiménez and Ribes 2007, Southwell et al. 2008b, Keesing et la. 2013) led to the central hypothesis that sponge respiration would be the dominant N source supplying the photosynthetic-associated demand by primary producers in the shallow, estuarine environment of Florida Bay. Organic-bound nutrient elements have a dominant role in this ecosystem (e.g., Boyer et al. 1997, Boyer et al. 2006), and by consequence, local recycling processes are expected to regulate the supply of inorganic nutrients in many locations (Rudnick et al. 2005, Boyer and Keller 2007, Boyer et al. 2009). Further, the shallow water column of this environment (typically < 3m water depth) and restricted physical exchange driven by the carbonate mud banks which compartmentalize this environment should improve our capability to quantify the importance of recycling processes, particularly those mediated by sponges. My research sites in Florida Bay were selected on the basis of habitat type, sponge abundance, and physical isolation that could emphasize the role of sponges.

The speciation and magnitude of effluent DIN from ecologically relevant sponge species was assessed using *in situ* benthic chambers. The rates of DIN release from sponge species common to Florida Bay were of the same magnitude as species observed in other coastal ecosystems (e.g, Corredor et al. 1988, Southwell et al. 2008b, Gibson 2011). This provided

further indication that these organisms have a capacity for driving local nutrient cycling in sponge-rich environments throughout the coastal ocean. In order to quantify the role of sponge recycled N in the budget of Florida Bay, an offshore basin (Mystery Basin) which was thought to be analogous to important sponge-rich sites throughout the bay as a whole was selected. Similar to other basins in the bay, the restricted water exchange at the selected site was hypothesized to allow local processes to dominate nutrient cycling. This restricted exchange, coupled with abundant sponge biomass, was thought to improve our capability to quantify the importance of recycled N from these organisms in the overall nutrient budget. The species-specific estimates of sponge effluent DIN generated from benthic chambers were used in conjunction with biomass surveys conducted within Mystery Basin to illustrate the relative importance of the sponge community and its ability to supply ample DIN to satisfy the majority of local photosynthetic N demand. I was able quantitatively demonstrate that observed C and N transformations are largely dependent upon the size and composition of the sponge community, and that the efflux of recycled N to the tested system represented the largest single source of DIN and approximately half of the inorganic N contributed to the water column. The dependence of this N flux on the structure and composition of the sponge community was hypothesized to predispose it to temporal instability given the susceptibility of these organisms to sudden mortality events in this environment (e.g., Butler et al. 1995, Peterson et al. 2006).

We observed a phytoplankton bloom that developed at our Mystery Basin study site, bloom that occurred simultaneously with the decimation of the vast majority of the sponge population. High microbial abundance (HMA) species appeared to be more severely impacted by elevated cyanobacterial populations than low microbial abundance (LMA) sponges. However, neither the underlying mechanism which led to the observed mass sponge mortality

nor the source of the differential response between HMA and LMA species is definitively known. A prevailing hypothesis based on previous studies is that the exceptionally high concentration of cells in the water column during blooms leads to mechanical blockage of sponge aquiferous canals, which in turn quickly contributes to a cessation in pumping and eventually leads to tissue necrosis or organismal death (Butler et al. 1995, Lynch and Phlips 2000, Wall et al. 2012). Physical obstruction may be enhanced in HMA species as they possess denser, more complex aquiferous structures than their LMA counterparts (Weisz et al. 2008), and this increased surface area to volume ratio of internal structures may raise their susceptibility to clogging. Blooming cyanobacteria in Mystery Basin were also associated with a rapid reduction in water column DIN to near trace concentrations, followed by a slow recovery to normative conditions that were not significantly different than pre-bloom values. The lack of a drastic shift in water column DIN concentrations was surprising given the mortality of the sponge community and previously quantified contribution of N from these organism. However, pre-bloom nutrient data for this location was temporally limited, and therefore the overall change to the long-term nature of the N cycle is unknown. Furthermore, we hypothesized that the decaying sponge biomass sourced from the observed die-off generated a considerable quantity of remineralized nutrients, potentially enhancing bloom density and duration. This source of recycled nutrients is not without precedent as the decaying biomass of fish killed during blooms of the toxic dinoflagellate *Karenia brevis* can supply quantities of regenerated nutrients that are capable of providing significant N and phosphorus (P) to the blooming organisms, thereby contributing to increased bloom duration (Vargo et al. 2008, Vargo 2009, Killberg-Thoreson et al. 2014). While fish mortality has not been noted during *Synechococcus* blooms in Florida Bay (Butler et al.

1995, Lynch and Phlips 2000), we calculated that the mass mortality of sponge biomass has the potential to provide a similar magnitude to regenerated nutrients.

The tested sponges have the capacity to alter local water quality through the observed C and N transformations mediated by the holobiont (sponge and associated microbiome), and the quantification of these processes across environmental gradients further suggests that sponges play a large role in ecosystems where their populations dominate. The potential stability of these organismal processes in varying environments may aid in the future incorporation of these organisms and their effluent into nutrient budgets of sponge-rich ecosystems throughout the Caribbean. Evidence from the natural, sponge exclusion event generated by mass organism mortality provides evidence for how sponge-rich areas within Florida Bay may respond to and possibly modulate recurring bloom phenomena. These results have implications for the management of blooms in sponge rich areas as the presence of these organisms may not only apply top-down pressure regulating cyanobacterial populations (Officer et al. 1982, Peterson et al. 2006), but their mortality may fuel explosive expansion of their populations.

Future Directions

Much of the data presented in this dissertation represents the work of conventional, campaign style sampling. However, the dominance of campaign-collected samples in this work belies the contemporaneous effort and progress made incorporating *in situ* instrumentation into measurements being made in the sampled environments. The ability to make continuous *in situ* measurements of environmental phenomena affords resolution and data quality that is impossible to match with discrete sampling. In the future, research in the coastal ocean and, more broadly, in field sciences will rely on these techniques. The use of these instruments has provided corroborating evidence to the nitrate plus nitrite (NO_x^-) release rates observed in chambered *C.*

nucula (Miniature Spectrophotometric Elemental Analysis System; MSEAS, USF-COT; Figure 7.1). Yet the ability to expand the efflux from a singular sponge oscula to the dozens of diffuse oscula on an individual organisms and then to the hundreds of individual organisms per square meter of benthos is only beginning to be understood. Furthermore, with the increasing availability of low-cost, small, and powerful electronics, the ability to make *in situ* measurements will increase rapidly with the development of sensors and autoanalyzers built by the scientists who intend to deploy them.

The future of the Florida Bay ecosystem is changing rapidly, particularly as the progress on the Comprehensive Everglades Restoration Plan (CERP) continues with the goal of restoring the ecological, hydraulic and water quality regime of the Everglades. Freshwater discharge from the Everglades will increase (as it is thought to have with the spring 2012 completion of the C-111 spreader diverting freshwater into Taylor Slough; US Army Corps of Engineers; UNESCO 2013), and so too will the role that this freshwater input has on the ecosystem as a whole. The rapidity with which this input returns may lead to unknown ecosystem response, as the system has evolved under the pressure of steadily reducing freshwater discharge (by as much as 60%) during the past century (Madden et al. 2009 and citations therein). The "return" of the freshwater endmember of this estuarine ecosystem may increase input of dissolved organic matter (DOM) from the Everglades, potentially enhancing the role of nutrient recycling processes like those mediated by sponges, particularly in basins near the outflow of Taylor Slough. Furthermore, if DOM loading increases with increasing freshwater input there may be a shift in the sponge community metabolism towards a reliance on dissolved organics rather than the particulates, assuming the community possesses the dietary plasticity to quickly respond to changes in the composition of nutritive organic matter.

This dissertation provides evidence of the role of sponge communities in the coastal cycles of C and N, which has implications for sponge-rich environments globally. The large role of these organisms as demonstrated herein and elsewhere coupled with the trend towards increasing anthropogenic change in the coastal zone, necessitates a furthered understanding of the response of sponges to environmental change as the future structure of C and N cycling in these environments may depend on these organisms.

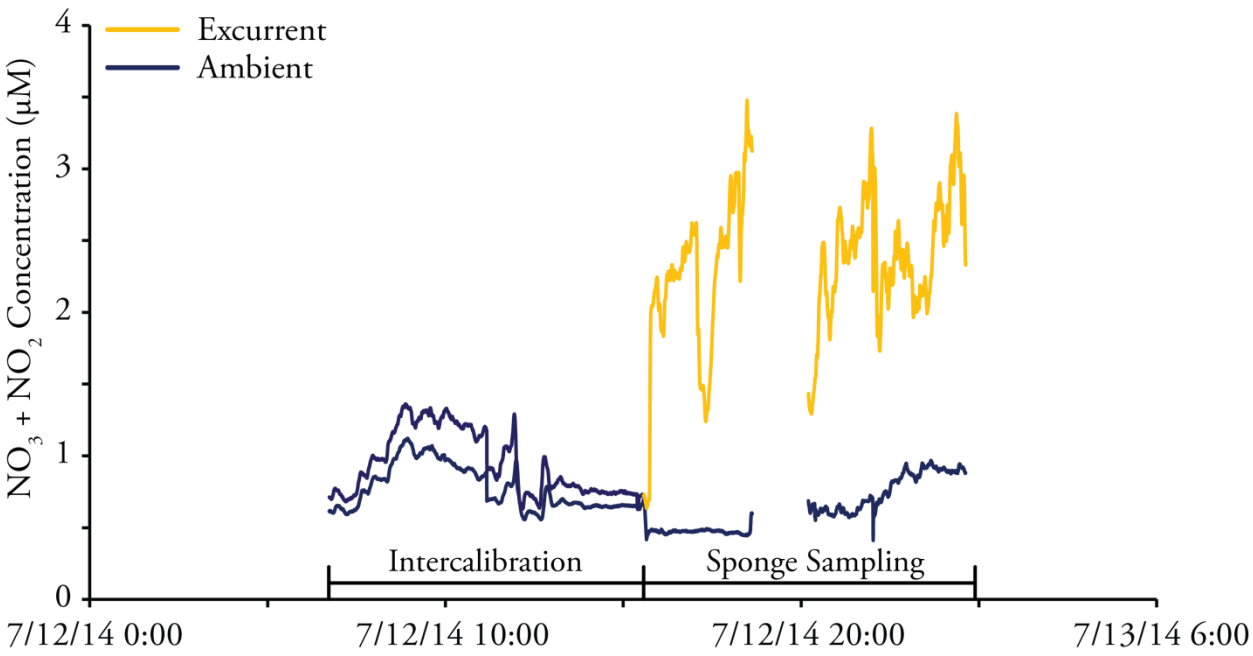

Figure 7.1: Sample of time-series data received from simultaneous measurement of ambient and excurrent NO_x^- (NO_3^- + NO_2^-) from *C. nucula* collected using prototype MSEAS instrumentation. The period marked "intercalibration" indicates when the inlets were sampling the same, ambient water mass, which is followed by separation of the sampling streams into ambient and excurrent water. Due to the small size of oscula on *C. nucula* individuals, excurrent represents water near the oscula of several *C. nucula* individuals.

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