Dissolved inorganic nitrogen fluxes from common Florida Bay (U.S.A.) sponges

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Abstract

Sponge biomass represents the largest heterotrophic component of benthic biota in the Florida Bay ecosystem. These organisms can significantly alter the water quality of their surrounding environment through biogeochemical transformations of nutrient elements resulting from their dynamic pumping, water filtration, and respiration processes. Ammonium (NH_4^+) and nitrate plus nitrite ($NO_3^- + NO_2^-$; NO_x^-) fluxes were obtained for 11 ecologically important species at three sites within Florida Bay, Florida (U.S.A.) utilizing chamber incubations on undisturbed individual sponges. Significant dissolved inorganic nitrogen (DIN) effluxes ranging between 9.0 ± 2.2 μmol N h^{-1} L_{sponge}^{-1} and 141 ± 26 μmol N h^{-1} L_{sponge}^{-1} were observed for eight of the 11 tested sponges; specifically, from six of eight tested high-microbial abundance (HMA) sponges, and from two of three tested low-microbial abundance (LMA) sponges. The abundant HMA species *Chondrilla nucula* showed the highest, volume-normalized rate of DIN release. These fluxes represent a continuation of the previously observed dichotomy in the chemical speciation of DIN in exhalent waters of LMA and HMA sponges, with NH_4^+ and NO_x^- dominating their respective exhalent jets. Surprisingly, we found that dissolved organic matter (DOM) appeared to make a negligible contribution to the total released N, but we hypothesize that the lack of DOM utilization or production was due to methodological limitations. Our flux data combined with sponge biomass estimates indicate that sponges, particularly HMA species, are a large, and potentially dominant, source of inorganic nitrogen to Florida Bay waters.

Florida Bay is a lagoonal estuary whose western boundary is open to the Gulf of Mexico. It is bordered to the north by peninsular Florida and to the south and east by the Florida Keys archipelagic island chain. The Bay is characterized by a shallow (< 3 m average water depth), oligotrophic water column (chlorophyll α levels typically < 1 μg L^{-1}; Phlips et al. [1999]) and a diverse benthic community of sponges, corals, seagrasses, and macroalgae. Oceanic exchange and circulation is limited within much of Florida Bay by an extensive network of carbonate mud shoals that form restricted basins with distinct chemical and biological characteristics (e.g., Fourqurean and Robblee 1999; Peterson et al. 2006; Zhang and Fischer 2014). The water column reservoir of nitrogen (N) is largely composed of dissolved organic matter (DOM) (Boyer et al. 1997), and N input from the Everglades is similarly dominated by dissolved organic nitrogen (DON) (Boyer et al. 1999, 2006; Childers et al. 2006). Sustaining a stable supply of inorganic N to primary producers therefore necessitates an efficient means of organic matter remineralization. Heterogeneously distributed seagrass beds have been highlighted as potentially important sites of DON remineralization (Maie et al. 2005; Yarbro and Carlson 2008), while also representing the largest component of phototrophic nutrient demand (e.g., Zieman et al. 1989; Fourqurean et al. 2001; Herbert and Fourqurean 2009). Sponges are known to be important sources for dissolved inorganic nitrogen (DIN) derived primarily from the remineralization of both particulate organic matter (POM) and DOM (e.g., Corredor et al. 1988; Jiménez and Ribes 2007; Southwell et al. 2008*b*), and are abundant components of the benthic biomass throughout Florida Bay (Peterson et al. 2006).

An extensive survey of sponge biomass in Florida Bay revealed sponge populations with densities between 0.02 individuals m^{-2} and 22 individuals m^{-2} at more than 70% of 207 sites surveyed (Peterson et al. 2006). Their abundant presence, enormous pumping rates, and efficiency as filter feeders (e.g., Reiswig 1971; Weisz et al. 2008; McMurray et al. 2014) make the sponge community the foremost benthic heterotroph in this ecosystem. The grazing pressure from sponges is

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enhanced by their ability to extract nutritive materials from both DOM and POM (e.g., Reiswig 1974; reviewed by Pawlik et al. 2015; Hoer et al. 2018). Due to its dietary plasticity, the sponge community may represent an important pathway for nutrient resupply through DOM remineralization in Florida Bay, given the demonstrated metabolic reliance of some sponges on DOM in reef ecosystems (e.g., de Goeij et al. 2008, 2013; Hoer et al. 2018). The DOM pool in Florida Bay is largely derived from unique allochthonous (FL Everglades runoff; e.g., Childers et al. 2006) and autochthonous sources (seagrass release; e.g., Fourqurean et al. 2001; Maie et al. 2005; Chen et al. 2013). The relative contribution from these may alter the bioavailability of the DOM pool to the sponge community and lead to enhanced consumption of POM.

Sponges feed by actively pumping large volumes of water through aquiferous canals in their tissues and out as an exhalent (excurrent) jet into the overlying water column. Their large pumping rates make sponges 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. 2008*a*; Keesing et al. 2013). Although they are distributed nonuniformly, sponges potentially represent an important source of N in Florida Bay, particularly considering previously measured rates of DIN release from two commonly occurring species, *Chondrilla nucula* and *Ircinia* sp. (Corredor et al. 1988; Diaz and Ward 1997; Southwell et al. 2008*b*). The chemical speciation of DIN released from various sponge species in tropical and temperate ecosystems ranges from entirely ammonium (NH_4^+) to entirely NO_x^- (nitrate plus nitrite; $NO_3^- + NO_2^-$) (e.g., Corredor et al. 1988; Jiménez and Ribes 2007; Southwell et al. 2008*b*). This range in chemical speciation of DIN in sponge effluent is known to be influenced by the presence of microbial communities within the sponge tissues (Diaz and Ward 1997; Taylor et al. 2005; Southwell et al. 2008*a,b*).

Dense microbial populations live within the tissue of high-microbial abundance (HMA) sponges (Hentschel et al. 2006; Weisz et al. 2008). These populations frequently occur in densities up to 10^8 to 10^{10} microorganisms per gram of sponge wet weight, which exceed seawater concentrations by as many as four orders of magnitude, and can represent ~ 40% of total sponge tissue volume (Hentschel et al. 2006, 2012; Freeman and Thacker 2011). Microbial metabolism occurring within HMA sponges contributes to the diversity of hosted nutrient element transformations, including nitrification (Southwell et al. 2008*a*; Hoffmann et al. 2009), and potentially broadens the scope of organic matter accessible to sponges (Reiswig 1974; Pawlik et al. 2015; Rix et al. 2017). Conversely, sponge species hosting low numbers of microorganisms, termed low-microbial abundance (LMA) sponges, (Hentschel et al. 2006) tend to produce ammonium-rich effluent with a chemical signature characteristic of animal-based metabolism (Southwell et al. 2008*b*). These LMA species contain only 10^5 to 10^6 microorganisms per gram of sponge wet weight, which is approximately the density of microbes in ambient seawater (Hentschel et al. 2006).

Of the many studies that have reported DIN release by sponges, very few (e.g., Southwell et al. 2008*b*; Fiore et al. 2013; Keesing et al. 2013) employed methodologies that did not involve significant physical disturbances of the sponge animal. Experimental manipulations of the surrounding environment or of the sponge animal can have large impacts on sponge pumping behavior as they have been proven to be sensitive to environmental and physical stressors (e.g., Gerrodette and Flechsig 1979; Tompkins-MacDonald and Leys 2008; reviewed by Maldonado et al. 2012). The effect of manipulations would be particularly pronounced for HMA species as environmental variability has been shown to significantly alter the function of the sponge holobiont (sponge animal and associated microbial consortia; Fan et al. 2013). Thus, manipulations could profoundly alter the N efflux from HMA species as the chemical speciation of released DIN is expected to be largely controlled by the activity of the hosted microbial consortia. Therefore, to minimize potential experimental artifacts, our investigation of the role of sponges in nitrogen cycling in Florida Bay utilized in situ, underwater methodologies to measure DIN composition and fluxes from undisturbed individuals representing 11 important species found in typical "hardground" areas of Florida Bay (Peterson et al. 2006). The flux measurements were made by briefly enclosing sponges representing each of these species still attached to their original substrate in well-oxygenated chambers and measuring DIN concentration changes over periods of several hours. We hypothesized differences in the chemical speciation of DIN in the exhalent waters of LMA vs. HMA sponges, with NH_4^+ and NO_x^- dominating their respective exhalent jets (Jiménez and Ribes 2007; Bayer et al. 2008; Southwell et al. 2008*b*).

Presented here are the results of in situ measurements of the chemical speciation and net flux of DIN from 11 sponge species commonly found throughout Florida Bay. We sought to determine the magnitude and relative importance of the selected sponges as focal sites of N remineralization and release, and to estimate the potential magnitude of their N fluxes based on a previous survey of Florida Bay sponge populations (Peterson et al. 2006). Further, we sought to compare calculated fluxes in Florida Bay with rates from other environments to assess any intraspecific variability in the rates of N efflux. Given the dominant presence of DOM in this environment, we hypothesized that DOM should be an important metabolic substrate for the examined sponges (de Goeij et al. 2008; Southwell et al. 2008*b*; Hoer et al. 2018), and sought to quantify the relative importance of DOM vs. POM for sponge metabolism in Florida Bay.

Methods

Sample sites

Three shallow sites (water depth < 3 m) representative of hardbottom environments that can be found within the Florida Bay ecosystem were sampled. The first site was

immediately adjacent to a dock operated by the Florida Fish and Wildlife Conservation Commission (FWC) South Florida Regional Laboratory in Marathon, FL (Site ID: FWC Dock, 24°42′45.31″N, 81°5′54.89″W; Fig. 1). The dock site was easily accessible from shore by snorkeling and SCUBA, featured dense populations of benthic macroalgae, and was characterized by a thin (~ 20 cm) layer of carbonate mud overlying Pleistocene limestone. The dock itself is stabilized by a rock jetty that has been colonized by a variety of boring and encrusting sponges, primarily *C. nucula*, with minimal contributions from nonboring and nonencrusting species. The water column was heavily influenced by strong alongshore tidal currents running to and from the large pass separating Knight's Key in the Middle Keys to Little Duck Key in the Lower Keys. Tidal current speeds observed at the dock site reached as high as 30 cm s^{-1} and corresponded to significant fluctuations in water quality parameters including ambient DIN, dissolved oxygen (DO), and temperature (D. Hoer, unpubl. data).

The second site, Burnt Point, was located within a semi-protected, cove on the northeast side of Long Point Key (Site ID: Burnt Point, 24°45′24.6″N, 80°58′55.0″W; Fig. 1). The Burnt Point site had a relatively thin veneer of carbonate mud (up to ~ 10 cm deep) overlying limestone, and featured a diverse, high-biomass group of sponges that included nonboring and nonencrusting species such as *Ircinia campana* and *Spongia* sp., in addition to seagrasses and macroalgae. Our Burnt Point site had a much lower degree of exposure to strong tidal currents than the FWC dock site.

The third site was 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; Fig. 1). Mystery Basin is effectively isolated from surrounding waters by an outer rim of shallow banks that shoal during low tides. The shoals forming the outer rim of the basin greatly restrict water exchange with the surrounding areas (Holmquist et al. 1989; Peterson et al. 2006) and are covered by seagrasses rooted in carbonate mud. Within Mystery Basin, the hardbottom had little (< 5 cm thick) or no carbonate sand and mud, and was characterized by a diverse population of sponges that included large populations of *Spheciospongia vesparium* and *Ircinia felix* in water depths averaging 1.5 m.

Sample collection and chamber methodology

Eleven sponge species were examined in this study: *Haliclona* sp., *Halichondria melanodocia*, *Cinachyrella* sp., *S. vesparium, I. felix, I. campana, Spongia* sp. (morphotype 1), *Spongia* sp. (morphotype 2), *Hippospongia lachne, Geodia gibberosa*, and *C. nucula* (photos of the sampled species are available in the Supporting Information; Supporting Information Fig. S1A–K). The sampled *Spongia* species are labeled simply as morphotypes 1 and 2 as members of this genera are notably difficult to characterize definitively at the species level (Supporting Information Fig. S1B,J; Cook and Bergquist 2002). The 11 species sampled were selected because they dominated sponge biomass both at our study sites and throughout Florida Bay (Peterson et al. 2006, B. Peterson pers. comm., January 2011). Furthermore, these choices allowed for a comparative analysis of the N efflux from HMA and LMA sponges. Of the 11 tested species, three were assigned as LMA sponges (*Haliclona* sp.: Sipkema et al. 2009; *Halichondria melanodocia*: Weisz et al. 2008; *Cinachyrella* sp.: genus level distinction based on Gloeckner et al. 2014), while the remaining eight 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; *Hippospongia lachne*; Ereskovsky et al. 2005; *G. gibberosa*: genus level distinction,

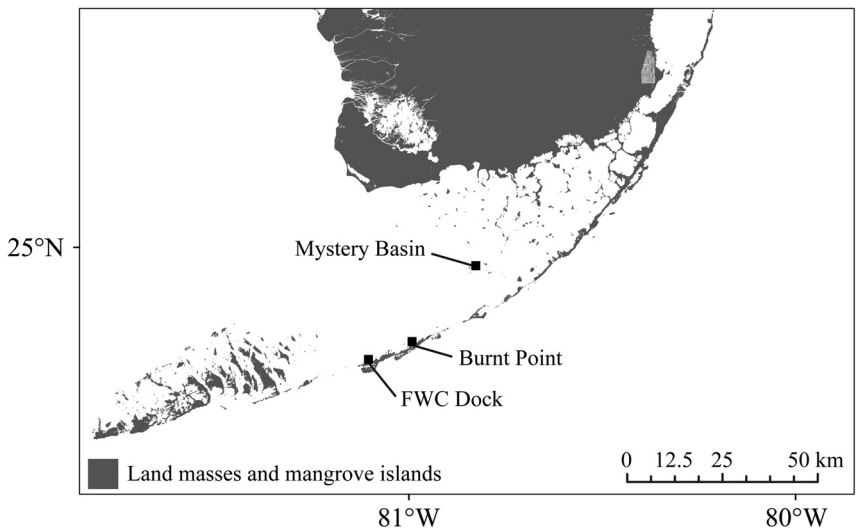

Fig. 1. Map of Florida Bay. Squares indicate locations where chamber experiments were performed.

Hoffmann et al. 2009; *C. nucula*: Hill et al. 2006). Each sponge species was examined at the site where the most substantial populations were found: *C. nucula* and *S. vesparium* were tested at the FWC Dock; *Spongia* sp. Morphotypes 1 and 2, *Hippospongia lachne*, *I. campana*, *I. felix*, and *S. vesparium* were tested at Burnt Point; and *G. gibberosa*, *Haliclona* sp., *Halichondria melanodocia*, and *Cinachyrella* sp. were tested at Mystery Basin.

"InEx" methodologies have been employed to measure sponge-mediated chemical changes in numerous previous studies (e.g., Southwell et al. 2008*a*,*b*; Fiore et al. 2013; Morganti et al. 2017). However, many of these studies focused on species or individuals with a single exhalent jet, which greatly simplifies the understanding of bulk transport of water through the sponge animal. Many of the sponge species that inhabit FL Bay are characterized by a multitude of small, diffuse oscula that cover the surface of single sponge individual (Supporting Information Fig. S1D–F,H–K). The size of these oscula was such that directly sampling them using InEx methodologies held considerable risk of contaminating exhalent samples by entraining ambient water. Consequently, a closed chamber incubation method was developed to determine fluxes and chemical speciation of released DIN at our Florida Bay sites. The use of a fully enclosed chamber allowed us to integrate the processes of the whole sponge animal, and thus attempt to develop an understanding of the large-scale N and C dynamics mediated by the selected species.

A simple benthic flux chamber was constructed from a 20-l polypropylene drinking water jug with its bottom excised. 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 animal. The final chamber volume was 16.5 L after modifications, which was sufficiently small to allow observation of DIN concentration increases over periods of 30–150 min that could be used to calculate net DIN fluxes. The chamber (Fig. 2) had inlets in place for water sample collection, for bubbling air introduction from a SCUBA regulator, and for an optical probe (HACH LDO101) for monitoring DO concentration in the chamber in real time. Healthy-looking sponge individuals were haphazardly selected for flux measurement by divers on SCUBA. Their active pumping was confirmed by fluorescein dye injected just above their oscula. After a targeted individual was confirmed to be pumping, the sponge was isolated from the surrounding substrate by slipping a slit-collared polypropylene plastic sheet around the base of the sponge, without contact with the sponge itself, to seal off sediment contact with water inside the chamber. An ambient water sample was collected, ambient DO concentration recorded, and then the chamber was carefully lowered over the individual. Triplicate samples were taken every 30 min for 2–2.5 h, and the chamber DO was monitored constantly with air being slowly bubbled into the chamber whenever it fell below 75% of ambient levels. One-way check valves were put in place to ensure that gas could escape during bubbling and to allow ambient water inflow during sample collection and air outflow. Oxygenation of our chambers allowed respiration to continue without initiation of hypoxic stress, but continued respiration allowed unabated carbon dioxide (CO_2) accumulation. This additional CO_2 likely reduced the pH of the chamber considerably over the course of the incubation, as was observed in similar chamber incubations in reef ecosystems (pH $\approx$ 8.0 to a pH $\approx$ 7.7; Gibson 2011). During our chamber experiments, pH was not measured and any impacts of lowered pH on the tested sponge holobionts remain unknown. However, previous work indicated that the microbiome of Mediterranean sponges was relatively stable in terms of abundance, richness, and diversity under comparable pH shifts (> 60 d at a pH $\approx$ 7.8; Ribes et al. 2016). The impact of this pH shift on the metabolic function of the microbiome is unknown, but we posit the brevity of the exposure to a large magnitude shift will temper any impact.

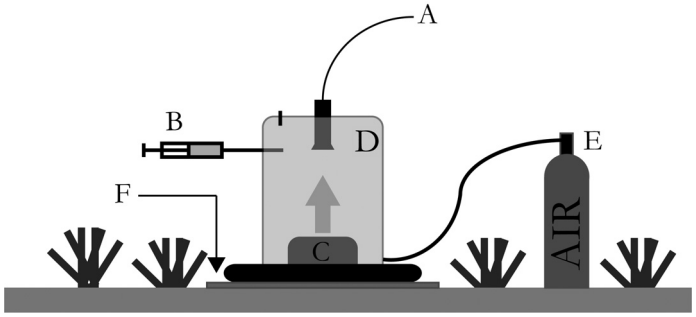

Fig. 2. Schematic representation of the benthic chamber utilized for determining sponge DIN production in situ. A–F: Oxygen optical probe with cable to surface datalogger; syringe outlet for discrete sample collection; enclosed sponge individual; 16.5-l polypropylene benthic chamber; SCUBA cylinder for aerating the chamber; 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. A photo of the chamber in use is available in the Supporting Information Fig. S2.

After the final sample was collected, the chamber was removed and the sponge was again checked with dye to ensure continued pumping activity. All the tested sponges were found to be pumping at the completion of chamber experiments. The sponge was then harvested for volume determination; sponge volumes were measured by water displacement, with each sponge being measured three times. A minimum of three replicate individuals per species were analyzed, and three chamber-only replicates were performed to observe any environmentally mediated phenomena in the absence of a sponge. Chamber experiments were performed during field excursions in May 2013, July 2013, 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 during sample collection using precombusted (baked at 450°C for > 6 h), 25 mm Whatman glass fiber filters (GF/Fs; pore size = 0.7 μm nominal) that were plumbed in-line. Whatman GF/Fs were selected for sample filtration and POM collection due to their ability to be combusted prior to use, and to allow comparisons with other reports of carbon and nitrogen cycling by sponges (e.g., Diaz and Ward 1997; Yahel et al. 2003; Southwell et al. 2008*b*). Prior to sample collection, the syringe, filter, and connected fittings were rinsed once with sample water, and the rinsing volume was discharged into the ambient water surrounding the chamber. Sample rinses were limited to minimize dilution of chamber water with ambient water introduced as sample was drawn out of the chamber. At each time point, 360 mL of water was removed from the incubation chamber (180 mL to rinse collection hardware, 180 mL for analysis). Syringes were slightly overdrawn (> 60 mL per syringe) during the collections for analysis so as to allow sufficient volume to sample rinse each vial as well as aliquot the sample to be analyzed. Syringes were immediately placed in a dark ice bath for transport to shore for subsampling and preservation (less than 8 h from collection to preservation or analysis).

Upon return to shore, samples were immediately divided into aliquots for DIN [NH_4^+ and $NO_2^- + NO_3^-$ (NO_x^-)], dissolved organic carbon (DOC), and total dissolved nitrogen (TN); DON was determined as the TN content less DIN. DOM (DOC and TN) samples were placed into three replicate, precombusted borosilicate glass scintillation vials. Each of the three collected syringes for each time point provided one sample for NH_4^+, NO_x^-, and DOM. Vials were rinsed with sample, filled with 20 mL 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 at –20°C until subsequent analysis. Ammonium (NH_4^+) samples (20 mL volume) were placed into sample-rinsed amber high-density polyethylene (HDPE) bottles. Ammonium samples were analyzed within 8 h of collection 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.

POM samples were collected from ambient water masses at all three study sites during chamber incubations to examine differences in particulate C and N composition. POM samples were collected daily at one or more of the sites during sampling campaigns in May 2013, July 2013, and September 2013 at three time points, 08:00 h, 12:00 h, and 16:00 h. Mystery Basin and FWC Dock sites were sampled during May 2013, Burnt Point was sampled during July 2013, and the FWC Dock was sampled again during September 2013. Peristaltic pumps were set up to pump ambient water (from ~ 30 cm off the bottom) through high-density polyethylene tubing to a shipboard GF/F (Whatman, 47 mm; pore size = 0.7 μm nominal), and the filtered water was discarded. Flow rates were set to 20 mL min^{-1} by adjusting pump speeds at the beginning of

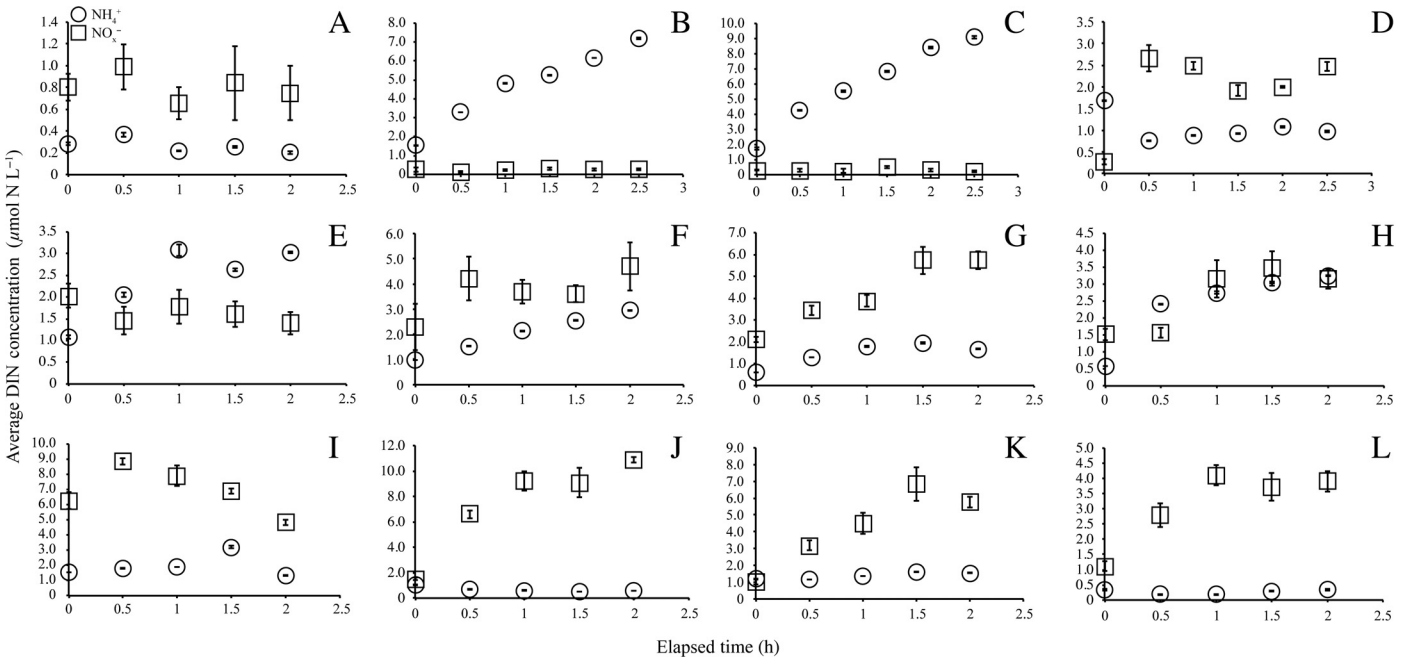

Fig. 3. The average NH_4^+ and NO_x^- concentrations from the performed chambers plotted vs. experimental time for all the tested species including the sponge-free control chambers. (**A**) Blank (sponge-free control), (**B**) *Haliclona* sp., (**C**) *Halichondria melanodocia*, (**D**) *Cinachyrella* sp., (**E**) *S. vesparium*, (**F**) *I. felix*, (**G**) *I. campana*, (**H**) *Spongia* sp. (Morphotype 1), (**I**) *Spongia* sp. (Morphotype 2), (**J**) *Hippospongia lachne*, (**K**) *G. gibberosa*, (**L**) *C. nucula.* Values represent the average concentration at a given time point for that species (μmol N L^{-1}; mean ± 1 SD).

each filter collection to ensure accurate collections. Samples were collected to give a 3 h, time-integrated sample of 3.6 L of filtered water. Sample inlets were covered with a mesh prefilter (polypropylene; pore size ≈ 100 μm) to exclude particles larger than those thought to be efficiently retained by sponges (Reiswig 1971; Yahel et al. 2003). Prefilters were replaced daily.

All plastics utilized in sample collection and processing were composed of polypropylene, high-density polyethylene, or polycarbonate and all were soaked in a 0.1 mol L^{-1} HCl bath for > 12 h 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 baking at 450°C for > 6 h to remove any residual organic matter. Combusted glassware was stored in combusted foil and bagged to minimize outside contamination prior to use. Utilized GF/Fs were baked at 450°C for > 6 h and stored in combusted foil. The sample bottles used for ammonium collections were incubated for 24 h with small aliquots of the o-phthalaldehyde working reagent following the wash protocol. This was performed to ensure removal of 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, 20 mL volumes were reacted with 5 mL of o-phthalaldehyde working reagent in 30 mL amber, HDPE sample bottles for 2.5 h. 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 detection limit of the method was 10 nmol L^{-1}, and was 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 along with experimental 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 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 and subsequent detection of DOC via nondispersive infrared spectroscopy and TN with chemiluminescence. The carbon values obtained are more accurately characterized as values of Nonpurgeable Organic Carbon due to the purging of volatile organics by vigorously bubbling during instrumental analysis. We assumed a negligible contribution to DOC from volatile organics, and henceforth the obtained values are referred to as DOC.

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 in a closed vessel overnight. Acid flushed filters were then dried at 80°C for 1 h and pulverized. Pulverized samples were placed into combusted foil boats and analyzed for C and N composition.

Data analysis

The regression of concentration vs. chambered time was used to calculate the rate of removal or release of the measured parameters by the tested sponges (mol h^{-1}). These rates of change were then normalized to organism biomass using the sponge volume measurements that were made following each incubation experiment (mol L_{sponge}^{-1} h^{-1}). The volume-normalized rates measured for replicate chambers from each species were checked against one another for quality control, and potential outlier rates were confirmed using the Grubbs Test (Grubbs 1969; $\alpha = 0.01$), and removed from the dataset. If the measured rate for one of the parameters (NH_4^+, NO_x^-, DOC, or DON) was determined to be an outlier by this test, none of the results from the flagged chamber was used in further data analysis as these spurious signals may have been indicative of experimental issues or erratic behavior in the organism. The reported release rate for each species represents the average of the sponge volume-normalized rates from each of the replicate chambers. Uncertainties in these measures were calculated based upon the deviation between replicates in the volume-normalized rate of change ($n = 3$–5).

The significance ($\alpha = 0.05$) of the calculated rate was determined using two metrics: a one-way ANOVA of the linear trend in concentration over time using the values measured in all replicate chambers, and a one-way *t*-test comparing the average calculated release rate for a given species (mol L_{sponge}^{-1} h^{-1}) vs. zero.

Spatial DIN flux estimates

Using our species-specific rates of DIN production and the volumetric biomass values obtained from the biomass surveys of Peterson et al. (2006), preliminary estimates of areal N fluxes from the sponge community were calculated. The volume of sponge biomass of each of the 11 species examined in this study was determined for each of the 206 sites surveyed by Peterson and coworkers during May 2001 and September 2001 (B. Peterson pers. comm., January 2011). This volume (L_{sponge} m^{-2}) was multiplied by the calculated sponge-normalized rate of DIN release (mol L_{sponge}^{-1} h^{-1}) to generate an N flux (mol DIN m^{-2} h^{-1}) for each of the 11 species; areal DIN fluxes were separately determined for NO_x^- and NH_4^+,

and the total DIN flux was subsequently calculated as the sum of the two estimates of N flux. The total, areal DIN flux from the surveyed sponge community was taken as the sum of the fluxes calculated for the chambered species.

Results

Limitations of fully enclosed chambers

With the enclosed system design of these experiments, the results should be regarded as approximations of natural rates as the enclosures will impact organismal behavior in both readily recognizable and unrecognizable ways. The use of fully enclosed chambers in this study allowed the tested sponges to reprocess the captured volume many times over the course of a single experiment (Supporting Information Tables S1, S2). Moreover, many of the tested species would have reprocessed the chamber volume several times before the first sample collection (Supporting Information Tables S1, S2). This excessive refiltration could have placed physiological stress on the test organism, altering its behavior over time. However, in chambers from individuals of the same species with vastly different clearance rates and reprocessing times, the increased refiltration did not appear to impact N release (i.e., *Hippospongia lachne* chambers 2 and 3; *Spongia* sp. [Morphotype 2] chambers 1, 2, and 3; Supporting Information Tables S1, S2, Supporting Information Fig. S3). There was little discernable evidence of disturbance elsewhere in the data, but most of the other examined species had comparable clearance rates across the tested individuals, and the refiltration stress would be expected to be similar across all chambered individuals. The variability that was observed was more pronounced between individuals of a given species and less over time in a single incubation (Supporting Information Tables S1, S2, Figs. S3–S7; Fig. 3). An additional result of refiltration is that the sponge can rapidly modify the concentration of reactants in the enclosed water (e.g., NH_4^+, organic matter, etc.), which can alter organism behavior as some sponges exhibit a nonlinear relationship between ambient concentration and sponge uptake or release (McMurray et al. 2016; Archer et al. 2017; Morganti et al. 2017). These behavioral alterations can be due to concentrations below the threshold for utilization (e.g., DOM), or concentrations of released waste accumulating to inhibitory excess (e.g., DIN). If a threshold value was being reached, one could expect drawdown followed by a relatively constant concentration maintained through the remainder of the experiment as the sponge produces and consumes the compound. This trend was not readily discernable in the tested parameters (Supporting Information Tables S3, S4, Figs. S4–S7; Fig. 3). However, it is also possible that the chambered individual removed the bioavailable C or N in the captured volume prior to collection of the first sample 30 min after sealing. This could explain apparent absence of production or consumption observed in DOM (Supporting Information Figs. S6, S7). The lack of evidence for some of these impacts in the data does not summarily preclude the influence of chamber incubation; it rather indicates that the impacts were manifest on a timescale or at a magnitude that was not well reflected in the collected data.

DIN release

The average ambient NH_4^+ concentrations when chamber experiments were performed were 0.4 ± 0.4 μmol L^{-1}, 1.5 ± 0.3 μmol L^{-1}, and 0.9 ± 0.7 μmol L^{-1} (mean $\pm$ 1 SD, n = 6, 13, and 25) for FWC Dock, Mystery Basin, and Burnt Point sites. The average ambient NO_x^- concentrations at those same sites were 0.9 ± 0.1 μmol L^{-1}, 0.4 ± 0.6 μmol L^{-1}, and 1.7 ± 1.4 μmol L^{-1} (mean $\pm$ 1 SD, n = 6, 13, and 25).

Many of the examined species were observed to alter N concentrations in the chamber water (Supporting Information Figs. S4, S5). Those species that modified chamber DIN exhibited roughly linear changes in N concentrations during each experiment, and these trends did not appear to diminish with time (Supporting Information Figs. S4, S5). Therefore, rates of DIN release in the chamber (mol h^{-1}) could be directly calculated as the linear regression of the concentration time-series data. Of the 48 performed chamber experiments, four generated anomalous rates that were flagged as outliers and these data were removed from further analysis (Grubbs 1969; α = 0.01). The control chambers performed without sponge biomass showed no significant linear trend in DIN over the sampled period (ANOVA of the performed linear regression; $F_{1,13} = 0.063$, $p > 0.8$ and $F_{1,13} = 0.37$, $p > 0.5$ for NO_x^- and NH_4^+, respectively; Supporting Information Tables S3, S4). This result provided confidence that any trends observed in the chambers containing sponges were the result of the sponge holobiont.

Eight of the 11 sampled sponge species exhibited statistical significance in both the trends in either NH_4^+ or NO_x^- over time (one-way ANOVA, α = 0.05; Supporting Information Tables S3, S4) and the average N flux for each DIN species (one-sample t-test vs. 0, $p < 0.05$; Fig. 4). Five of these eight sponge species generated significant, mean rates of NO_x^- release (one-way t-test vs. 0; $p < 0.05$), ranging from 48 ± 6 μmol NO_x^- h^{-1} L_{sponge}^{-1} to 141 ± 26 μmol NO_x^- h^{-1} L_{sponge}^{-1} with an average of 90 ± 5 μmol NO_x^- h^{-1} L_{sponge}^{-1} (mean $\pm$ 1 SE; Table 1) and with *C. nucula* showing the highest NO_x^- release (Table 1; Fig. 4). Significant ammonium release was characteristic of three species, *Haliclona* sp., *H. melanocoia*, and *S. vesparium*, which showed release rates of 52 ± 7 μmol NH_4^+ h^{-1} L_{sponge}^{-1}, 52 ± 5 μmol NH_4^+ h^{-1} L_{sponge}^{-1}, and 9 ± 2 μmol NH_4^+ h^{-1} L_{sponge}^{-1}, respectively (Fig. 4; Table 1).

The sponges thought to host active nitrifying microorganisms (those with significant, positive release of NO_x^-; Fig. 4; Table 1) had a greater total DIN production than those exhibiting only NH_4^+ release (unpaired t-test, df = 10, $p < 0.01$), where total DIN released was taken as the sum of the NH_4^+ and NO_x^- fluxes.

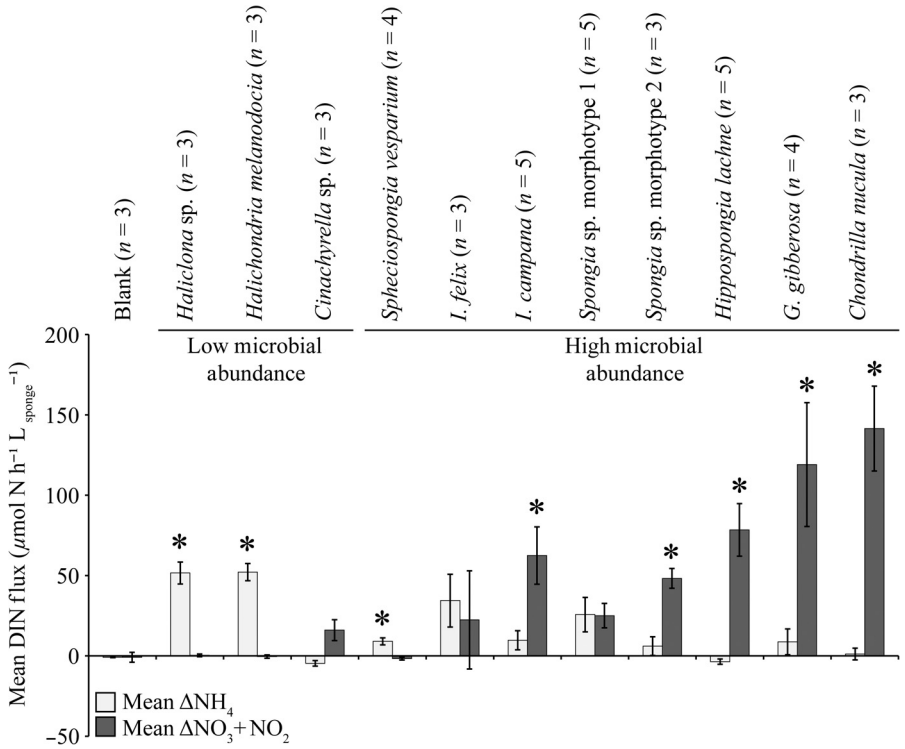

Fig. 4. Mean volume-normalized DIN fluxes for the 11 tested species in Florida Bay. Error bars represent 1 SE and asterisks (*) indicate significance for both the linear regression of concentration vs. incubation time (one-way ANOVA; $\alpha = 0.05$) as well as for the average release rate of replicate individuals (one-sample *t*-test vs. 0; $p \leq 0.05$). Data from individual chambers can be found in the Supporting Information Tables S3, S4.

DOM fluxes

The average ambient DOC concentrations when chamber experiments were performed were 175 ± 17 μmol C L^{-1}, 550 ± 30 μmol C L^{-1}, and 241 ± 48 μmol C L^{-1} (mean $\pm$ 1 SD, $n = 6$, 13, and 25) for FWC Dock, Mystery Basin, and Burnt Point sites. The average ambient DON concentrations at the same sites were 16 ± 6 μmol N L^{-1}, 38 ± 14 μmol N L^{-1}, and 29 ± 21 μmol N L^{-1} (mean $\pm$ 1 SD, $n = 6$, 13, and 25).

None of the 11 sponge replicates showed significant DON uptake or production at the 95% confidence level (Fig. 5). Among all sponge species tested, only *G. gibberosa* showed DOC uptake (one-way ANOVA; $F_{1,14} = 18$, $p = 0.001$; Supporting Information Tables S5, S6) at the rate of 1.0 ± 0.2 mmol C h^{-1} $L_{sponge}{}^{-1}$ (mean $\pm$ 1 SE; $n = 4$; one sample *t*-test vs. 0, $p < 0.05$; Fig. 5). The trends in DOC for *G. gibberosa* were best characterized by linear fits. Nonlinear models were also tested with this and other species for DOC and DON changes (de Goeij and van Duyl 2007; de Goeij et al. 2008), but this produced no significant improvement in uncertainties.

Particulate organic matter

The average ambient particulate organic carbon (POC) concentrations when chamber experiments were performed were 14 ± 1 μmol C L^{-1}, 23 ± 5 μmol C L^{-1}, and 8 ± 1 μmol C L^{-1} (mean $\pm$ 1 SE, $n = 35$, 10, and 16) for FWC Dock, Mystery Basin, and Burnt Point sites. The average ambient particulate organic nitrogen (PON) concentrations at the same sites were 2.3 ± 0.3 μmol N L^{-1}, 3.2 ± 0.6 μmol N L^{-1}, and 1.2 ± 0.1 μmol N L^{-1} (mean $\pm$ 1 SE, $n = 35$, 10, and 16). There were no significant differences in mean POC and PON levels at the FWC Dock site among 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, $p > 0.05$). The average C : N elemental ratio of the POM was 6.1 ± 0.2, 7.0 ± 0.2, and 7.1 ± 1.2 (mean $\pm$ 1 SE, $n = 35$, 10, and 16) for FWC Dock, Mystery Basin, and Burnt Point, respectively. Overall, the POM observed at the tested sites was not significantly different from the Redfield C : N ratio (6.5 ± 0.1; mean $\pm$ 1 SE; one sample *t*-test vs. Redfield (1958) C : N; df = 60; $p > 0.4$).

Spatial DIN flux estimates

Seventy-seven of the 207 sites surveyed by Peterson et al. (2006) featured the sponge species we studied, and at each location our selected species represented 49% $\pm$ 39% (mean $\pm$ 1 SD) of the surveyed biomass. There were 38 sites surveyed by Peterson et al. (2006) that did not show any contribution from our examined species, and in total, these sites represented 14% of the total biomass surveyed bay-wide (Peterson et al. 2006). The areal DIN flux estimated using the sponge community biomass reported by Peterson et al. (2006) and our

Table 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. unpubl. data).

Species	*N*	NH_4^+ flux (μmol L^{-1} sponge h^{-1})	NO_x^- flux (μmol L^{-1} sponge h^{-1})	Source
Control	3	–0.9±0.2	–0.9±3.1	This study
Haliclona sp.	3	52±7*	0.3±0.8	This study
Halichondria melanodocia	3	52±5*	–0.4±1.0	This study
Cinachyrella sp.	3	–4.7±1.8	16±7	This study
S. vesparium	4	9.0±2.2*	–1.7±0.9	This study
I. felix	3	34±17	22±31	This study
I. felix		41±16	270±63	Southwell et al. (2008*b*)
I. campana	5	9.7±6.0	62±18*	This study
I. campana		220±50	90±20	Southwell et al. (2008*b*)
Spongia sp. (morph. 1)	5	26±11	25±8	This study
Spongia sp. (morph. 2)	3	6.1±5.8	48±6*	This study
Hippospongia lachne	5	–3.6±1.6	78±16*	This study
G. gibberosa	4	8.7±8.0	119±38*	This study
C. nucula	3	1.1±3.6	141±26*	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; $\alpha = 0.05$) and significance of average DIN production (one-sample *t*-test vs. 0, $p \leq 0.05$).

chamber flux values (Table 1) ranged from 0 μmol N m^{-2} h^{-1} at sites where no sponge biomass was observed to 540 ± 100 μmol N m^{-2} h^{-1} where the sponge population was dominated by *C. nucula* (Fig. 6).

Discussion

We found clear evidence of DIN release from many of the sponge species common to Florida Bay. The N efflux from these organisms ranged from 9.0 ± 2.2 μmol N h^{-1} L_{sponge}^{-1} to 141 ± 26 μmol N h^{-1} L_{sponge}^{-1} (Table 1). The range of observed N release rates across the tested species provides evidence that the composition of the sponge community will have a large impact on the total predicted flux of DIN from these organisms. Similar to what has been shown on reef environments, sponge communities dominated by HMA species, and primarily *C. nucula*, will likely produce the greatest N flux. Further, these results provide important benchmark values for comparisons between localized sources of N to Florida Bay.

POM and DOM remineralization by Florida Bay sponge holobionts

We hypothesized that the HMA and LMA sponges examined in Florida Bay would exhibit divergent behavior in organic matter uptake because of the diverse metabolic capabilities of these organisms observed in other environments (e.g., Reiswig 1974; van Duyl et al. 2008; Pawlik et al. 2015). Surprisingly, the observed DOM utilization was minimal (Fig. 5). On average, none of the tested species exhibited significant trends in DON production or removal (Fig. 5, Supporting Information Fig. S7), and the only species that exhibited significant changes in DOC was *G. gibberosa*. This species showed DOC uptake at a rate that was similar to other HMAs found on reefs (e.g., Yahel et al. 2003; de Goeij et al. 2008; Fig. 5). Recent work has implicated ambient conditions in the variable behavior of sponges (McMurray et al. 2016; Archer et al. 2017; Morganti et al. 2017), but our results have no discernable correlation with their starting conditions. This is most likely to be a function of the refiltration of the water by the enclosed sponge, as their starting conditions are quickly modified by the sponge effluent (Supporting Information Figs. S4–S7). However, the only sponge to consistently remove DOC (*G. gibberosa*) was the only HMA species that was tested in Mystery Basin, which had notably higher ambient DOC concentrations than the other sites (550 ± 30 μmol C L^{-1} in Mystery Basin compared to 175 ± 17 μmol C L^{-1} and 241 ± 48 μmol C L^{-1} for FWC Dock and Burnt Point, respectively). Species in Florida Bay may have an elevated threshold concentration at which point DOC removal is favorable, but future nonincubation sampling will be required to effectively examine this hypothesis. The lack of DOM utilization could implicate POM as a primary food source for Florida Bay sponges. However, the POM data that was collected only serves to characterize the ambient water masses, and we lack

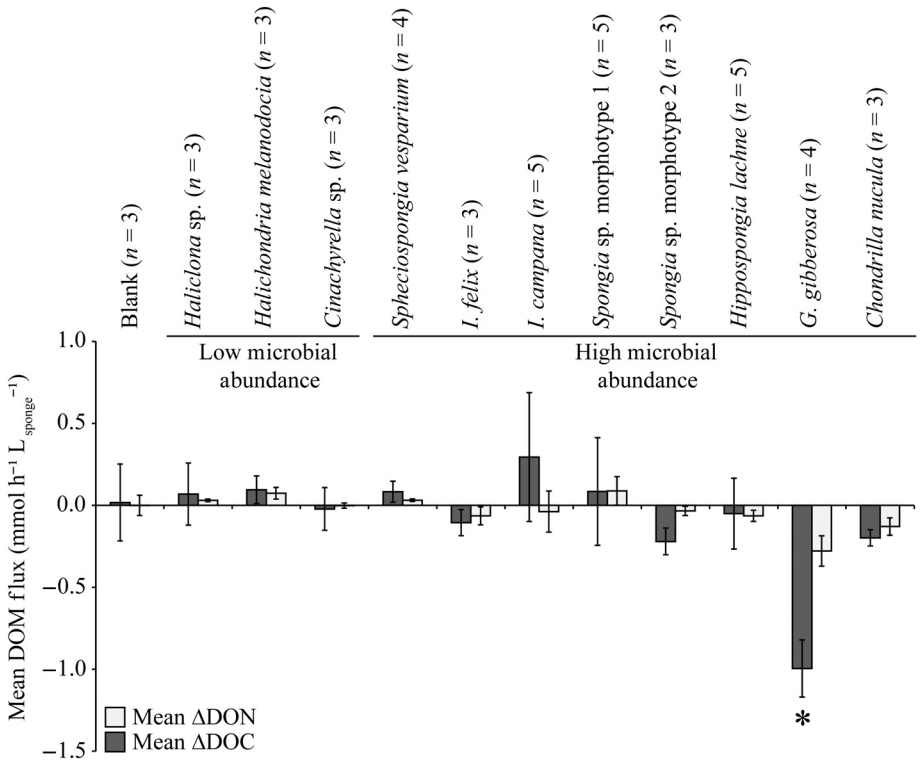

Fig. 5. Mean volume-normalized DOC and DON fluxes for the 11 tested species in Florida Bay. Error bars represent 1 SE and asterisks (*) indicate significance for both the linear regression of concentration vs. incubation time (one-way ANOVA; $\alpha = 0.05$) as well as for the average release rate of replicate individuals (one-sample *t*-test vs. 0; $p \leq 0.05$). Data from individual chambers can be found in the Supporting Information Tables S3, S4.

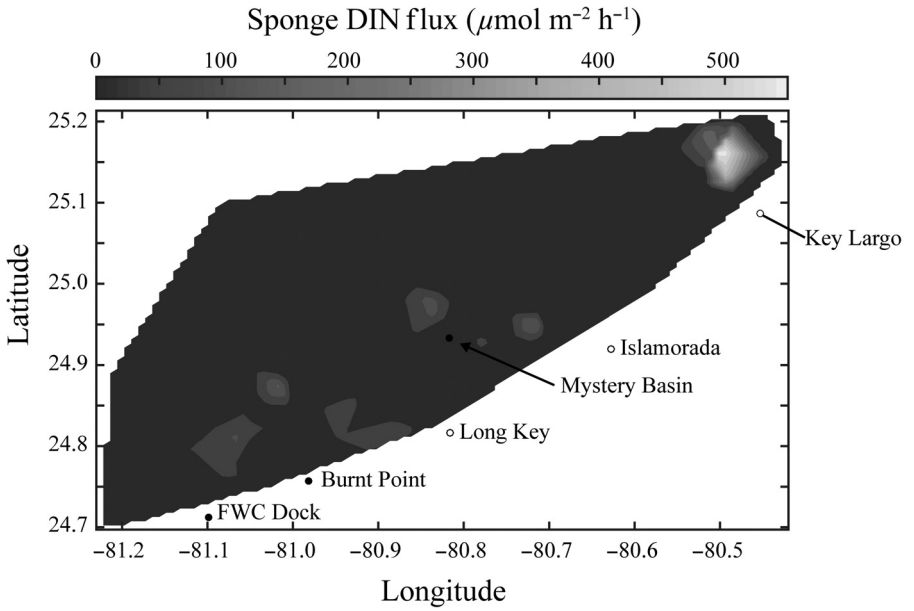

Fig. 6. Contour plot of the estimated DIN flux from the sponge community in Florida Bay. Fluxes were calculated using bay-wide biomass surveys from Peterson et al. (2006) and calculated rates of N efflux from the 11 species examined herein.

these measurements altogether from within the chamber experiments. This precludes us from assessing the relative importance of POM and DOM for Florida Bay species.

The absence of DOC and DON utilization or release is most parsimoniously explained by the previously highlighted methodological limitations (*see* "Limitations of fully enclosed

chambers" section). These factors likely acted in concert, leading an underestimation of the ingested or produced DOM (as in Jiménez and Ribes 2007). Of note in this context is that sponges, including one of the species analyzed herein, *I. felix*, can be both sources and sinks of organic matter (e.g., Archer et al. 2017). This continual production and consumption in the chambered water mass would obscure overall trends in behavior and could similarly stymie attempts to determine uptake or production over time.

Interspecific and intraspecific variability in the rate of DIN release

Our observations lend further evidence to the idea that sponges may be important agents of N cycling in coastal environments, particularly those with large populations of HMA sponges (e.g., Corredor et al. 1988; Southwell et al. 2008*b*, Keesing et al. 2013). The DIN released by the tested sponges represents the combined influence of modification of ambient DIN, remineralized N from ingested organic matter, as well as potentially "new" N from N_2 fixation (e.g., Lesser et al. 2007; Zhang et al. 2014). We hypothesize that N_2 fixation will contribute minimally to sponge-released DIN (e.g., Ribes et al. 2015), however, from the present data, we cannot assess if or what proportion is contributed by sponge-hosted diazotrophs. Regardless whether it is "new" or recycled, sponge-sourced bioavailable DIN should be particularly important in the western areas of Florida Bay where primary production is often N limited (Fourqurean et al. 1993; Phlips et al. 1999). Sponge-mediated production of NO_x^- is thought to be due to microbes within the sponge tissue mediating the nitrification of available ammonium (*see* Fiore et al. 2013). The NH_4^+ removed by NO_x^- producing species is insufficient to account for rate of nitrification observed (Table 1; Fig. 4, Supporting Information Figs. S4, S5), thus necessitating ammonium production by the sponge (either by ammonification of organic matter or N_2 fixation). The accumulation of NO_x^- did not appear to trail ammonium production in those species thought to be hosting nitrifying populations (Fig. 3). This indicates that NO_x^- production was not likely to be due to refiltration and nitrification of sponge exhaled ammonium, but rather that rapidly coupled ammonium production and subsequent microbial nitrification occur within sponge tissues. This provides additional evidence for a phenomenon that has been observed in both incubation and nonincubation sampling (e.g., Corredor et al. 1988; Southwell et al. 2008*b*; Fiore et al. 2013).

Eight of the 11 chambered species exhibited significant DIN efflux, with the largest component of N release occurring as NO_x^- (Fig. 4; Table 1). The spectrum of N release rates observed among the 11 species agrees with the results of other incubation-style measurements for a variety of sponges in the Caribbean and Mediterranean Seas (Jiménez and Ribes 2007; Southwell et al. 2008*b*; Schläppy et al. 2010). However, of the tested species, only *C. nucula*, *I. felix*, and *I. campana* have been previously examined for sponge-volume normalized rates of DIN release (e.g., Corredor et al. 1988; Diaz and Ward 1997; Southwell et al. 2008*b*). These previous assessments were performed on sponge individuals collected from oceanic coral reefs that normally exhibit profoundly different environmental characteristics as compared to our sampled sites in Florida Bay (e.g., temperature, salinity, concentrations of dissolved and particulate constituents, pH, etc.; e.g., Boyer et al. 1999; Stokes et al. 2011). The environmental divergence predicted between previously tested reef sponges and the same species in Florida Bay will facilitate an examination of intraspecific variability across habitat types. However, these comparisons could be additionally confounded by methodological differences.

The individuals of *C. nucula* tested herein yielded the highest sponge-volume normalized rate of N efflux of all the tested species (Fig. 4; Table 1). The rates of NO_x^- release observed for *C. nucula* were similar to a previous assessment of this species by Diaz and Ward (1997), but were much higher than the rates reported by Corredor et al. (1988) (Table 1). The discrepancy between these was likely due to alleviating the inhibiting effects of chamber volume that were thought to have contributed to the relatively diminished rates observed by Corredor et al. (1988) (Diaz and Ward 1997). The broad comparability between the measured rates despite differences in the incubation methodology may be due to increased resilience to perturbation or rapid healing as this species is frequently noted as an aggressive competitor for space, often found overgrowing neighboring organisms (Vicente 1990; Aronson et al. 2002). It also suggests minimal intraspecific variability in the N release in this species and implies a broad applicability of these N release rates for use determining nutrient regeneration in environments with large populations of *C. nucula*.

The rate and speciation of N release from the tested *Ircinia* species (*I. felix* and *I. campana*) exhibited a considerable deviation from previous assessments on Florida Keys reefs (Southwell et al. 2008*b*; Table 1). For *I. campana*, this difference is most pronounced for NH_4 release rates, yet there are no apparent differences between the NO_x^- release rates in this species (Table 1). In *I. felix*, the NO_x^- are similar, but the NH_4^+ are drastically different (Table 1). The incongruities observed in the rates of N release between this and the experiments of Southwell et al. (2008*b*) may be due methodological deviations or natural intraspecific variability in response to differences in environmental conditions (e.g., Bayer et al. 2008; Archer et al. 2017; Morganti et al. 2017). Large differences in the factors that have been hypothesized to generate the observed changes (temperature, salinity, DO, and DIN availability; Bayer et al. 2008; Archer et al. 2017; Morganti et al. 2017) also occur between the FL Keys reef tract sites tested in Southwell et al. (2008*b*) to locations in FL Bay (e.g., Boyer et al. 1999; Stokes et al. 2011). It is plausible that differences in one or more of these environmental parameters

may produce intraspecific variability in DIN release by the tested *Ircinia* individuals.

Additionally, these species have been shown to possess phototrophic symbionts as indicated by high concentrations of chlorophyll *a* in its tissues (Erwin and Thacker 2007; Southwell et al. 2008*b*; Archer et al. 2017). The activity of the photosymbionts in the sponge tissues could have a large impact on DIN dynamics, particularly those of ammonium, due to demand from photoautotrophic utilization. Disruption of the photosynthetic biomass in sponge tissues through experimental manipulation, seasonal or local differences in photosymbiont abundance, or shading during incubations could have significantly impacted the measured rates of DIN release.

DIN speciation and production rates vs. sponge holobiont composition

The differences in the chemical speciation of DIN released by HMA and LMA sponges provides further evidence for the importance of sponge-hosted microbial communities in N recycling processes (e.g., Jiménez and Ribes 2007; Bayer et al. 2008; Southwell et al. 2008*b*; Fig. 4). DIN released from the LMA species *Haliclona* sp. and *Halichondria melanodocia* was exclusively NH_4^+ (Table 1; Fig. 4), and was likely a straightforward result of organic matter respiration and ammonification mediated by the sponge animal (Diaz and Ward 1997). By contrast, HMA species rapidly nitrify this heterotrophically produced ammonium, leading to a preponderance of NO_x^- release (e.g., Bayer et al. 2008 and citations within, Hoffmann et al. 2009; Schläppy et al. 2010).

When comparing the species with significant N fluxes, the rates observed in HMA sponges were typically larger than those from the LMAs (Fig. 4; Table 1). HMA sponges generally exhibit slower water pumping rates and have a higher surface area to volume ratio of their internal aquiferous structures than LMA species (Weisz et al. 2008). These characteristics should combine to enhance chemical exchange and reactions of inorganic and organic materials throughout the internal canals of HMA sponges. We hypothesize that this contributes to concentrated DIN release in HMA sponges, particularly those that host active, nitrifying microbes. It is possible that the difference between the DIN release rates observed for HMAs and LMAs was due to a more pronounced underestimation of N flux in LMAs. The faster pumping rates in LMA species could have led them to deplete the metabolic resources in the enclosed volume more quickly than tested HMA species. However, the rates of DIN release for the tested LMAs did not appear to diminish over the course of the experiment (Fig. 3, Supporting Information Fig. S4), which argues against resource limitation leading to suppressed rates in these species.

One of the three tested LMA species and two of the eight HMA species did not exhibit significant DIN flux (Fig. 4; Table 1). Individual chamber experiments with these three species yielded variable trends in DIN release; however, the replicates from these species did not feature the consistent behavior that was present in the other sponge species. The variability in their behavior may have been a result of enhanced sensitivity to repeated filtration of the chamber water, which could have led to physiological stress during incubations and to the correspondingly erratic behavior.

The species *S. vesparium* is an oddity among sponges classified as HMA given that it was observed to release only NH_4^+ (this study; Southwell et al. 2008*a*), and it has the lowest volume-normalized DIN efflux of all the sampled species (Fig. 4; Table 1). There have been conflicting reports (e.g., LMA, Poppell et al. 2014 vs. HMA, Gloeckner et al. 2014) about the microbial density of this putative HMA species after the initial classification (Weisz et al. 2008). It is unknown which other tested species are similarly characterized by inconsistencies in the density and diversity of their tissue microbial consortia. This is particularly true for distinctions made at the genus level, as marked differences have been demonstrated in the microbial consortia of sponges in the same genus, including one of the genera we studied here (*Cinachyrella* sp.; Cuvelier et al. 2014). Therefore, the HMA/LMA distinctions we have presented herein should be viewed as preliminary in the absence of species-specific analyses.

Estimated sponge N fluxes to overlying waters

Our results indicate the potential for the sponge community to represent the largest source of DIN in Florida Bay. Field surveys by Peterson et al. (2006) revealed sponges at more than 70% of the sites analyzed, with biomass contributions ranging from 0.5 g sponge dry weight m^{-2} to 1443 g sponge dry weight m^{-2}; areal coverage of sponges was focused on the hard-bottom regions along the southern edge of the bay as well as the eastern and western margins (Peterson et al. 2006). Applying measured N efflux rates for the 11 species in this study to these extensive biomass surveys illustrates that the quantity of released N depends not only on the total biomass of sponges, but also on the species composition of the community (Fig. 6). The largest calculated flux of N was not found associated with the greatest total sponge biomass, but instead was found at a site in the northeastern corner of the Bay that was dominated by large communities of *C. nucula*. The calculated flux of N at this site (540 ± 100 μmol N h^{-1} m^{-2}; Fig. 6) is roughly comparable to previous estimates of DIN contributions from the sponge community on Caribbean reefs (Corredor et al. 1988; Diaz and Ward 1997; Southwell et al. 2008*b*). In addition to local peaks in N flux, we also found that the calculated N fluxes from the sponge community exceeded other locally important sources of DIN at almost half of the sites where the tested species were found (31 of the 77 sites; locally important sources of DIN include N flux from seagrass/sediment, 15 ± 11 μmol N h^{-1} m^{-2} [Yarbro and Carlson 2008], groundwater discharge, $< 13 \pm 2$ μmol N h^{-1} m^{-2}, [Corbett et al. 1999], and discharge from the Everglades, ~ 5 μmol N h^{-1} m^{-2}, [Rudnick et al. 1999]). However,

the importance of the sponge population in localized nutrient cycles (especially the relative significance of HMA and LMA species) will depend on both on their rate of N release as well as their pumping rate, as this will dictate how efficiently the sponge population can effect change on water column chemistry. Therefore, directly measuring pumping rate and N efflux using nondisruptive techniques will be required to generate the most accurate models of sponge population impacts on local water quality.

These preliminary estimates required considerable extrapolation using biomass data that is over a decade old, and as a result should be regarded cautiously. Florida Bay is subject to mass sponge mortality events (e.g., Butler et al. 1995), that continue to reshape Bay-wide sponge biomass distributions. However, to our knowledge, the data collected by Peterson et al. (2006) represents the most complete sponge biomass survey of Florida Bay to date, and provides a means to view how sponge N release can impact local nutrient budgets throughout this environment. Future quantifications will require species-specific biomass estimates at a high spatial resolution to accurately determine the magnitude and ecological significance of the N contributed by the sponge community.

Conclusion

Our results indicate a large contribution of N to the water column by sponge communities living in Florida Bay. While the calculated areal flux is patchy and locally dependent upon sponge community composition and density, the sponge-mediated N flux 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 seagrass population adjacent to sponge-rich hardbottoms. In addition to spatial variability, these flux contributions are subject to temporal variability associated with the demonstrated crashes in the sponge population in this environment (Butler et al. 1995; Peterson et al. 2006; Wall et al. 2012). Decimation of sponge biomass during hypoxia or other damaging events can be expected to have a significant, long-term impact on the water column N content and speciation.

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Conflict of Interest

None declared.

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