Consumption of dissolved organic carbon by Caribbean reef sponges

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Abstract

Sponges are conspicuous and abundant within the benthic fauna on Caribbean reefs. The ability of these organisms to efficiently capture carbon from particulate sources is well known and the importance of dissolved organic carbon (DOC) uptake has been recognized for several species. We surveyed DOC ingestion by seven sponge species common to Florida Keys reefs using non-disruptive sampling methods on undisturbed individuals. Three of the seven species exhibited significant DOC removal ranging from 13 to 24% of ambient concentrations. The tested species that removed DOC host large microbial consortia within their tissues, while the converse was observed for those that did not. This divergent behavior may suggest an important role for sponge associated microbes in the utilization of DOC by these species. The feeding behaviors of individuals of *Xestospongia muta* were then monitored over time to investigate its respiratory consumption of particulate and dissolved organic C. The uptake rates of dissolved oxygen (DO) and organic carbon by two undisturbed individuals revealed that DOC represented 96% of removed C, and that the tested individuals removed approximately equal quantities of C and DO. This demonstrates that *X muta* largely satisfies its respiration demands through DOC consumption, and that DOC likely represents the dominant C source for biomass production and cell overturn in this species. These results further illustrate the metabolic importance of DOC to sponges, and suggest that these organisms are an important pathway for remineralizing organic matter on Caribbean reefs.

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, Schutte et al. 2010, Perry et al. 2013). Concomitant with this decline, sponge density has increased, and sponges now dominate benthic biomass on some reef ecosystems (Aronson et al. 2002, McMurray et al. 2010, McMurray et al. 2015). Shallow water Caribbean sponge communities have large water filtering capacity (Corredor et al. 1988, Weisz et al. 2008, McMurray et al. 2014) and their exhaled water carries with it the imprint of a wide variety of biogeochemical transformations mediated by the sponge animal and its associated microbial and macrofaunal communities, henceforth referred to as the sponge holobiont (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) species; Hentschel et al. 2006) that drive diverse nutrient element transformations. In contrast, species with low numbers of associated microorganisms (termed low microbial abundance (LMA); 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).

Caribbean sponge species derive much of their required metabolic C through heterotrophic feeding, which contrasts with Indo-Pacific species that rely heavily on photoautotrophy (e.g., Wilkinson 1983, 1987, Powell et al. 2014). Heterotrophic sponges efficiently feed across a wide range of particle sizes (Reiswig 1971); however, early studies of sponge energetics (Reiswig 1971, 1974, 1981) revealed an apparent discrepancy between their uptake of particulate organic carbon (POC) and metabolic C demands as indicated by their rates of dissolved oxygen (DO) removal. Many species of sponge have since been shown to fill this metabolic C gap through dissolved organic carbon (DOC) utilization (Yahel et al. 2003), and nutritional use of this C source has been demonstrated in both HMA and LMA species (e.g., de Goeij et al. 2008a, de Goeij et al. 2013, Mueller et al. 2014b). However, many investigations of

food limitation in sponge communities focus solely on the nutritional use of particulate C (e.g., Lesser 2006, Trussel et al. 2006, Lesser and Slattery 2013). These prominent studies of resource limitation in Caribbean sponges are a central part of the larger debate concerning the relative importance of "bottom-up" versus "top-down" processes in controlling sponge ecology (e.g., Pawlik et al. 2015a, Slattery and Lesser 2015, Pawlik et al. 2015b). Understanding what species utilize DOC as a metabolic C source and to what extent this satisfies their energetic requirements will be critical to fully elucidating the role of bottom-up processes in sponge ecology.

Non-encrusting sponge species have been shown to utilize DOC (e.g., Yahel et al. 2003, Mueller et al. 2014b, McMurray et al. 2016), but compared to cryptic species, evidence of DOC uptake in satisfaction of their metabolic C requirements is limited. Investigations of encrusting sponges commonly found in coral cavities showed that ~40% of their C retention is accounted for by respiration; the remainder is hypothesized to be assimilated to account for rapid cell turnover in the sponge animal (de Goeij et al. 2008a, 2008b, 2009). This high rate of DOC uptake and cell turnover is thought to yield tremendous amounts of cellular debris that is expelled by the sponge and subsequently available for consumption by detritivores, forming the putative "sponge loop" (de Goeij et al. 2013). At the reef scale, the rate of sponge DOC removal is thought to be equivalent to the rate of C fixation from gross primary productivity (de Goeij et al. 2013). Given the abundance of sponges on Caribbean reefs and the magnitude of their ability to consume and recycle dissolved organics, these processes may prove to be critical to energy and nutrient exchange by making the resources within the DOM pool available to higher trophic levels through detrital release or fixation as sponge biomass.

In this study, we tested seven non-boring, non-encrusting reef species for evidence of DOC uptake to determine the extent of both interspecific and intraspecific variability in the use

of this C source, as well as to examine differential DOC removal in HMA and LMA species. We then examined DOC removal and respiration oxygen demand by the HMA species *Xestospongia muta*, commonly called the giant barrel sponge, to quantify the relative importance of DOC versus POC in satisfying the metabolic demand of the sponge. The pumping rates of *X. muta* as well as removal of DOC, POC, and DO were simultaneously measured in situ. All measurements were performed using undisturbed individuals attached to their original substrate to minimize any physiological changes resulting from physical manipulation.

Methods

Water samples for this study were collected 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. 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).

Sample collection: DOC uptake survey

Seven sponge species were sampled in this study: *Callyspongia vaginalis, Ircinia strobilina, Mycale laxissima, Niphates digitalis, Spheciospongia vesparium, Xestospongia muta, and Verongula gigantea*. These all have an erect morphology, distinct oscula for expelling filtered water, and are abundant in the Florida Keys reef ecosystem (Weisz et al. 2008, McMurray et al. 2010). *Xestospongia muta* and *V. gigantea* have a massive barrel morphology with a large central cavity that receives the outflow of multiple small oscula distributed across its interior surface. *Mycale laxisima* commonly has smaller, barrel-shape lobes, each with a single exhalent opening. *Callyspongia vaginalis* and *N. digitalis* are generally elongated tube or vase

shaped with a single orifice for expelling filtered water. *Ircinia strobilina* and *S. vesparium* are round and squat with multiple distinct oscula collected into a central osculum group. Individual sponges were haphazardly selected by divers among healthy looking, actively pumping sponges. These species cover a range of population sizes, pumping rates, tissue densities, and microbial abundance (Table 1). Of the sponges investigated, *I. strobilina*, *S. vesparium*, *V. gigantea*, and *X. muta* are classified as HMA species, while *C. vaginalis*, *M. laxisima*, and *N. digitalis* are classified as LMA species (Gloeckner et al. 2014).

Water samples were collected by saturation divers working from ARB and surface SCUBA divers at depths ranging from 12 m to 30 m during a range of dates in July and October 2008, in May, July, and September 2009, and in July and October 2010. Ambient waters near the exterior walls of the sponge (<20 cm from the sponge) and excurrent waters pumped out of 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, "InEx" 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). Each water sample was simultaneously collected and filtered (Whatman GF/F; 0.7 μm nominal pore size) using a 60 mL polypropylene syringe connected to a 3-way polycarbonate stopcock. One arm of the attached stopcock had an in-line filter and 15 cm of small-diameter (4.5 mm ID), 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 (baked at 450°C for >6 h), 25 mm Whatman GF/F filter was used 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, Mueller et al. 2014b, McMurray et al.

2016). During sample collection, the syringe, filter, and sample collection tubing were rinsed three times 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 s^{-1}) to ensure the collected samples were representative of the desired water mass. This sample collection rate is more than two orders of magnitude lower than the previously measured pumping rate values of the tested sponges on Conch Reef (e.g., Reiswig 1974, Weisz et al. 2008, McMurray et al. 2014). This provided confidence that the sampled water represented exhalent water from the sampled sponges.

After sample collection was completed, the stopcock was closed and the sample was returned to the surface at the completion of the dive or to ARB to await transport to the surface in a dark ice bath. Samples were stored on ice in darkness prior to and during transport to shore for processing and preservation (< 12 hrs from collection to shore-based processing and storage). At the shore-based lab, samples were immediately divided into triplicate borosilicate glass scintillation vials. Vials were first rinsed with the collected water, they were then filled with 20mL of sample, and 100 μL of 50% H_3PO_4 was added. After the acid addition, the sample was stored at 4°C until analysis.

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 h 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 h and stored in combusted foil until use.

Sample collection: metabolic utilization of DOC

Xestospongia muta was selected for further examination as it represents 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, 2014). *Xestospongia muta* is an HMA species and has tissue bacterial densities of up to 8 x 10^9 microorganisms per gram of sponge wet weight (Hentschel et al. 2006). We hypothesized that its tissue microbial consortia could enhance metabolic DOC demand and utilization in the holobiont relative to species without dense tissue microbiomes. Further, this species has been demonstrated to remove DOC from seawater (McMurray et al. 2016).

During August 2011, two healthy-looking individuals of *X. muta* were chosen for in situ respiration measurements and water sample collection. Two large specimens were used due to limited availability of in situ instrumentation as well as to maximize the temporal coverage of sampling for the tested sponges. The large size and barrel morphology of this species 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 hand-measured using rulers by saturation divers to calculate their estimated tissue volumes without contact. The sponge volumes were then calculated using the formula $V_{sponge} = 28.514 \times \text{osculum diameter}^{2.1}$ (McMurray et al. 2010), which generated volumes within 5% of those generated using geometric

approximations of the sponge. The volumes calculated using the method of McMurray et al. (2010) will be used for this discussion.

Sponge excurrent and ambient water samples were collected by saturation divers working out of ARB from August 9 to 16, 2011 employing the same, "InEx" methodology as was used for those samples collected during the broader reef survey. Samples were collected at three time points every day, 07:00, 12:00, and 17:00 (AM, Noon, and PM, respectively), to reflect any changes from morning to evening resultant from light-associated alterations in the sponge holobiont behavior; *X. muta* hosts dense populations of cyanobacteria in its ectoderm (Erwin and Thacker 2007). Water samples were collected at 19 different times on both of the 2 individuals (k = 19 time points, n = 2 individuals). Upon completion of sample collection, the stopcock was closed and the sample was stored in a dark ice bath inside ARB. Samples were stored on ice less than 8 hours prior to being taken to the surface and transported to shore for subsampling and preservation (< 12 hrs from collection to shore-based processing, preservation, and storage). Shore-based processing was performed identically to the samples from the broader survey.

POC sample collection

In situ samples were also collected to examine particulate organic matter (POM) uptake by one of the two instrumented individuals of *X. muta*. POM samples were collected repeatedly from the same individual (sponge 2) during the mission using a passive in situ vacuum filtration apparatus (N. Lindquist pers. comm. 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. This methodology was employed due to the exceedingly low particle loading in reef water, which necessitated large volumes of water be filtered to obtain an analytically robust sample. As such, filters used for the filtration of DOC

samples contained insufficient POM for this purpose and were discarded after use. Flow rates on the in situ apparatus were controlled to draw approximately 6 $L_{seawater}$ h^{-1} to match the sampling flow rate for water sampling by syringe. As with the syringe samples, this collection rate is more than three orders of magnitude lower than the pumping rate found in individuals of *X. muta* (e.g., Southwell et al. 2008, Fiore et al. 2013, McMurray et al. 2014). During the 2011 POC sample collections, additional safeguards of this fact were provided by the availability of real-time pumping rate data that provided confirmation that the 6 $L_{seawater}$ h^{-1} sampling rate represented less than 0.01% of the instantaneous fluid flux from the sponge.

Samples were collected simultaneously from ambient and excurrent water masses giving a 2 hour, time-integrated sample of 13 L of filtered water. The collections were initiated during times (AM or PM sample periods) when discrete DOC samples were being collected, such that there would be temporal overlap between these collections. 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 survey samples were collected from 12 haphazardly selected, healthy-looking, and actively pumping individuals of *X. muta*. There samples were collected over several days in July, September, and October 2007.

Sample Analysis

DOC samples were analyzed using high-temperature catalytic oxidation (HTCO) and non-dispersive infrared spectroscopy (NDIR) using Shimadzu TOC-5000 (2008, 2009, 2010

samples) and TOC-L CPH/CPN (2011 samples) organic carbon analyzers. 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 for additionally quality assurance and control. 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 vigorously 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 as Non-Purgeable Organic Carbon (NPOC). We assume a negligible contribution to DOC in this environment 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 duplicate analysis tubes was 2.3 μmol C $L_{seawater}^{-1}$, 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 content.

In situ Instrumentation

The water pumping speeds and respiration rates of the two selected specimens of *X. muta* were continuously measured from May 25 to August 17, 2011, however for the purposes of this study, only the period where discrete water samples were collected (August 9 to 17, 2011) will be considered. The water pumping speed was determined using Nortek Vector acoustic Doppler velocimeters (ADVs) and respiration rates were measured using an Aanderaa Data Instruments (AADI) SeaGuard system with dual oxygen optode sensors placed to measure ambient versus excurrent waters. 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 AADI SeaGuard system were cabled to the ARB Life Support Buoy 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" at 4 Hz, every 5 minutes, for the duration of the deployment. 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 sponge had 26 of these sample blocks (19 daytime periods where discrete DOC samples were collected, 7 nighttime) in which data were averaged; each block of data represented 12 sampling "bursts" totaling to approximately 1200 individual measurements of vertical fluid speed. Similarly, pumping velocity was averaged for sponge 2 during each of the two-hour time periods when POC samples were collected; similar to those for DOC collections, each of these blocks represented 24 ADV sampling "bursts" and comprised 2400 individual measurements of 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. Using fluorescein dye injections across the oscular plane of the tested sponges, velocity distributions were observed to decrease moving radially from the center, and thus the excurrent plumes were determined to be poorly approximated as plug flows and were rather assumed to be parabolic (Vogel 1994, Savarese et al. 1997, McMurray et al. 2014). Therefore, the average velocity of excurrent flow across the planar area of the osculum ($\bar{W}$) was taken as one-half of W_{exc}, which represents the measured centerline velocity of the excurrent flow from the tested sponges

($\overline{W}$=0.5W_{exc}; Vogel 1994, Savarese et al. 1997, McMurray et al. 2014). Volumetric flow (V) was then calculated as $V=A_{osc}(0.5 \times W_{exc})$, where A_{osc} is the area of the osculum. Oscular openings for both sponges were approximately elliptical and the areas were approximated as such using diver-obtained measurements of their major and minor axes.

To measure DO uptake by the sponges, a AADI SeaGuard system equipped with a digital optode string for continuous measurements of dissolved oxygen (DO) was deployed simultaneously with the ADVs. During the deployment, the foil surfaces of the optode DO sensors were periodically cleaned by gently wiping the surface of the sensor with a gloved finger to minimize the growth of photosynthetic algae. Pairs of DO optode sensors were positioned to simultaneously sample ambient and effluent waters of the targeted individuals of *X. muta* to determine DO drawdown and calculate a respiration rate for the sponge holobiont. Each of the DO sensors collected DO concentrations every 30 seconds for the duration of the deployment. These data were treated similarly to the ADV data in that they were subsampled into blocks based on the dates and times that discrete sample collection occurred (k = 19 time points). 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* (k = 7 overnight sample blocks). Each of the 26 sample blocks represented approximately 120 paired ambient and excurrent data points from the tested sponges.

Results

DOC uptake by surveyed species

The mean DOC concentration of ambient reef water from all samples during the survey was 89 ± 31 μmol C $L_{seawater}^{-1}$ (mean ± 1SD). Three of the four HMA sponges sampled in this study were found to significantly reduce the concentration of DOC in the waters they processed

(matched pairs; one sample t-test vs. 0; Figure 1). *Ircinia strobilina* had the greatest removal with a mean DOC uptake of 27 ± 10 μmol C $L_{seawater}^{-1}$ (mean ± 1SE, $n = 9$, $p = 0.02$). The two common HMA barrel sponges also showed an uptake significantly different from zero: *V. gigantea* had a mean uptake of 23 ± 8 μmol C $L_{seawater}^{-1}$ (mean ± 1SE, $n = 6$, $p = 0.04$) while *X. muta* retained 10 ± 2 μmol C $L_{seawater}^{-1}$ (mean ± 1SE, $n = 65$, $p < 0.0001$). The remaining HMA sponge tested, *S. vesparium*, did not show a significant DOC uptake (-1 ± 7 μmol C $L_{seawater}^{-1}$; mean ± 1SE; $n = 6$). Of the three LMA sponges studied, *C. vaginalis*, *M. laxissima*, and *N. digitalis*, no significant change in DOC concentration between the ambient and the exhalant water was observed (Figure 1, Table 1). It bears noting that the samples collected during the survey of DOC removal represent approximately 10 minutes of sponge feeding due to the amount of time required to collect each sample. Therefore, they represent instantaneous measurements of uptake mediated by the sponges, and do not factor in the effects of sponge size and water pumping rate. Despite the limitations, these samples provide important information about the interspecific differences in the ability to process DOC, and indicate the magnitude of intraspecific variability of DOC removal.

Metabolic utilization of DOC by X. muta

Sponge volumes

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 led 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 of the osculum. These volumes agreed with values

calculated through geometric approximations of the shape of the sponge (126 L and 42 L for sponges 1 and 2, respectively).

Pumping velocity and volumetric flow

The two individuals of *X. muta* showed significantly different excurrent velocities over the measured period (Wilcoxon signed rank test; k = 19 time points, Z = -3.34, p = 0.001). Sponge 1 produced an average excurrent velocity (W_{exc}) of 5.3 ± 0.9 cm s^{-1} (mean ± 1 SD, k = 19), and sponge 2 showed an average of 4.3 ± 0.9 cm s^{-1} (mean ± 1 SD, k = 19). Individual, nighttime excurrent velocities did not differ from the corresponding daytime values nor did the averages differ between day and nighttime measurements (nighttime averages: 4.9 ± 1.4 and 4.3 ± 0.4 cm s^{-1}; mean ± 1 SD, k = 7; sponges 1 and 2, respectively). Volumetric flowrates were then calculated using the excurrent velocities and the planar area of the sponge osculum. These calculations were made assuming a parabolic velocity distribution across the area of the osculum as indicated by fluorescein injections over the tested sponges, and results in the literature (e.g., McMurray et al. 2014; $V=A_{osc}(0.5 \times W_{exc})$). Sponge 1 had a planar area of 2787 cm^2 and this generated an average volumetric flow rate of 7.4 L s^{-1}, while sponge 2 had a planar area of 929 cm^2 that produced an average volumetric flow rate of 2.0 L s^{-1}. For sponges 1 and 2, these flow rates were equivalent to filtering water more than 4,100x and 3,600x their body volume daily (0.048 ± 0.008 and 0.042 ± 0.009 $L_{seawater}$ s^{-1} L_{sponge}^{-1}; mean ± 1 SD, for sponges 1 and 2, respectively). The water pumping rates measured for the observed specimens fall on the lower end of a spectrum of values previously observed for this species (e.g., Weisz et al. 2008, Fiore et al. 2013, McMurray et al. 2014), but were very similar to the average rate observed by Lewis and Finelli (2015) at sites in the Florida Keys and the Bahamas (0.044 ± 0.007 L sec^{-1} L_{sponge}^{-1}). The discrepancy between our results and the range of others for this species was likely due to our

approximation of the velocity distribution as parabolic across the planar area of the oscula rather than as a plug that is uniformly distributed across the oscular opening.

Volumetric flow rates were individually calculated for each discrete collection period using the corresponding ADV data "block." These individual volumetric flowrates were used in conjunction with ΔDOC and ΔDO measurements to generate chemical fluxes for each time period where chemical samples (DOC, POC, and DO) were collected. Sponge 2 experienced total pumping cessation during the observed period, which lasted approximately 2.5 h (Figure 2). This cessation event overlapped with the beginning of a morning discrete sample period, and generated anomalously high difference in DO concentrations in ambient and excurrent water masses (Figure 2). As a result, DOC measurements performed over this time period were not included in the averages of measured difference between ambient and excurrent water masses. However, these measurements were included in averages of chemical flux, as calculation for these used the quantified volumetric flowrate that appropriately accounted for the altered pumping rate at the time of collection.

DOC uptake by X. muta

The average DOC concentration in ambient water surrounding the test sponges was 89 ± 1 μmol C $L_{seawater}^{-1}$ (mean ± 1 SE), and there was no significant difference between the ambient water sampled at sponges 1 and 2 (Wilcoxon signed rank test; k = 19 time points, Z = -0.684, p = 0.49). A significant difference between ambient and excurrent water masses was seen for both sponges (Wilcoxon signed rank test; k = 19 and 18, Z = -3.783 and -2.940, $p < 0.001$ and 0.003 for sponges 1 and 2, respectively; Table 2), indicative of DOC uptake. Of the 38 ambient/excurrent pairs that were collected, 33 showed DOC uptake greater than the 2.3 μmol C $L_{seawater}^{-1}$ estimated analytical precision, three indicated an increase of DOC from ambient to

excurrent, and two featured a change that did not exceed the estimated precision. Sponge 1 showed an average DOC uptake of 12 ± 1 μmol C $L_{seawater}^{-1}$ from ambient water (mean ± 1 SE, k = 19 time points; Table 2) while sponge 2, exhibited an average DOC uptake of 11 ± 3 μmol C $L_{seawater}^{-1}$ (mean ± 1 SE, k = 18; Table 2). This yielded an average DOC uptake for the two analyzed individuals of 12 ± 1 μmol C $L_{seawater}^{-1}$ (mean ± 1 SE, k = 37; Table 2). The obtained uptake values were converted to chemical fluxes using the temporally appropriate volumetric pumping rates determined by the ADV measurements and normalized to calculated sponge volumes. This yielded average DOC uptake fluxes of 2.1 ± 0.3 mmol C h^{-1} L_{sponge}^{-1} for sponge 1 and 1.7 ± 0.4 mmol C h^{-1} L_{sponge}^{-1} for sponge 2 (mean ± 1 SE, k = 19 time points for each sponge; Figure 3), and produced an average DOC uptake flux for the two sampled sponges of 1.9 ± 0.2 mmol C h^{-1} L_{sponge}^{-1} (mean ± 1 SE, k = 19 per individual, n = 2 individuals; Table 2). There were no significant differences in DOC uptake flux between the two test sponges (Wilcoxon signed rank test, p = 0.717), nor did DOC uptake fluxes exhibit any significant temporal variability over the sampled period (Friedman's ANOVA; $X^2_{(df = 18,\, n = 2)} = 23.937$, $p \approx 0.15$)

POC Uptake by X. muta

Ambient POC concentrations around sponge 2 were found to be 2.4 ± 0.3 μmol C $L_{seawater}^{-1}$ (mean ± 1 SE, k = 4 time points). The measured POC content represents only ~3% of the total organic carbon (TOC; 91 ± 1 μmol C $L_{seawater}^{-1}$; mean ± 1 SE) in ambient reef water, where TOC is defined as the sum of POC and DOC (Yahel et al. 2003).

Sponge 2 data revealed consistent removal of POC from ambient water, yielding an average uptake of 0.43 ± 0.11 μmol C $L_{seawater}^{-1}$ (mean ± 1 SD, k = 4). As with the DOC, the POC uptake values were converted to chemical fluxes using the volumetric flow rate at the time of sample collection and the calculated sponge volume, which yielded an average POC uptake

flux of 0.06 ± 0.01 mmol C h^{-1} L_{sponge}^{-1} (mean ± 1SD, k = 4; Table 2, Figure 3). Comparatively, the 12 sample pairs from 2007 showed a mean ambient POC content that was slightly higher than was observed in 2011 (3.9 ± 1.3 μmol C $L_{seawater}^{-1}$; mean ± 1 SD, n = 12; Table 2), and the 2011 sponges also retained slightly more POC (0.96 ± 0.28 μmol C $L_{seawater}^{-1}$; mean ± 1 SD, n = 12; Table 2). The 2007 collections were performed in the absence of measurements of excurrent flow velocities, which negated the ability to generate chemical fluxes for comparison with those from the 2011 samples.

DO uptake by X. muta

Both sponges consistently removed DO from pumped ambient water during the measurement period, however, in early July the ambient oxygen sensor on sponge 1 failed. For the six weeks prior to the instrumental failure (May 25 to July 3), the measured DO concentration in the ambient water was 193 ± 9 μmol DO $L_{seawater}^{-1}$ (mean ± 1 SD) at sponge 1 and 187 ± 13 μmol DO $L_{seawater}^{-1}$ (mean ± 1 SD) at sponge 2. Based on this similarity between the measured values and the proximity of the tested individuals, the ambient oxygen data from sponge 2 was used for calculating oxygen uptake by both sponges. During the period when discrete water samples were being collected both individuals showed significant differences between the DO concentration in ambient and excurrent water masses (Wilcoxon signed rank test; k = 19 and 18, Z = -3.823 and -1.982, $p < 0.001$ and $p < 0.05$ for sponges 1 and 2, respectively). However, diurnal variability was observed in the DO uptake in the target sponges, particularly for sponge 1 (Figure 4). This was likely due to either photosynthetic biomass in the tissues of the sponge (e.g., Yahel et al. 2003) or to fouling algae on the surface of the oxygen optodes. To avoid the potentially confounding influence of oxygenic photosynthesis on the observed respiration rates, the overnight values of DO uptake were used to calculate net fluxes

(Figure 3). The overnight DO uptake values for both individuals showed significant differences between ambient and excurrent water masses (Wilcoxon signed rank test; $k = 7$, $Z = -2.36$, $p < 0.02$ for both sponges 1 and 2; Table 2).

As with the DOC and POC uptake, the DO removal values were converted to chemical fluxes using temporally appropriate volumetric flow rates and calculated sponge volumes. Sponge 1 showed an average uptake of 1.4 ± 0.3 mmol DO h^{-1} L_{sponge}^{-1} and sponge 2 showed uptake of 1.7 ± 0.4 mmol DO h^{-1} L_{sponge}^{-1} (nighttime collections only; mean ± 1 SD, $k = 7$ time points for each sponge; Figure 3).

Respiration balance for X. muta

There was no difference between the uptake fluxes of DOC and DO for sponge 2 (paired t-test, $p > 0.5$, Figure 3), while there was a difference observed for sponge 1 (paired t-test, $p < 0.02$; Figure 3). However, the quantity of C consumed by the holobiont is dependent on the chemical composition of the absorbed C as well as the physiological state and cellular composition of organism itself (e.g., Koopmans et al. 2010 and citations therein). As a result, the respiration balance was quantified for the tested *X. muta* using a pair of respiratory quotients (RQ; mole $C_{respired}$ mole DO^{-1}) that span the range of what has been shown for marine invertebrates (0.7 and 1.0 mole $C_{respired}$ mole DO^{-1}; Hatcher 1989, Koopmans et al. 2010). Assuming a RQ = 1, sponge 2 exhibits balance between the absorbed DO and C whereas sponge 1 shows C removal in excess of its respiratory DO demand (Figure 3). The non-respiratory C demand for this individual was 0.7 ± 0.4 mmol C h^{-1} L_{sponge}^{-1} (mean ± 1 SE). With RQ = 0.7, sponge 2 retains the approximate balance observed with RQ = 1, and the excess C retained by sponge 1 increased proportionally to the change in the respiratory quotient (1.1 ± 0.4 mmol C h^{-1} L_{sponge}^{-1}; mean ± 1 SE).

Discussion

Of the tested sponges, HMA species exhibited significant DOC uptake whereas LMA species did not (Table 1, Figure 1). This observation conditionally supports our hypothesis that the microbial associations present in some HMA sponges found on Conch Reef directly use or facilitate the use of DOC as a metabolic C source. Further, our results indicate that DOC is the dominant organic C source for the giant barrel sponge *X. muta* (Table 2), and that metabolic DO demand in this species can be accounted for exclusively by DOC-fueled respiration (Figure 3). These results lend further support to the growing body of direct measurements showing that C utilized by many sponge species and their associated microbial consortia comes predominantly from the dissolved organic matter pool (e.g., Yahel et al. 2003, de Goeij et al. 2013, and McMurray et al. 2016).

Both the present study and the work of a host others (e.g., Yahel et al. 2003, de Goeij et al. 2013, and McMurray et al. 2016) indicate the profound importance of DOC removal by sponges on Caribbean reefs and illustrate a need for furthered understanding of this mechanism of C cycling. Three of the four HMA species in our study removed DOC from filtered water, while none of the three tested LMAs exhibited significant removal. *Spheciospongia vesparium* was the only tested HMA species that was not observed to remove DOC from filtered water (Figure 1). The specific mechanism for this difference in behavior is not immediately clear. However, there have been conflicting reports (e.g., LMA, Poppell et al. 2014, versus HMA, Gloeckner et al. 2014) about the tissue microbial density in *S. vesparium* subsequent to the initial classification as an HMA made by Weisz and co-workers (2008). This difference by no means indicates that the classification of a species as LMA summarily precludes the use of DOC. Evidence exists in abundance for LMA species that have a demonstrated ability to take up DOC

(e.g., de Goeij et al. 2008, Mueller et al. 2014b, Rix et al. 2016), and recent work on sponges in the Red Sea has implicated both the sponge animal and its associated microbial consortia in the uptake and utilization of reef derived organic matter (Rix et al. 2017). Further, it is possible that the dichotomy observed in HMA/LMA uptake of DOC is simply due to the higher residence time of water in the tissues of the tested HMA species (Reiswig 1974, Weisz et al. 2008, McMurray et al. 2014). This higher residence time would increase the potential for filtered water to experience sponge-mediated chemical transformation, and could lead to the enhanced DOC removal observed in HMAs versus LMAs. This could help explain the behavior of *S. vesparium* whose pumping rate falls on the lower side of the tested LMAs but higher than the tested HMAs (Weisz et al. 2008). It is possible that there is a relationship between tissue residence time and DOC removal (or even a threshold where DOC processing becomes viable), but effective interrogation of this hypothesis would require direct assessments of pumping rate and DOC utilization across a range of species and pumping rates.

Nevertheless, it is plausible that the C metabolism and the magnitude of DOC utilization of some Caribbean species is affected, at least in part, by the quantity and composition of sponge tissue-hosted microbial biomass. It is therefore similarly plausible that interspecific changes in the composition of this microbial community could contribute to the observed variability in uptake magnitude observed in those HMA sponges that did remove DOC from filtered water (Figure 1). Despite the presence of considerable interspecific differences, intraspecific change in DOC uptake was observed to be relatively small (Table 1, Figure 1). This preliminarily indicates conservation of this behavior, which is promising for enhanced understanding of the metabolic behavior of these organisms at the reef scale. Further understanding of commonalities between sponge species that do and do not consume DOC will help further elucidate potentially predictive

factors for C metabolism in these organisms, and would enhance our understanding of the potential for and role of resource limitation in their ecology.

The concentration of POC available in ambient reef water and the observed DO demand from *X. muta* strongly suggested an alternative source of C to feed the metabolic demand of this species (Table 2). Specifically, if the tested individuals of *X. muta* removed all the POC available in the ambient reef water, the C obtained would only account for ~20-30% of their respiratory DO demand (assuming RQ = 1.0 and 0.7 for minimum and maximum estimates, respectively). Low POC uptake observed in our 2007 survey of *X. muta* further suggests relatively conserved metabolic behavior. Ambient POC and ΔPOC were higher in 2007 than 2011, yet the tested sponges in 2007 removed the same proportion of filtered POC (~20%). Furthermore, even at these elevated ambient POC concentrations, particulate removal alone is insufficient to account for the observed metabolic DO demand of the tested individuals. Assuming the sponges observed in 2011 removed all of the ambient POC observed in 2007, the removed C would account for a maximum of 50% of their respiration DO demand (conservatively assuming RQ = 0.7).

The insufficiency of available POC to satisfy DO demand, coupled with the observed DOC removal, indicates that these sponges were likely utilizing DOC as their primary metabolic C source. Increased utilization of DOC by benthic organisms in this and similar environments may be caused by abundant autochthonous, bioavailable organic matter from the extensive soft coral and macroalgal communities (Haas et al. 2011, Nelson et al. 2013). Organic matter sourced from 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). Recent stable isotopic work Red Sea sponges corroborated these observations and demonstrated differential processing and incorporation of

algal- and coral-derived DOC by the sponge holobiont; higher rates of algal-derived DOC incorporation was found in microbe-specific fatty acids while coral-derived DOC was primarily assimilated in to sponge-specific fatty acids (Rix et al. 2017).

Our measured DOC uptake agrees well with published values for non-manipulated "InEx" ΔDOC sampling (Yahel et al. 2003, Mueller et al 2014b, and McMurray et al. 2016; Table 2). DOC uptake by *X. muta* during our time-series respiration measurements of sponges 1 and 2 was equivalent to those sampled during the broader reef survey (Tables 1 and 2). However, the DOC removal by the *X. muta* we examined fall on the low end of the range previously measured for this species (McMurray et al. 2016). The agreement of our ΔDOC results with the in situ work of Mueller et al. (2014b) may indicate a degree of metabolic similarity between the species tested herein and excavating species. However, without the ability to normalize to sponge biomass, it is difficult to further compare the observed fluxes. Our volume-normalized DOC removal rates were significantly lower than both those from de Goeij et al. (2008a) and from the ^{13}C-labeled DOC respiration and assimilation rates of de Goeij et al. (2008b, 2013). Differences in the measured rates of DOC uptake between the present study and those performed by de Goeij et al. (2008a, 2008b, 2013) could be attributed to a variety of factors. The assessments of de Goeij et al. (2008a, 2008b, 2013) employed chamber methodologies where experimental sponges were removed from their original substrate and enclosed in chambers for monitoring. This manipulation can potentially have 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). As a result of this sensitivity, the sponges tested in this study were undisturbed so as to remove any potentially confounding factors resultant from individual or environmental manipulations. Additionally, the

^{13}C labeled incubations of de Goeij et al. (2008b, 2013) were performed with elevated concentrations of labile, diatom DOM, and that would be expected to accelerate the rate of DOC uptake relative to natural composition DOC if the labile fraction is the primary target of sponge respiration. However, this may be a reflection of natural conditions as ambient DOC concentrations naturally present on the reefs of Curaçao are often higher than were observed on Conch Reef (values ranged from ~90-160 μmol C $L_{seawater}^{-1}$; de Goeij et al. 2008a, Mueller et al. 2014a). Additionally, physiological deviations between encrusting species studied by de Goeij et al. (2008a, 2008b, 2013) and non-encrusting species like *X. muta* could also be cause for the observed disparity, yet the specific mechanisms that would be responsible for these differences are unclear.

The observed DO demand (1.4 ± 0.3 and 1.7 ± 0.4 mmol DO h^{-1} L_{sponge}^{-1}, for sponges 1 and 2, respectively) falls within the range of previously reported respiration rates of undisturbed sponges in situ. Reiswig (1974, 1981) reported a range from 0.5 to 4.7 mmol DO L_{sponge}^{-1} h^{-1} for *Tethya crypta* and *Verongia fistularis*, respectively, and Yahel et al. (2003) reported a rate of 1.38 ± 0.78 mmol DO L_{sponge}^{-1} h^{-1} for *T. swinhoei*. Additionally, our 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 DO 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 sponge cells (de Goeij et al. 2009). This DOC-fueled cellular overturn 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. In the examined individuals of *X. muta*, respiration

accounted for 60-100% of the C acquired, depending upon the assumed respiratory quotient (Figure 3). However, the metabolic utilization of the absorbed C is expected to be subject to temporal variability associated with changes in food composition and the physiological state of the holobiont (Maldonado et al. 2012). A lack of additional information regarding effluent CO_2 concentrations precludes direct calculation of the respiratory quotient for each sample collection event. Nevertheless, the absence of a large discrepancy between C uptake and respiration suggests that *X. muta* respires the majority of the ingested C as opposed to using it for cellular overturn and maintenance to the degree that was observed in *H. caerulea* (de Goeij et al. 2008a, de Goeij et al. 2009). This strongly suggests that *X. muta* only marginally participates in the "sponge loop," if it does so at all, despite its demonstrated ability to feed on DOC. Further evidence to this end is the absence of observed detrital production from *X. muta*. Neither of the POC datasets that were collected provided evidence for the export of particulate C concomitant to the import of DOC. However, the 100 μm pre-filter installed on the POC collection inlet may have precluded the collection of sticky, mucosal strands of detrital material ejected by the sponge. It is also possible that the absence of POC was 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, Mueller et al. 2014a, Haas et al. 2016).

Sponge 1 did show a small discrepancy between DOC uptake and respiration O_2 demand indicative of a minor amount of non-respiratory C use (Figure 3). Carbon retention calculations for the tested individuals of *X. muta* were restricted to nighttime rates of DO removal to avoid the impact of the observed phototrophic DO subsidy. In these calculations sponge 2 exhibited

approximate balance between removed C and DO whereas sponge 1 was observed to retain between 0.7 ± 0.4 and 1.1 ± 0.4 mmol C h^{-1} L_{sponge}^{-1}. The observed C accumulation from sponge 1 is much less than the quantity of C retained by *H. caerulea* for cell turnover and shedding (13.8 mmol C L_{sponge}^{-1} h^{-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 minimal (de Goeij et al. 2008a) whereas the same assumption cannot be made for *X. muta* as it shows considerable annual growth (McMurray et al. 2008, 2015). Cryptic habitats are typically characterized by severe space limitation, and the assumption of low sponge growth in these environments is likely safe. Therefore, cryptic sponges are driven by this limitation to allocate more energy towards functions other than growth, but *X. muta* does not experience the same degree of spatial pressure. In addition to growth, non-respired C could be allocated to other organismal functions such as modest 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). It is also important to consider the potential for enhanced variability as the monitoring techniques utilized herein are applied to a larger sponge population. While a degree of corroboration of the time-series data comes from the broader survey, it is likely that as the sample size expands further the allocation of the absorbed DOC within the sponge holobiont will become clearer. Therefore, it is important to view the calculations performed using the data from these two sponges as a preliminary step towards a complete understanding of the metabolic capacity of these organisms.

Population densities of *X. muta* at Conch Reef increased by up to 46% from 2000 to 2006 (McMurray et al. 2010); in 2006, the abundance of this speices 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 70 ± 50 mmol C m^{-2} d^{-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} d^{-1} as DOC). Estimates of gross primary productivity for reef environments are approximately 200 – 500 mmol C m^{-2} d^{-1}, with conservative evaluations suggesting that 10% of C fixed by macroalgal photosynthesis is exuded as DOC (Hatcher 1990, Haas et al. 2011). If all non-encrusting HMA sponge biomass on Conch Reef absorbs DOC at the same rate as the HMA species tested here, the total community flux is approximately 150 mmol C m^{-2} d^{-1} (using mean HMA biomass data from Southwell et al. 2008). This rough estimate of C flux approaches the 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 many HMA species on Caribbean reefs have 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 biological community within the benthic boundary layer. The bioavailable N contribution from sponge DOM remineralization may result in locally enhanced photosynthetic

production, which would lead to increased DOC concentrations, directly enhancing the sponge's primary C feedstock. This could provide a competitive advantage to seaweeds and sponges over corals, further reinforce coral depletion, and lead to reduced reef resilience (Pawlik et al. 2016). The Indo-Pacific, by contrast, is dominated by phototrophic species (e.g., Wilkinson 1983, 1987, Powell et al. 2014) that are net primary producers whose nutrients are likely retained and cycled internally as opposed to expelled in the excurrent jet (Pawlik et al. 2016). These reefs would be less susceptible to the feedback between sponges and seaweeds, and thus could exhibit higher overall resilience than those where sponge assemblages are dominated by heterotrophic species possessing the capacity to feed on dissolved organics (Pawlik et al. 2016).

The observed C cycling by *X. muta* and other sponge species serves to illustrate the fate of a large proportion of DOC available in reef waters and further implicates sponges in the processing of dissolved organic matter in reef ecosystems. Preliminary calculations suggest the HMA sponge population on Conch Reef can remove a large proportion of the DOC produced through reef primary productivity. This behavior further demonstrates a metabolic adaptation that allows some sponge species to utilize an abundant resource in productive reef waters. 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. Further in situ studies with non-manipulated sponges will provide additional insight into the native behavior of these organisms, and will serve to significantly advance our understanding of their role in coral reef ecosystems.

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Figure 1: Mean DOC uptake as the difference between paired ambient and excurrent water samples for the surveyed sponges from Conch Reef. Numbers above the bar represent sample size, error bars are 1 SE, and * denotes a significant difference from zero.

Figure 2: Time-series datasets of excurrent velocity and oxygen concentrations in the ambient and excurrent waters of sponge 2 leading up to, during, and following a cessation event. Excurrent velocity (W_{exc}) values represent the mean value of each 30 second collection "burst," which occured every 5 minutes. Dashed vertical line indicates sunrise; solid vertical line indicates sunset.

Figure 3: Average uptake fluxes of DOC, DO, and POC for the two tested sponges. 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, DO, and POC. These values were then normalized to the volume of the tested sponge. DO uptake values are those from nighttime collections to avoid the influence of oxygenic photosynthesis. Error bars represent 1 SE and *p* values (paired t-test) indicate the level of significance of the difference between DOC and DO fluxes.

Figure 4: The average concentration of oxygen in ambient and excurrent water masses of the two specimens of *X. muta* during the AM (07:00), Noon (11:30), PM (17:00), and Nighttime (23:00) sampling periods. The plotted time indicates the beginning of the period across which data were averaged to form sampling blocks. The top bar indicates daylight (open) and darkness (solid) time periods. Error bars represent 1 SE of the measured dates (9 – 16 August 2011; $k = 6$ for PM and Noon, $k = 7$ for AM and Nighttime).

837 **Table 1**: DOC uptake for the seven species examined during the broader survey of sponge C
838 uptake on the reef (mean ± 1 SD). DOC uptake represents the mean concentration difference
839 between paired samples from ambient and excurrent waters. Negative DOC uptake values
840 represent a release of DOC relative to the ambient water column, and an * indicates a significant
841 flux relative to zero.

Species	**HMA/LMA**	**n Individuals**	**ΔDOC (μmol C $L_{seawater}^{-1}$)**	**ΔDOC % Ambient Removed**
X. muta (Survey)	HMA[a]	65	9.8 ± 13.1 *	15
X. muta (2011 Samples)	HMA[a]	2	11.8 ± 8.5 *	13
I. strobilina	HMA[a]	8	26.8 ± 27.0 *	21
V. gigantea	HMA[a]	6	22.7 ± 20.5 *	24
S. vesparium	HMA[a], LMA[b]	6	-1.3 ± 17.7	-2
N. digitalis	LMA[a]	10	-1.8 ± 5.0	-3
C. vaginalis	LMA[a]	7	2.4 ± 7.5	3
M. laxissima	LMA[a]	2	1.4 ± 5.9	1

[a]Gloeckner et al. (2014)

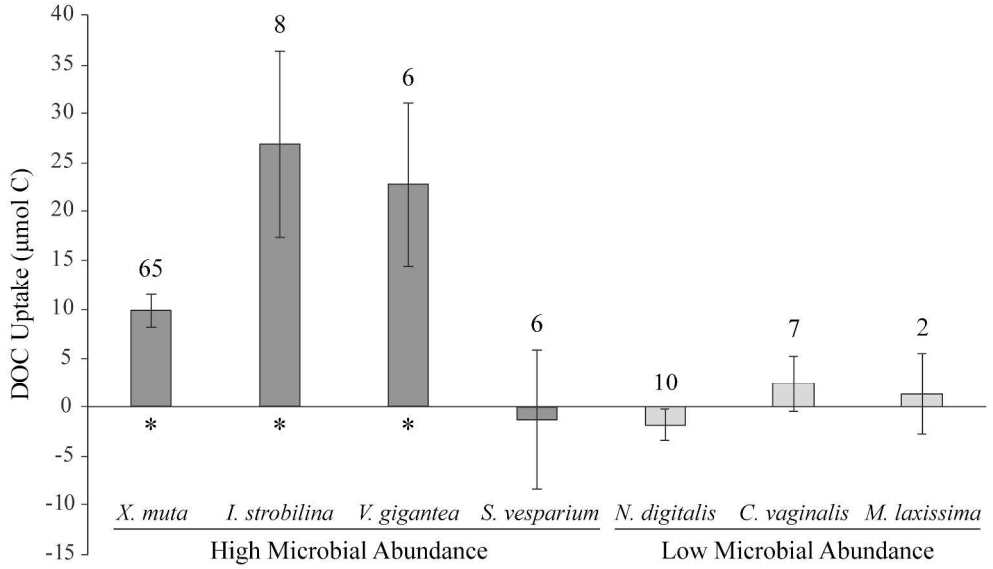
[b]Poppell et al. (2014)

842 **Table 2**: Comparison of published directly measured in situ carbon uptake and respiration
843 activity for sponge species along with the mean ambient values for each parameter (mean ± 1
844 SD). Results of studies conducted in chambers are not included due to possible manipulation
845 artifacts. Ambient C and DO concentrations are reported as µmol C $L_{seawater}^{-1}$ for DOC and POC
846 and µmol O_2 $L_{seawater}^{-1}$ for DO. Carbon and DO uptake rates are reported as µmol C $L_{seawater}^{-1}$ for
847 DOC and POC or µmol O_2 $L_{seawater}^{-1}$ for DO. Listed flux measurements appear italicized and
848 were normalized to the volume (L) of sponge biomass (mmol C L_{sponge}^{-1} hr^{-1} for DOC and POC,
849 mmol O_2 L_{sponge}^{-1} hr^{-1} for DO). Oxygen data from this study represents only nighttime sample
850 periods.

Species	**Ambient DOC**	**ΔDOC** ***DOC Flux***	**Ambient POC**	**ΔPOC** ***POC Flux***	**Ambient DO**	**ΔDO** ***DO Flux***	**Source**
X. muta	89 ± 5	11.8 ± 8.5 *1.9 ± 1.5*	2.4 ± 0.6	0.4 ± 0.1 *0.06 ± 0.01*	177 ± 9	10.0 ± 2.6 *1.6 ± 0.4*	a
X. muta	89 ± 31	9.8 ± 13.1	3.9 ± 1.3	0.96 ± 0.28			b
X. muta	61.9 – 123.5	0.63 to 11.3	7.9 – 12.6† 2 – 15*	0.09 to 6.4† *0.18 to 3.2**			c
T. swinhoei	81 ± 11	10 ± 8 *1.56 ± 1.1*	2.5 ± 1.3*	2.1 ± 1.0* *0.24 ± 0.18**	200 ± 10	9 ± 5 *1.38 ± 0.78*	d
Siphonodictyon sp.	110 ± 18	13 ± 17	4 ± 1	3 ± 1			e
Cliona delitrix	95 ± 5	10 ± 12	4 ± 1	3 ± 1			e

Sources: a: This study (2011 Samples); b: This study (Survey Samples); c: McMurray et al. (2016); d: Yahel et al. (2003); e: Mueller et al. (2014b)

*POC represents LvPOC, or living particulate organic matter (Yahel et al. 2003, McMurray et al. 2016), † represents detrital POC (McMurray et al. 2016).



Mean DOC uptake as the difference between paired ambient and excurrent water samples for the surveyed sponges from Conch Reef. Numbers above the bar represent sample size, error bars are 1 SE, and * denotes a significant difference from zero.

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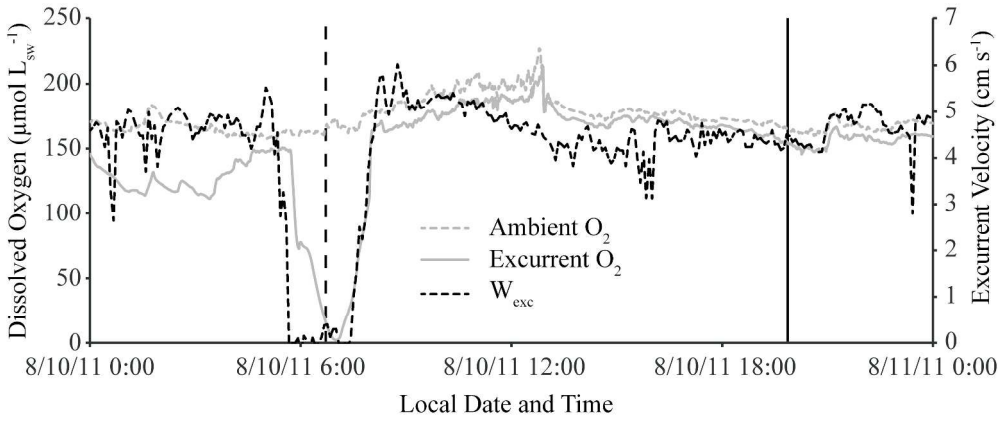

Time-series datasets of excurrent velocity and oxygen concentrations in the ambient and excurrent waters of sponge 2 leading up to, during, and following a cessation event. Excurrent velocity (W_{exc}) values represent the mean value of each 30 second collection "burst," which occured every 5 minutes. Dashed vertical line indicates sunrise; solid vertical line indicates sunset.

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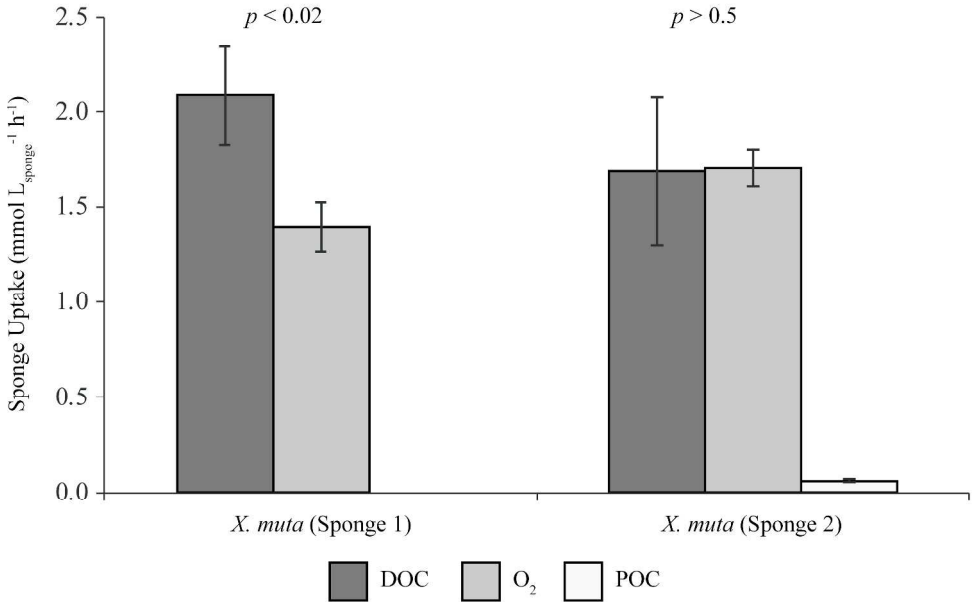

Average uptake fluxes of DOC, DO, and POC for the two tested sponges. POC was only sampled for sponge 2. Flux calculations were performed using the average, volumetric pumping rate for each individual and the average uptake of DOC, DO, and POC. These values were then normalized to the volume of the tested sponge. DO uptake values are those from nighttime collections to avoid the influence of oxygenic photosynthesis. Error bars represent 1 SE and *p* values (paired t-test) indicate the level of significance of the difference between DOC and DO fluxes.

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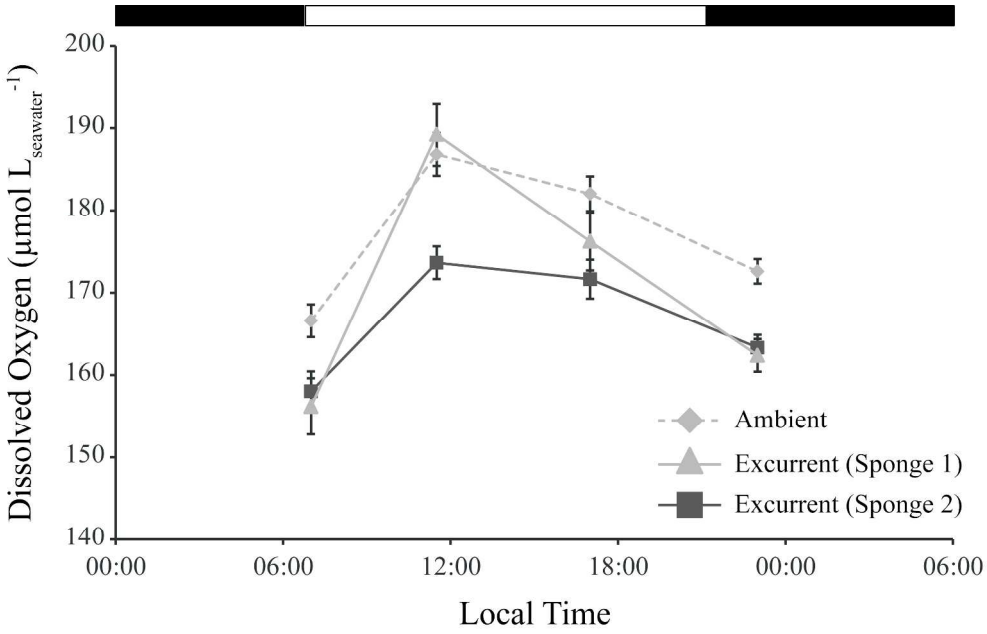

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