



FEATURE ARTICLE

A test of the sponge-loop hypothesis for emergent Caribbean reef sponges

Steven E. McMurray^{1,*}, Amber D. Stubler^{1,2}, Patrick M. Erwin¹,
Christopher M. Finelli¹, Joseph R. Pawlik¹

¹Department of Biology and Marine Biology, University of North Carolina Wilmington, Wilmington, North Carolina, USA

²Biology Department, Occidental College, 1600 Campus Road, Los Angeles, California, USA

ABSTRACT: The sponge-loop hypothesis proposes that coral reef sponges convert the dissolved organic matter (DOM) released by benthic primary producers into particulate detritus available to higher trophic levels. The hypothesis was developed and subsequently supported from studies of cryptic, encrusting sponges; however, the sponge-loop has yet to be considered for massive, emergent sponge species that dominate the surface of Caribbean reefs and represent a large proportion of sponge community biomass. We tested the generality of the sponge-loop for emergent sponge fauna by using direct In-Ex methods combined with acoustic Doppler velocimetry and sponge volume calculations to quantify carbon flux for 9 sponge species representing a variety of functional types. The diet of 5 species hosting abundant symbiotic microbes (HMA) primarily consisted of dissolved organic carbon (DOC), while 4 species with low microbial abundances (LMA) primarily consumed detritus and picoplankton. None of the sponge species studied were found to produce significant quantities of detritus. We conclude that, instead of releasing assimilated carbon in the form of detritus, as originally proposed by the sponge-loop for encrusting, cryptic sponges, emergent sponge species likely retain assimilated carbon as biomass. Given the high rates of DOC uptake observed for HMA species, we propose an additional pathway by which the sponge-loop fuels higher trophic levels via predation by fish, turtle, and invertebrate spongivores.

KEY WORDS: Benthic–pelagic coupling · Suspension feeding · Dissolved organic carbon · DOC · Particulate organic carbon · POC · Porifera · Detritus



Analyses of seawater collected before and after passage through emergent sponge species reveal that they are not net producers of detritus.

Photo: Joseph R. Pawlik

INTRODUCTION

The microbial loop (Azam et al. 1983) transformed our understanding of trophic interactions in the ocean and finally unraveled the paradox of abundant planktonic life in the oligotrophic open ocean (Pomeroy et al. 2007). It is now understood that the structure of plankton communities is a web of trophic interactions, rather than a simple linear chain, largely driven by the cycling of carbon and nutrients by microbes (Fenchel 2008). By consuming the dissolved organic matter (DOM) released by phytoplankton that would otherwise be lost to the environ-

*Corresponding author: mcmurrays@uncw.edu

ment, microbes remineralize nutrients that fuel primary production and return assimilated DOM to the planktonic food web via protists (Fenchel 2008).

More recently, a process analogous to the microbial loop has been hypothesized to explain a similar paradox: how highly productive and diverse coral reef ecosystems occur in desert-like tropical seas. Specifically, it was proposed that sponges, and their symbiotic microbes, may sustain the high productivity of coral reef ecosystems by mediating the recycling of primary production released by reef-building corals and macroalgae in the form of DOM (de Goeij et al. 2013). Through this process, termed the 'sponge-loop', sponges consume dissolved organic carbon (DOC), and up to 40% of this carbon is subsequently released as particulate organic carbon (POC) in the form of shed cellular detritus that is then assimilated by benthic detritivores and suspension feeders and subsequently passed to higher trophic levels via predation (de Goeij et al. 2013, Rix et al. 2016, 2017). Although the underlying mechanism of detritus production in the sponge-loop has yet to be resolved, it is hypothesized that there is a trade-off between sponge growth and detritus production, with the latter linked to rapid cell turnover, primarily of choanocytes, through cell proliferation and cell shedding (de Goeij et al. 2009, 2013, Alexander et al. 2014, 2015; but see Kahn & Leys 2016).

Like the microbial loop, the sponge-loop has the potential to explain an important link in the cycling of carbon in coral reef ecosystems. There is good evidence that primary producers on coral reefs (seaweeds and corals) release up to 50% of their fixed carbon as mucus and other exudates, and that most of these exudates become part of the DOC pool (Wild et al. 2004, Haas et al. 2010). Further, sponges have the capacity to turn over huge quantities of seawater in the process of feeding. Reiswig (1974) estimated that the sponge community of Discovery Bay, Jamaica, could process the water-column above the reef each day to a depth of 15.5 to 40.0 m; more recently, work with the giant barrel sponge *Xestospongia muta* from the Florida Keys and Bahamas indicates that this species alone can overturn a volume of water equivalent to a layer of 1.7 to 12.9 m each day (McMurray et al. 2014). Therefore, the sponge-loop could be a dominant and previously unrealized component in the cycling of carbon for coral reef ecosystems. Indeed, it has already been invoked to explain phase shifts in fish biomass on impacted reefs (Silveira et al. 2015). Moreover, DOM consumption by sponges may contribute to a reciprocal positive interaction with macroalgae, whereby remineralized nutrients fuel the growth of macroalgae that subse-

quently results in enhanced levels of macroalgal-derived DOM; a process termed the 'vicious circle hypothesis' because of the detrimental effect that enhanced sponge and macroalgal growth has on the ability of reef-building corals to compete for space in benthic reef communities (Pawlik et al. 2016).

As originally developed, the sponge-loop hypothesis was based on studies of 4 species of cryptic, encrusting Caribbean sponge species using augmented DOC in the form of laboratory-produced diatom extract (de Goeij et al. 2013). Notably, an increasing number of sponge species have been found to feed on DOC (Yahel et al. 2003, de Goeij et al. 2008b, Ribes et al. 2012, Mueller et al. 2014, McMurray et al. 2016, 2017, Archer et al. 2017, Hoer et al. 2018, Morganti et al. 2017), and a link between DOC uptake and detritus production has been established for additional cryptic sponge species (Alexander et al. 2014, Rix et al. 2016, 2017). While it was generally thought that sponge species with dense assemblages of symbiotic microbes—i.e. high microbial abundance (HMA) sponges (Gloeckner et al. 2014)—derive a majority of their dietary carbon from DOC, and low microbial abundance (LMA) sponges derive it from filtering POC (Maldonado et al. 2012, Hoer et al. 2018), there is increasing evidence that some LMA sponges consume DOC (Mueller et al. 2014, Rix et al. 2016, 2017, Morganti et al. 2017) and that sponge cells are directly involved in DOC uptake (de Goeij et al. 2008a, Rix et al. 2016, 2017). The sponge-loop has been supported in recent studies of encrusting species using more ecologically-relevant DOC types, including coral- and algal-derived DOC (Rix et al. 2017), and assimilation rates for these DOC types are comparable to those for diatom-derived DOC (de Goeij et al. 2013). Further, the sponge-loop has been found to function for sponge species spanning a broad geographic range, including the Red Sea, temperate Mediterranean, and deep-water North Atlantic (Alexander et al. 2014, Rix et al. 2016).

Despite growing support for the sponge-loop, the ecological significance and generality of the hypothesis across the Porifera remains unclear. To date, the sponge-loop has been exclusively studied using incubation methods, both in the laboratory and *in situ*, with isotopically-enriched, and generally labile, DOM as a tracer to follow sponge DOM uptake and detritus production (de Goeij et al. 2013, Rix et al. 2016, 2017) and subsequent detritus transfer to higher trophic levels (de Goeij et al. 2013). However, the hypothesis has yet to be tested *in situ* using the natural pool of DOM found in seawater over coral reefs. The DOM pool available on coral reefs is highly heterogeneous

(Hansell & Carlson 2002), consisting of a mixture of refractory and labile forms that likely vary in their bioavailability to sponges (Yahel et al. 2003, de Goeij et al. 2008b). Additionally, both the quality (Rix et al. 2017) and concentration (Mueller et al. 2014, McMurray et al. 2016, 2017, Archer et al. 2017, Morganti et al. 2017) of DOC are known to affect rates of sponge-mediated DOC flux. Even more striking is that work on the sponge-loop has thus far been limited to sponge species with thinly encrusting growth forms that typically live in reef interstices. Massive and emergent sponge species, which dominate the surface of Caribbean coral reefs (Loh & Pawlik 2014), represent a large proportion of total coral reef sponge biomass and are therefore expected to represent a substantial contribution to carbon cycling if they participate in the sponge-loop. While DOC uptake has been demonstrated for a few emergent sponge species (Yahel et al. 2003, McMurray et al. 2016, 2017, Archer et al. 2017, Hoer et al. 2018), potential detritus production has yet to be quantified for these species (but see McMurray et al. 2016).

The purpose of this study was to test the generality of the sponge-loop *in situ* with emergent species spanning a variety of functional types. A total of 9 sponge species were chosen for this study from a range of taxonomic groups and including both HMA and LMA sponge types; all chosen species exhibit morphologies (tube, vase, and barrel) that separate incurrent from excurrent seawater flow. In addition to differences in the abundance and diversity of microbial symbiont communities (Gloeckner et al. 2014), a number of other functional traits may influence sponge-mediated carbon flux for HMA and LMA species, with the latter having more porous tissue and higher rates of pumping activity, greater densities of choanocyte chambers, and shorter and wider water canals (Weisz et al. 2008, Poppell et al. 2014). We used direct In-Ex methods (Yahel et al. 2005) combined with acoustic Doppler velocimetry to measure sponge pumping and geometric estimates of sponge volume to quantify the natural flux of detritus from unmanipulated individual sponges. We conducted our studies at 2 locations expected to vary in the composition and concentration of particulate and dissolved food resources available to sponges.

MATERIALS AND METHODS

Carbon flux was quantified for sponges on Conch Reef, Key Largo, Florida (24° 56.9' N, 80° 27.2' W), and reefs off Carrie Bow Cay, Belize (16° 56.9' N,

80° 27.2' W), in June and July 2016, respectively. At each location, a total of 2 to 7 individuals each of 8 large and abundant emergent sponge species, common throughout the Caribbean, were haphazardly selected for study at between 15 and 20 m depths. Sponge species were chosen that exhibit morphologies that distinctly separate incurrent from excurrent flow; these include barrel, vase and tube-forming species. Of the species investigated, *Agelas tubulata* (cf. *conifera*), *Verongula gigantea*, *V. reisiwigi*, and *Xestospongia muta* are considered HMA species and *Callyspongia plicifera*, *C. vaginalis*, *Mycale laxissima*, and *Niphates digitalis* are considered LMA species (Weisz et al. 2008, Maldonado et al. 2012, Gloeckner et al. 2014). An additional species, the HMA sponge *Ircinia strobilina*, was selected for study on Conch Reef only. With the exception of *A. tubulata*, only individuals with a single osculum were studied for each species. Additionally, only sponges with no obvious signs of disease or tissue damage and not fouled with algae or colonized by epibionts (e.g. zoanths) were included. For photographs and descriptions of each species, see Zea et al. (2014).

Paired 1.5 l incurrent (ambient) and excurrent seawater samples were collected from each sponge with 100 ml syringes (5 mm diameter tip opening) as previously described (McMurray et al. 2016) for measurements of live particulate organic carbon (LPOC), total POC, and DOC. Briefly, incurrent seawater samples were collected adjacent to the ostia lining the external sponge surface and excurrent samples were slowly collected from approximately 5 cm below the osculum within the atrium (inner empty space) of each sponge and at a rate lower than the excurrent water velocity to avoid contamination from ambient seawater. Samples thus represent an integration of approximately 10 to 20 min of sponge feeding. Following seawater collection, the velocity of excurrent seawater at the centerline of the osculum of each sponge was measured using a Sontek Micro acoustic Doppler velocimeter (ADV) mounted on a tripod for 3 min at 2 Hz (McMurray et al. 2014). The dimensions of each sponge were subsequently measured using a flexible measuring tape and sponge volume, excluding the spongocoel, was calculated by approximating the morphology of each individual as an appropriate geometric solid (McMurray et al. 2008).

To quantify the flux of LPOC in the form of picoplankton, 5 ml of both incurrent and excurrent seawater samples were preserved in electron microscopy grade glutaraldehyde at a final concentration of 0.1% in cryovials for 10 min in the dark and subsequently frozen in liquid nitrogen and stored at -80°C

until flow cytometry analysis. Phytoplankton (*Prochlorococcus* [Pro], *Synechococcus* [Syn], and photosynthetic pico- and nanoeukaryotes [Euk]) and bacterioplankton (high nucleic acid bacteria [HNA] and low nucleic acid bacteria [LNA]) in seawater samples were enumerated using a BD FACSCelesta flow cytometer and populations were characterized as previously described (McMurray et al. 2016). Briefly, cells were excited with a 488 nm laser and forward scatter, side scatter, green fluorescence (530 ± 30 nm), orange fluorescence (575 ± 26 nm), and red fluorescence (695 ± 40 nm) emissions were measured. Phytoplankton were analyzed for 10 min at high flow rate and heterotrophic bacteria were stained with Sybr Green-I as previously described (Marie et al. 1997) and analyzed at low flow rate for 5 min. Picoplankton were classified based on their characteristic flow cytometric signatures relative to standard fluorescent microspheres following standard population gating schemes (Cavender-Bares et al. 1998). Carbon content of each type of picoplankton was estimated using standard cell conversions used in previous studies of sponge feeding (e.g. Pile 1997, Lesser 2006, Morganti et al. 2017): 53 fg C cell⁻¹ for Pro (Morel et al. 1993), 470 fg C cell⁻¹ for Syn (Campbell et al. 1994), 1496 fg C cell⁻¹ for Euk (Zubkov et al. 1998), and 20 fg C cell⁻¹ for HNA and LNA bacteria (Ducklow et al. 1993).

To quantify sponge-mediated flux of POC and DOC, the remaining seawater from each sample was filtered through a 100 µm mesh that excluded particles greater than the size of incurrent ostia and subsequently through a precombusted GF/F glass fiber filter under low pressure. Filters were individually wrapped in aluminum foil and frozen until analysis of POC; 20 ml of the filtrate from each sample was transferred to an EPA precleaned glass vial, acidified with 100 µl of 50 % phosphoric acid, and stored at 4°C until analysis of DOC. POC was measured using a CE Elantech NC2100 combustion elemental analyzer after filters were dried at 50°C and subsequently exposed to hydrochloric acid fumes for 24 h. DOC was measured using high temperature catalytic oxidation with a Shimadzu TOC 5050 analyzer (McMurray et al. 2016). Calibration was achieved with standards diluted from a stock solution of potassium hydrogen phthalate and both standards and deep seawater consensus reference material (Batch 9, Lot #09-09 from the Hansell Laboratory, Rosenstiel School of Marine and Atmospheric Science, University of Miami) were interspersed with samples for quality assurance and control. Each seawater sample was run in duplicate and each analysis tube was injected 3 to 5 times for a coefficient of variance <1.5 %.

The approximate analytical precision of the instrument was 2 µmol C l⁻¹ seawater. All plastic used for sample collection was soaked in a 0.5 M HCl bath for at least 24 h and subsequently thoroughly rinsed in ultrapure water before use and all glassware and aluminum foil used to process samples were combusted at 450° for >4 h prior to use (Tupas et al. 1994). Some samples were discarded due to potential contamination while processing in the field; therefore, a small number of individuals are lacking flux estimates for 1 or 2 of the 3 carbon pools investigated (see Table 1 below in 'Results' for sample sizes).

Detrital carbon in incurrent and excurrent seawater samples was estimated as the portion of total POC not accounted for by LPOC (i.e. Detritus = POC – LPOC) (Ribes et al. 1999, Hadas et al. 2009, McMurray et al. 2016). Sponge specific filtration rates, or carbon flux (µmol C s⁻¹ l⁻¹ sponge), of DOC, LPOC, and detritus were calculated as:

$$C \text{ flux} = \frac{(C_{in} - C_{ex}) \times Q}{V_{sponge}} \quad (1)$$

where C_{in} and C_{ex} are the incurrent and excurrent concentrations of each carbon pool (µmol C l⁻¹ seawater), V_{sponge} is sponge tissue volume (l), and Q is the volume flow or pumping rate for each sponge (l s⁻¹). Positive and negative flux estimates therefore represent consumption and production of a particular carbon pool, respectively. For Q , we assumed that the mean excurrent velocity for each sponge was equivalent to the velocity of seawater measured at the osculum centerline with an ADV (i.e. plug flow), and volume flow was calculated as the product of the centerline excurrent velocity and the osculum area; for *X. muta*, the mean excurrent seawater velocity for each sponge was corrected for the uneven velocity distribution across the osculum due to the morphology of the spongocoel (Eq. 3 in McMurray et al. 2014).

For all analyses, assumptions of normality and homogeneity of variances were checked with box and residual plots and data were transformed as needed or nonparametric tests were used. Log₁₀-transformed incurrent carbon concentrations were compared between locations (Conch Reef and Carrie Bow Cay) and carbon pools (DOC, LPOC, detritus) with a 2-way ANOVA and significant interactions were evaluated by tests of simple main effects. Specific filtration rates were compared between locations, sponge species, and carbon pools using the Scheirer-Ray-Hare extension of the Kruskal-Wallis test (Sokal & Rohlf 1995); *V. gigantea* and *V. reiswigi* were excluded from this analysis due to insufficient replication for these species at Carrie Bow Cay and

Conch Reef, respectively. To test the hypothesis that sponges are net producers (or consumers) of detritus, paired t -tests were used to compare the concentrations of detritus in incurrent and excurrent seawater for each species. Statistical analyses were conducted with SAS version 9.1.3 for Windows (SAS Institute) and SPSS Statistics version 22 for Windows (IBM) statistical software.

RESULTS

The concentration of incurrent (ambient) carbon available to sponges over the study period significantly differed between locations ($F_{1,222} = 48.99$, $p < 0.001$) and between carbon pools ($F_{2,222} = 2192.92$, $p < 0.001$). Additionally, differences in the concentrations between carbon pools significantly varied between study locations (carbon pool by location interaction: $F_{2,222} = 14.19$, $p < 0.001$; Fig. 1). Comparisons of simple main effects indicated that the concentration of incurrent carbon significantly differed between carbon pools at both Conch Reef ($F_{2,222} = 1058.30$, $p < 0.001$) and Carrie Bow Cay ($F_{2,222} = 1138.87$, $p < 0.001$). Pairwise comparisons revealed that there was more carbon in the form of DOC relative to detritus and LPOC ($p < 0.001$ for both tests),

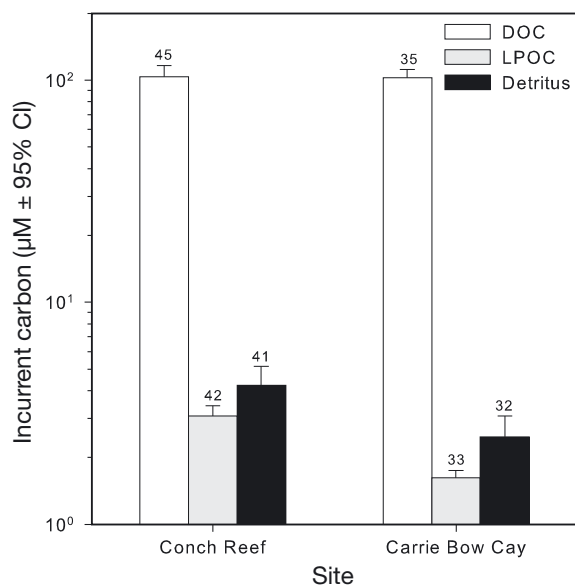


Fig. 1. Mean (\pm 95% CI) concentration of dissolved organic carbon (DOC), live particulate organic carbon (LPOC), and detritus in incurrent (ambient) seawater on Conch Reef, Key Largo, FL, and Carrie Bow Cay, Belize, over the study period. Numbers above the bar represent sample sizes; these were variable because some samples were discarded due to potential contamination while processing in the field

and more detritus relative to LPOC ($p = 0.023$) at Conch Reef; the same pattern held at Carrie Bow Cay, with more DOC than detritus and LPOC ($p < 0.001$), and more detritus than LPOC ($p = 0.006$). The concentrations of LPOC and detrital carbon were significantly greater at Conch Reef relative to Carrie Bow Cay (LPOC: $F_{1,222} = 42.80$, $p < 0.001$; detritus: $F_{1,222} = 32.03$, $p < 0.001$), but DOC concentrations were similar between locations ($F_{1,222} = 0.05$, $p = 0.817$). Mean \pm 95% CI concentrations of ambient DOC, detritus, and LPOC were 103.4 ± 12.7 , 4.23 ± 0.89 , and 3.07 ± 0.34 μM at Conch Reef and 102.7 ± 8.8 , 2.48 ± 0.57 , and 1.62 ± 0.12 μM at Carrie Bow Cay, respectively (Fig. 1).

Of the 59 sponges at both locations for which detrital carbon flux was measured, 11 individuals were found to have net production of detritus (negative flux); all others had net consumption of detritus (positive flux). Detrital production for these 11 individuals was generally small, with 7 emitting <0.009 $\mu\text{mol detrital C s}^{-1} \text{ l}^{-1}$ sponge, 3 emitting 0.03 to 0.05 $\mu\text{mol C s}^{-1} \text{ l}^{-1}$ sponge, and 1 emitting 0.20 $\mu\text{mol C s}^{-1} \text{ l}^{-1}$ sponge. The 11 individuals with detrital production were spread over 7 different species. Individuals of *Ircinia strobilina* and *Niphates digitalis* only exhibited net detritus consumption (positive flux).

In terms of mean responses for the 9 species investigated at Conch Reef and the 8 species investigated at Carrie Bow Cay, only *Mycale laxissima* at Carrie Bow Cay was found to have mean detritus production (-0.0004 ± 0.003 $\mu\text{mol C s}^{-1} \text{ l}^{-1}$ sponge, mean \pm 95% CI), however, the concentration of detritus in excurrent seawater was not significantly different from that in ambient seawater ($t = -0.12$, $df = 4$, $p = 0.91$) and conspecifics at Conch Reef had mean consumption of detritus (Fig. 2, Table 1). Only 1 individual each of *Verongula reiswigi* at Conch Reef and of *V. gigantea* at Carrie Bow Cay were measured and both were found to have negative detrital carbon flux (-0.008 and -0.20 $\mu\text{mol C s}^{-1} \text{ l}^{-1}$ sponge, respectively), but conspecifics of *V. reiswigi* at Carrie Bow Cay and *V. gigantea* at Conch Reef were found to have mean positive detrital carbon flux (Fig. 2, Table 1). Conversely, at Conch Reef, significant consumption of detritus was found for *Callyspongia plicifera* ($t = 3.74$, $df = 6$, $p = 0.010$) and *I. strobilina* ($t = 7.60$, $df = 2$, $p = 0.017$), while detritus uptake for all other species was marginally significant or nonsignificant (*Agelas tubulata*: $t = 2.34$, $df = 5$, $p = 0.067$; *C. vaginalis*: $t = 2.01$, $df = 4$, $p = 0.115$; *M. laxissima*: $t = 2.23$, $df = 5$, $p = 0.076$; *Niphates digitalis*: $t = 2.59$, $df = 3$, $p = 0.081$; *V. gigantea*: $t = 2.48$, $df = 4$, $p = 0.069$; *Xestospongia muta*: $t = 1.78$, $df = 3$, $p = 0.173$) (Fig. 2a,

Table 1. Mean (\pm 95% CI) sponge volumes, pumping rates, and carbon fluxes mediated by Caribbean coral reef sponges studied *in situ* at Conch Reef, Key Largo, Florida, USA and Carrie Bow Cay, Belize. Sponges are identified as high microbial abundance (HMA) and low microbial abundance (LMA) species. Food types considered were dissolved organic carbon (DOC), detritus (DET), and live particulate organic carbon (LPOC). Negative values indicate net excretion and positive values indicate net consumption of a particular food type. '% total carbon uptake' is the percent contribution of each food type to the specific filtration rate for total organic carbon. n: number of samples. Significant differences between incurrent and excurrent concentrations of detritus are indicated by an asterisk: * $p < 0.05$, paired *t*-test

Location and species	Sponge volume (l)	Pumping rate ($l\ s^{-1}\ l^{-1}$ sponge)	Food type	n	Carbon consumed ($\mu\text{mol}\ l^{-1}$ seawater)	Specific filtration rate ($\text{nmol}\ C\ s^{-1}\ l^{-1}$ sponge)	% total carbon uptake
Conch Reef							
<i>Agelas tubulata</i> (HMA)	2.38 \pm 1.47	0.01 \pm 0.003	DOC	6	13.1 \pm 27.7	196 \pm 406	75
			DET	6	2.84 \pm 2.38	36 \pm 40.1	14
			LPOC	6	3.20 \pm 1.08	30.3 \pm 10.4	12
<i>Ircinia strobilina</i> (HMA)	2.86 \pm 3.65	0.005 \pm 0.006	DOC	2	17 \pm 17	135 \pm 188	75
			DET	3	7.17 \pm 1.85	30 \pm 26.8*	17
			LPOC	3	3.15 \pm 0.40	15.8 \pm 17	9
<i>Verongula gigantea</i> (HMA)	9.95 \pm 7.22	0.055 \pm 0.025	DOC	6	9.68 \pm 23.9	529 \pm 1395	71
			DET	5	1.45 \pm 1.15	70.3 \pm 43.5	10
			LPOC	5	2.86 \pm 1.44	140 \pm 105	19
<i>V. reiswigi</i> (HMA)	9.52 \pm 15.5	0.001 \pm 0.001	DOC	2	-13.5 \pm 16.9	-7.27 \pm 0.66	0
			DET	1	-5.98	-8.44	0
			LPOC	1	4.07	5.75	100
<i>Xestospongia muta</i> (HMA)	31.5 \pm 25.3	0.027 \pm 0.017	DOC	5	-5.94 \pm 38.6	191 \pm 731	74
			DET	4	0.94 \pm 1.03	31.3 \pm 45.7	12
			LPOC	4	1.79 \pm 0.24	37.7 \pm 25.3	14
<i>Callyspongia plicifera</i> (LMA)	0.39 \pm 0.16	0.104 \pm 0.031	DOC	7	-0.70 \pm 15.6	2.43 \pm 1615	1
			DET	7	1.12 \pm 0.59	123 \pm 78.5*	30
			LPOC	7	2.66 \pm 1.12	278 \pm 126	69
<i>C. vaginalis</i> (LMA)	0.07 \pm 0.02	0.05 \pm 0.051	DOC	5	-4.85 \pm 20.9	8.69 \pm 438	4
			DET	5	1.15 \pm 1.13	94.1 \pm 173	41
			LPOC	5	2.33 \pm 0.5	128 \pm 147	55
<i>Mycale laxissima</i> (LMA)	0.49 \pm 0.23	0.003 \pm 0.003	DOC	6	-5.31 \pm 17	-13.5 \pm 43.1	0
			DET	6	1.03 \pm 0.9	2.83 \pm 2.37	36
			LPOC	6	1.84 \pm 0.32	5.11 \pm 1.46	64
<i>Niphates digitalis</i> (LMA)	0.49 \pm 0.22	0.127 \pm 0.053	DOC	6	-22.3 \pm 24.6	-2389 \pm 2791	0
			DET	4	1.49 \pm 1.13	192 \pm 162	35
			LPOC	5	2.29 \pm 0.31	358 \pm 146	65
Carrie Bow Cay							
<i>A. tubulata</i> (HMA)	3.67 \pm 2.38	0.005 \pm 0.003	DOC	6	7.63 \pm 19.4	36.4 \pm 80.6	80
			DET	5	0.47 \pm 0.47	1.72 \pm 2.37	4
			LPOC	6	1.61 \pm 0.21	7.33 \pm 3.46	16
<i>V. gigantea</i> (HMA)	24.5 \pm 35.9	0.055 \pm 0.001	DOC	2	7.98 \pm 9.07	439 \pm 499	92
			DET	1	-3.66	-201	0
			LPOC	1	0.67	36.8	8
<i>V. reiswigi</i> (HMA)	7.68 \pm 3.77	0.004 \pm 0.003	DOC	3	17.9 \pm 28.5	116 \pm 208	93
			DET	3	0.61 \pm 0.17	2.68 \pm 2.13*	2
			LPOC	3	1.58 \pm 0.002	6.67 \pm 4.3	5
<i>X. muta</i> (HMA)	40 \pm 16.6	0.008 \pm 0.006	DOC	5	-7.45 \pm 24.1	-91.3 \pm 180	0
			DET	4	0.56 \pm 0.17	4.31 \pm 2.61*	27
			LPOC	4	1.36 \pm 0.10	12 \pm 9.3	73
<i>C. plicifera</i> (LMA)	0.87 \pm 0.48	0.095 \pm 0.032	DOC	5	-3.61 \pm 16.1	-741 \pm 1743	0
			DET	5	1.11 \pm 1.4	138 \pm 207	53
			LPOC	5	1.36 \pm 0.26	122 \pm 30.5	47
<i>C. vaginalis</i> (LMA)	1.7 \pm 1.04	0.057 \pm 0.046	DOC	5	-12.7 \pm 15.9	-897 \pm 655	0
			DET	5	0.78 \pm 0.47	44.9 \pm 30.8*	38
			LPOC	5	1.36 \pm 0.28	73.5 \pm 61.2	62
<i>M. laxissima</i> (LMA)	1.29 \pm 0.54	0.002 \pm 0.0004	DOC	5	-1.55 \pm 10.1	-8.14 \pm 17.8	0
			DET	5	-0.08 \pm 1.39	-0.43 \pm 3.37	0
			LPOC	5	1.01 \pm 0.33	2.38 \pm 1.01	100
<i>N. digitalis</i> (LMA)	1.79 \pm 1.07	0.082 \pm 0.083	DOC	4	-3.2 \pm 29.6	-134 \pm 820	0
			DET	4	1.34 \pm 1.72	214 \pm 404	74
			LPOC	4	1.41 \pm 0.47	73.6 \pm 67.7	26

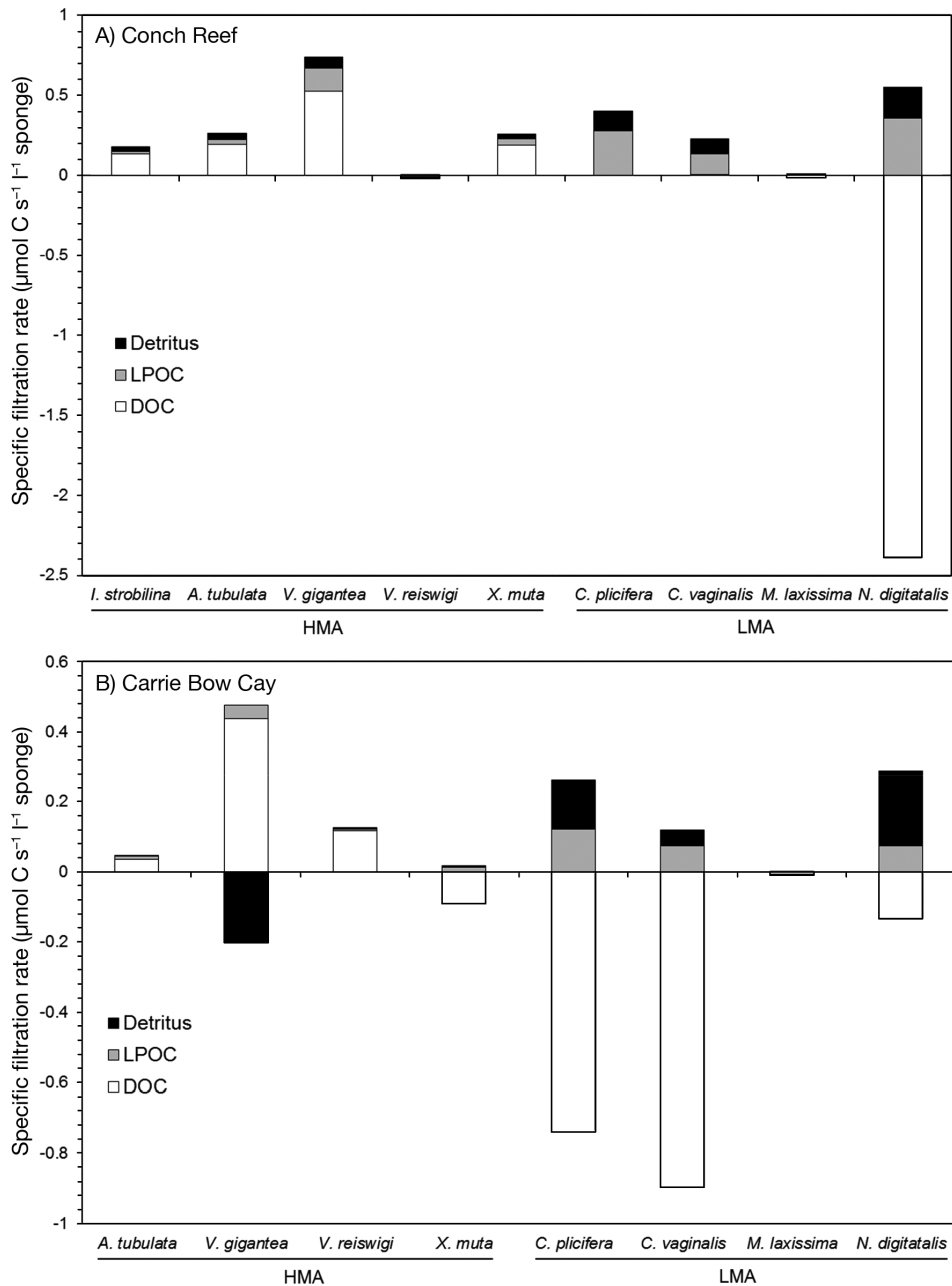


Fig. 2. Mean specific filtration rates for dissolved organic carbon (DOC), live particulate organic carbon (LPOC), and detritus by high microbial abundance (HMA) and low microbial abundance (LMA) sponge species at (A) Conch Reef, Key Largo, FL, and (B) Carrie Bow Cay, Belize. See Table 1 for full species names and sample sizes

Table 1). At Carrie Bow Cay, detritus consumption was significant for *C. vaginalis* ($t = 3.24$, $df = 4$, $p = 0.032$), *V. reiswigi* ($t = 7.06$, $df = 2$, $p = 0.019$), and *X. muta* ($t = 6.49$, $df = 3$, $p = 0.007$), but was not significant for *A. tubulata* ($t = 1.96$, $df = 4$, $p = 0.122$), *C. plicifera* ($t = 1.55$, $df = 4$, $p = 0.196$), and *N. digitalis* ($t = 1.53$, $df = 3$, $p = 0.223$) (Fig. 2b, Table 1).

Specific filtration rates were significantly greater at Conch Reef relative to Carrie Bow Cay ($H = 8.4$, $df = 1$,

$p < 0.01$), and significantly differed between carbon pools ($H = 16.9$, $df = 2$, $p < 0.001$) and between species ($H = 25.8$, $df = 5$, $p < 0.001$). There was particularly high inter- and intraspecific variability in sponge-mediated flux of DOC, especially for the LMA sponges *C. plicifera*, *C. vaginalis*, and *N. digitalis*; however, all 2- and 3-way interactions between variables were nonsignificant ($p > 0.05$; Fig. 2, Table 1). HMA sponges generally obtained carbon in the form of

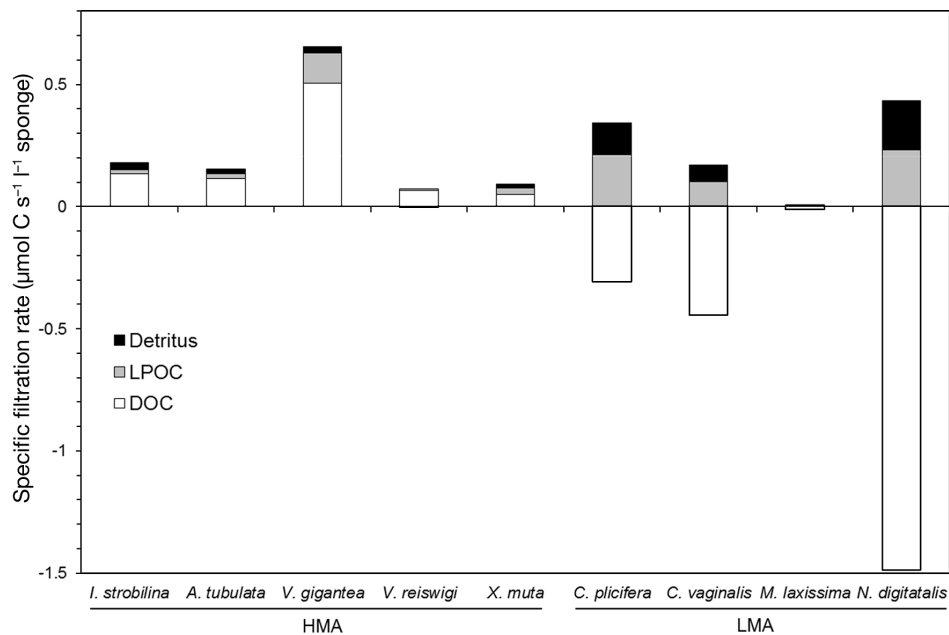


Fig. 3. Mean specific filtration rates at both study locations (Conch Reef, Key Largo, FL, and Carrie Bow Cay, Belize) for dissolved organic carbon (DOC), live particulate organic carbon (LPOC), and detritus by HMA and LMA sponge species (see Fig. 2 legend for definitions). See Table 1 for full species names and sample sizes

DOC, while LMA sponges were generally net sources of DOC, with a diet that primarily consisted of LPOC and detritus (Fig. 3). Considering both locations combined, DOC accounted for 75, 75, 77, 91, and 54% of mean total carbon uptake for the HMA sponges *A. tubulata*, *I. strobilina*, *V. gigantea*, *V. reiswigi*, and *X. muta*, respectively, while the proportions of mean total carbon uptake in the form of LPOC and detritus by LMA sponges were, respectively, 62 and 38% for *C. plicifera*, 59 and 41% for *C. vaginalis*, 74 and 26% for *M. laxissima*, and 53 and 47% for *N. digitalis*.

Ordinary least squares regression was used to assess the variability of DOC flux by examining the relationship between DOC specific filtration rates and \log_e -transformed incurrent DOC concentration. There was a significant direct relationship between the flux of DOC and the concentration of DOC in ambient seawater for the HMA sponges *A. tubulata*, *V. gigantea*, and *V. reiswigi* (Fig. 4) and the LMA sponge *C. plicifera* (Fig. 5), but not for the sponges *X. muta*, *C. vaginalis*, *N. digitalis*, or *M. laxissima*.

DISCUSSION

Just as the functional role of microbes in recycling DOM was found to explain the paradox of abundant planktonic life in oligotrophic seas (Pomeroy et al. 2007), another long-overlooked group, the Porifera,

may underpin the high productivity of biodiverse coral reefs in desert-like tropical seas via the sponge-loop (de Goeij et al. 2013). In fact, it has been suggested that DOM turnover by encrusting coral reef cavity sponges alone is on par with estimates of the daily gross primary production of the entire reef ecosystem (de Goeij et al. 2008b), and that a large portion of assimilated DOM is subsequently released as detritus (de Goeij et al. 2013, Alexander et al. 2014, Rix et al. 2016, 2017). The putative ecological significance of the sponge-loop is even more impressive when one considers that, with one exception (McMurray et al. 2016), DOM turnover and detritus production has yet to be considered for the massive, emergent sponge species that dominate the surface of Caribbean coral reefs and represent a large proportion of sponge community biomass. While DOC uptake by emergent sponges was observed in the present study, particularly by HMA sponge species, no sponge species was found to produce significant quantities of detritus; thus, the sponge-loop, as originally proposed, may be limited to encrusting sponges found in the interstices of coral reefs.

In retrospect, these findings may not be especially surprising considering the proposed mechanism and high rates of sponge detritus production observed for encrusting sponge species. The growth of cryptic, encrusting sponge species is thought to be negligible, limited by high competition for available sub-

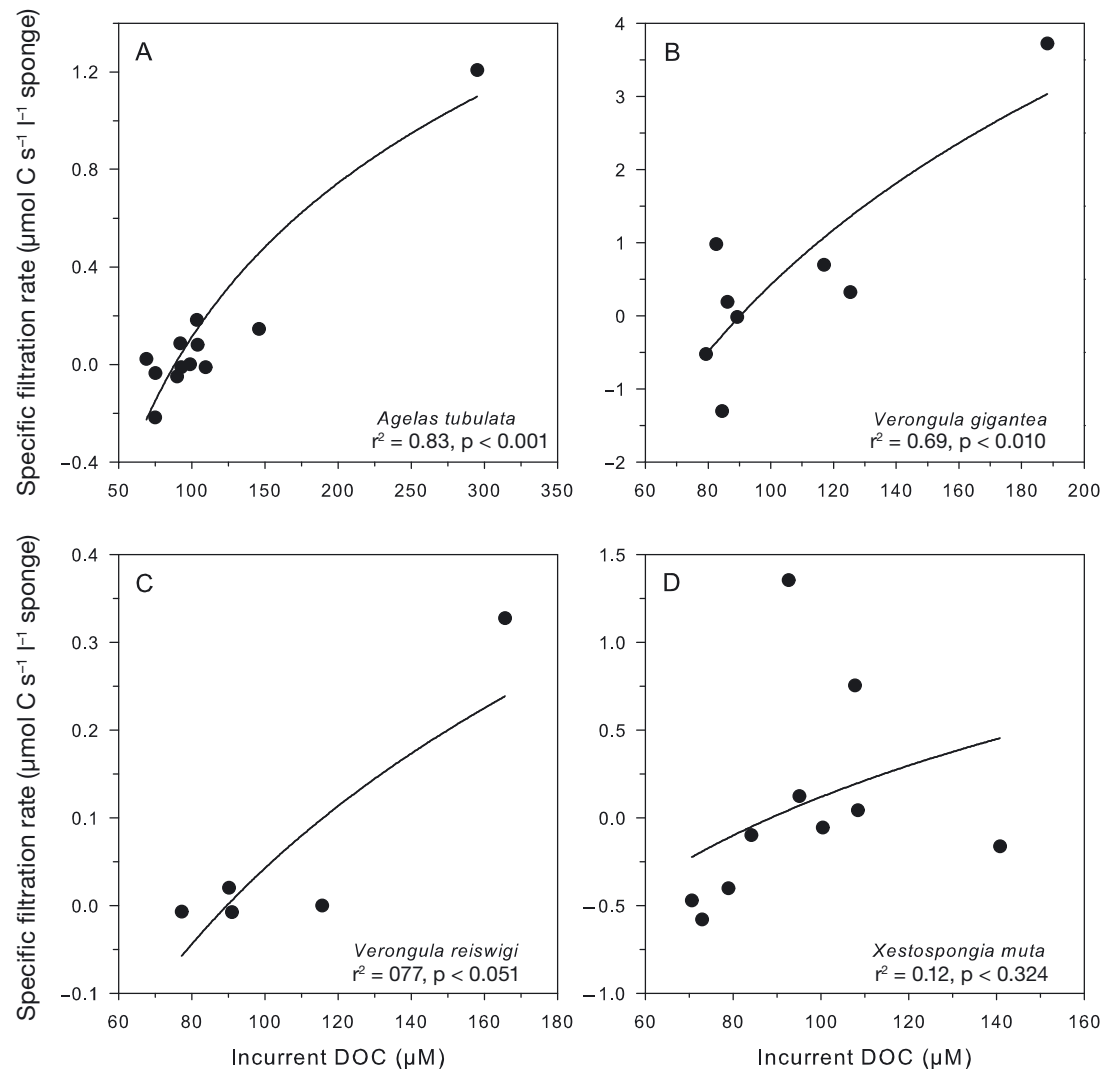


Fig. 4. Relationship between specific filtration rate and the abundance of dissolved organic carbon (DOC) for HMA sponges (see Fig. 2 legend for definition): (A) *Agelas tubulata*, (B) *Verongula gigantea*, (C) *V. reiswigi*, and (D) *Xestospongia muta*

strata; yet, these species consume DOC at high rates and DOC frequently constitutes >90% of total organic carbon uptake (de Goeij et al. 2008b, 2013). Instead of growing, it is hypothesized that detritus production results from the rapid proliferation and shedding of cells, primarily choanocytes (de Goeij et al. 2009, 2013, Alexander et al. 2014, 2015), but may also include digestive waste products (Kahn & Leys 2016, Maldonado 2016). This process is energetically costly, and may represent 75% of the total organic carbon (DOC and POC) assimilated (Kahn & Leys 2016). Moreover, it has been estimated that up to 40% of the DOC consumed by cryptic sponges is released as detritus (Rix et al. 2016). Therefore, if massive, emergent sponge species exhibit similarly rapid cell proliferation and shedding, one would expect to observe: (1) minimal sponge growth; and, if

rates of detritus production for encrusting species are scaled up to the much larger biomass of emergent species, (2) discharge of detritus in the excurrent seawater of emergent species. We maintain that there is little evidence to support these predictions. Massive, emergent sponges often display substantial growth (e.g. Reiswig 1973, McMurray et al. 2008); moreover, in contrast to detritus production by encrusting species at rates sufficient to permit daily collection via pipette (Alexander et al. 2014), we have not observed the discharge of detritus from massive species in the field. Detritus production has been found to decrease as sponges regenerate from tissue damage (Alexander et al. 2015); however we employed In-Ex methods that do not require sponge manipulation and only included individuals with no obvious signs of disease or tissue damage. We hypothesize that, un-

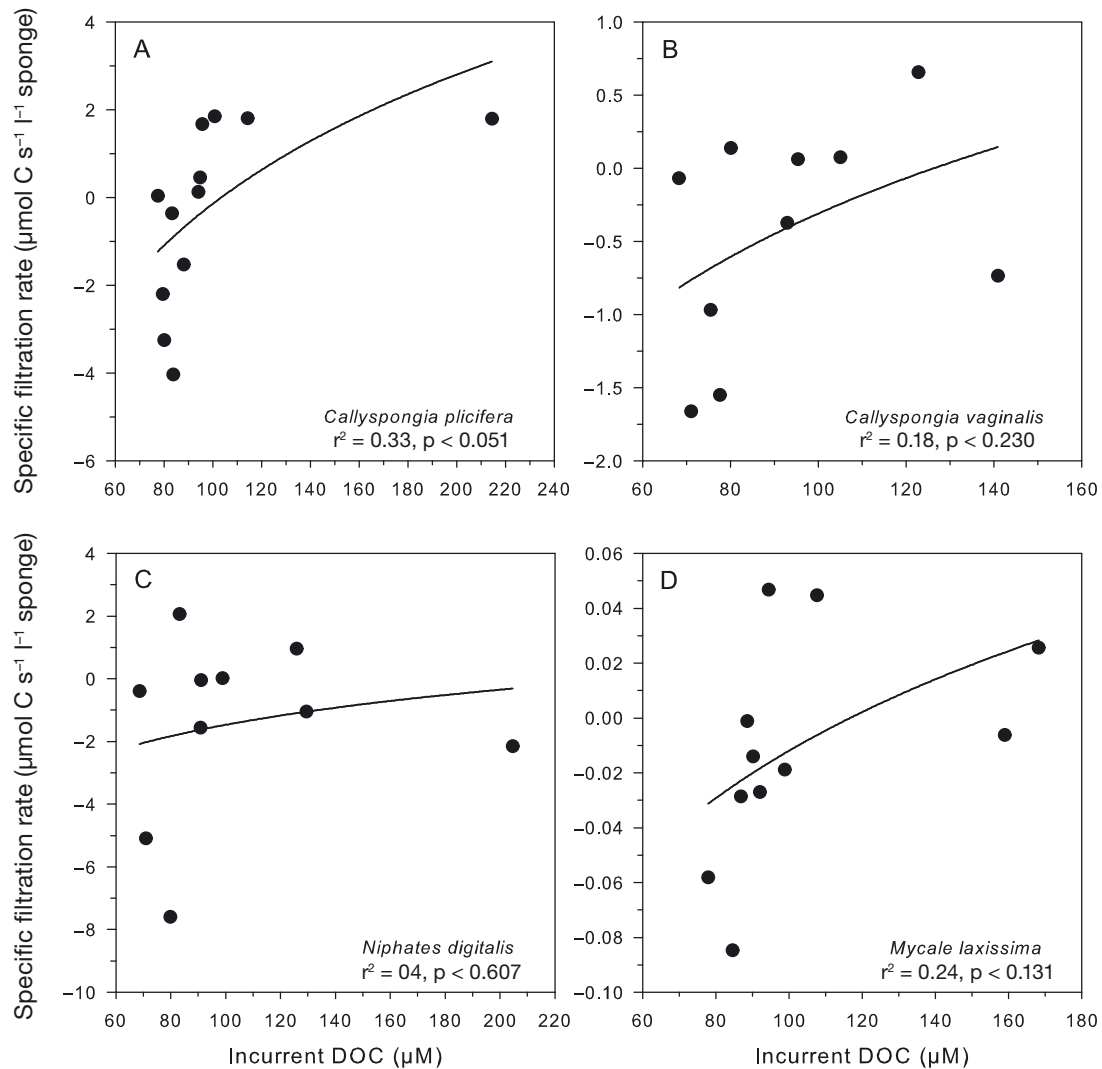


Fig. 5. Relationship between specific filtration rate and the abundance of dissolved organic carbon (DOC) for LMA sponges (see Fig. 2 legend for definition): (A) *Callyspongia plicifera*, (B) *C. vaginalis*, (C) *Niphates digitalis*, and (D) *Mycale laxissima*

like encrusting species which are largely limited to growth in 2 dimensions, emergent species divert assimilated carbon from DOC to biomass production instead of cell turnover and detritus production.

Low DOC uptake by emergent sponges, relative to encrusting species, is likely an additional factor that may explain the lack of detritus production observed in this study. Our estimates of DOC flux for *Xestospongia muta* on Conch Reef ($0.19 \pm 0.73 \mu\text{mol C s}^{-1} \text{ l}^{-1} \text{ sponge}$, mean \pm 95% CI; Fig. 2a) agree with those found for the same species at the same location ($0.53 \pm 0.42 \mu\text{mol C s}^{-1} \text{ l}^{-1} \text{ sponge}$, mean \pm SE) by Hoer et al. (2018), and DOC flux for the emergent species *Theonella swinhoei* ($0.43 \pm 0.30 \mu\text{mol C s}^{-1} \text{ l}^{-1} \text{ sponge}$, mean \pm SD; Yahel et al. 2003) is within the range we found for the HMA species investigated (Figs. 2 & 3); however, these estimates are approxi-

mately an order of magnitude lower than those reported for cryptic, encrusting species that produce detritus (3.64 ± 0.69 , 4.22 ± 0.25 , and $3.78 \pm 0.67 \mu\text{mol C s}^{-1} \text{ l}^{-1} \text{ sponge}$, mean \pm SD, for *Halisarca caerulea*, *Mycale microsigmatosa*, and *Merlia normani*, respectively; de Goeij et al. 2008b, 2013). The estimates of DOC flux for emergent species reported here also appear lower than those found for encrusting species inhabiting temperate Mediterranean reefs, although DOC fluxes were standardized to sponge area and not volume, making it problematic to compare with the data presented here (Morganti et al. 2017). Although sponge-mediated DOC uptake is commonly reported in the literature, there is unfortunately limited information on rates of sponge-mediated DOC flux standardized to sponge tissue volume, making further comparisons difficult.

The concentration and composition of DOC available affects rates of DOC uptake and transformation by sponges and may partly explain conflicting findings for encrusting versus emergent sponge species. It is thought that sponges primarily consume labile, rather than refractory, forms of DOC (Yahel et al. 2003, de Goeij et al. 2008b), and higher rates of uptake and detritus production have been found for sponges incubated with algal- versus coral-derived DOC (Rix et al. 2017). Evidence suggests that there may be a direct relationship between ambient DOC concentrations and DOC uptake, and there may be a threshold for DOC uptake (Mueller et al. 2014, McMurray et al. 2016, 2017, Archer et al. 2017, Morganti et al. 2017). Our results only support such a relationship for the HMA species *Agelas tubulata*, *Verongula reiswigi*, *V. gigantea*, and the LMA species *Callyspongia plicifera*, although additional data are needed to further examine this putative relationship for the species investigated. A direct relationship between DOC availability and consumption may indicate that microbes are primarily involved in DOM uptake (Middelburg 2015); however, microbes and sponge cells may also differentially assimilate different DOC fractions (Rix et al. 2017), which may explain the lack of a relationship for the other 4 species considered. Finally, given lower rates of DOC uptake by emergent sponge species, and approximately equivalent dissolved oxygen demand for emergent and encrusting species (Reiswig 1974, 1981, Yahel et al. 2003, de Goeij et al. 2008b, Hoer et al. 2018), a larger proportion of consumed DOC may be used to satisfy respiration demands by emergent species relative to encrusting species, allowing for carbon accumulation and cell turnover by the latter (see Hoer et al. 2018 for discussion).

Is it possible that significant detritus production by the emergent sponges sampled in this study was somehow overlooked as a consequence of using In-Ex methods? For example, it is currently unknown if detritus production varies temporally; if detritus was released by sponges episodically, then it may have been missed within the relatively short sampling interval that was employed in this study (de Goeij et al. 2017). This seems unlikely, given the number of replicates that have been analyzed in this study, and in previous studies of *X. muta* in which excurrent detritus was also not detected (McMurray et al. 2016). Further, Yahel et al. (2003) was able to detect excurrent detritus from the Red Sea sponge *T. swinhoei* using similar In-Ex sampling methods. Other techniques that have been used to study sponge detritus production (de Goeij et al. 2017) involve the place-

ment of sponges in open pots or flow chambers and the subsequent collection of detritus from the sponge surface and the bottom surface of open pots via pipette (Alexander et al. 2014) or by filtering the volume of seawater in the chamber through a GF/F filter after a sufficient interval of time (de Goeij et al. 2013, Rix et al. 2016, 2017). The deposition of sponge-produced detritus in aquaria or incubation chambers (e.g. de Goeij et al. 2013, Alexander et al. 2014) might suggest that it is emitted by sponges as clumps or larger particles that exceed 100 μm in size, potentially larger than the pre-filter used in this study. However, seawater samples analyzed in the present study were expelled from syringes through 100 μm mesh suspended in air within a filtration funnel, resulting in a great deal of shear that would break-up any aggregated or flocculated detritus that might be present, but retain potential contaminants such as zooplankton. Further, no aggregates of detritus were ever observed on the surface of the white mesh while processing samples for this study. It should also be noted that even relatively small particles (<50 μm) sink and contribute to the vertical flux of detrital carbon in marine systems (Gowing & Silver 1985). Bacterial colonization and the aggregation of detritus emitted by sponges may further contribute to detrital deposition (Biddanda & Pomeroy 1988), and the nitrogen contribution by colonizing bacteria has been invoked to explain the lower C:N ratio of sponge-produced detritus relative to the DOM consumed by sponges (de Goeij et al. 2017). There is little information on the physical nature of sponge-produced detritus; however particles <8 μm have been detected in excurrent seawater using flow cytometry (Yahel et al. 2003, McMurray et al. 2016), and Wolfrath & Barthel (1989) reported that the sponge *Halichondrea panacea* excreted detritus in the form of spherical fecal pellets with diameters that ranged from 10 to 55 μm when fed microspheres and unicellular algae. Examinations of detritus within the lumen of sponge excurrent canals have additionally revealed that the detritus has a complex composition and includes choanocytes, archeocytes, spherulous cells, granular cells, and exocytosed vesicles, all generally <10 μm in diameter (Maldonado 2016), and that, closer to the oscula, this detritus may occur as mucal sheets of cellular debris (Alexander et al. 2014).

While we did not detect significant net detritus production, it is important to note that some individual sponges did produce measurable detritus. Sponge detritus includes both debris resulting from cell turnover and digestive wastes (Maldonado 2016). For the

few individuals with negative detrital flux, the concentration of carbon emitted was generally small; therefore, we hypothesize that, similar to conclusions reached by Kahn & Leys (2016) for work on explants of 4 cold-water sponge species, the detritus released by massive species is likely produced as waste, rather than cellular debris resulting from rapid cell turnover. Given the indirect method of quantifying detritus used here, it is impossible to distinguish sponge-generated detritus from incurrent detritus. Therefore, it is possible that individuals that are net consumers of detritus also produce detritus, but at rates lower than detritus consumption. Some sponge species are also known to harbor invertebrate symbionts such as shrimps and brittle stars (e.g. Henkel & Pawlik 2005), and it is unknown whether endosymbiotic macrofauna consume sponge-produced detritus in excurrent canals before it can be released from the sponge. However, these symbiotic relationships are highly variable across sponge species (and are often species-specific); if detrital consumption within the sponge was taking place, it is likely that divergent results would have been obtained across the sponge species studied herein. In any case, the ecological relevance of detritus production by emergent sponge species is questionable if there is overall net removal of detritus from the water-column and production does not approach the levels originally suggested for encrusting species (de Goeij et al. 2013).

Our findings support the general paradigm that the diet of HMA species primarily consists of DOC (e.g. Hoer et al. 2018), while LMA species have greater reliance on dead and live POC in the form of picoplankton (Maldonado et al. 2012). However, we note that this may not hold for LMA species with encrusting morphologies, as significant amounts of DOC uptake have been found for a number of species (de Goeij et al. 2008b, 2013, Rix et al. 2016, 2017, Morganti et al. 2017; but see Ribes et al. 1999, 2012). HMA species have a variety of traits, including slower pumping rates and denser tissue, which increase the time of seawater contact within the aquiferous system and may aid in higher DOC uptake relative to LMA species (Weisz et al. 2008). Intraspecific rates of DOC flux were especially variable; this may be due to variability in the concentration and composition of ambient DOC (see above), but may also reflect differences in the sponge microbiome (Fiore et al. 2013). Although the relative role of microbes versus sponge cells in DOC processing remains unresolved, higher rates of DOC uptake by HMA versus LMA species is consistent with the view that DOC processing is, at least in part, mediated by symbiotic

microbes (Hoer et al. 2018). Nonetheless, the ability of LMA species to process DOC, albeit at lower and variable rates, may also indicate that sponge cells have a direct role; and tracer experiments have shown that both sponge cells and microbes assimilate DOC (de Goeij et al. 2008a, Rix et al. 2016, 2017). While a correlation between picoplankton abundance and sponge growth (as tube elongation) has been used to suggest that food is limiting for Caribbean coral reef sponges (Lesser & Slattery 2013, Slattery & Lesser 2015), this conclusion has been contested (Pawlik et al. 2013, 2015a,b). Considering that the diets of both the HMA and LMA species examined here were found to be broader than initially assumed under the food limitation hypothesis, and include both DOC and detritus, we maintain that the abundance of picoplankton alone is not an appropriate metric with which to assess food limitation.

Despite a lack of evidence for the production of detritus by massive, emergent sponge species investigated here, the rates of DOC uptake by HMA sponges likely signify a similarly important, but different, trophic pathway from that originally described for the sponge-loop hypothesis. The bioavailable product of DOC assimilation by cryptic sponge species is detritus that is available to detritivores and suspension feeders (de Goeij et al. 2009, 2013, Alexander et al. 2014, 2015), while emergent sponge species return carbon to the benthos in the form of biomass that feeds spongivorous fishes, turtles, and invertebrates. Not all of the sponge biomass produced by emergent sponge species is available to higher trophic levels, as some species invest resources into the production of secondary metabolites that deter predation; however, species that lack chemical defenses grow and reproduce at faster rates and are grazed upon by spongivorous predators (Pawlik 2011). In contrast, chemically defended individuals that grow relatively slowly and may be long-lived (McMurray et al. 2008) may be important for the sequestration and storage of carbon as biomass.

Our evidence of detritus uptake by a number of emergent sponge species, and mean production of DOC by emergent LMA species, may indicate even further complexity in the trophic relationships mediated by sponges. For example, it remains unknown whether detritus production by cryptic species may be feeding emergent sponge species, and whether DOC released by LMA species may be feeding emergent HMA and cryptic LMA species in a manner similar to the partial trophic niche separation of HMA and LMA species recently proposed on the basis of sponge nitrogen cycling (Morganti et al. 2017). Given

the contrasting trophic pathways of DOC cycling by cryptic versus emergent coral reef sponges, and the diversity of trophic groups that may be ultimately fueled by this production, we conclude that the complexity and ecological importance of the sponge-loop may be even greater than originally proposed by de Goeij et al. (2013).

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