Carbon Flux Through the Giant Barrel Sponge *Xestospongia testudinaria* in the Red Sea

Thesis by

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The thesis of Michael Wooster is approved by the examination committee.

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Sponges have important ecological functions on coral reefs because they are regionally abundant, competitively dominant, and process large volumes of seawater. The sponge loop hypothesis proposes that sponges consume dissolved organic carbon (DOC) and then releases the carbon as shed cellular detritus back to the reef benthos. Within this context, we examined the carbon flux mediated by the giant barrel sponge, *Xestospongia testudinaria*, on reefs in the Red Sea, where sponge abundance is comparatively low relative to coral reefs elsewhere, such as the Caribbean. Seawater samples were collected from the incurrent and excurrent (In-Ex) flow of 40 sponges from inshore, mid-shelf, and offshore reefs between 18° and 22°N latitude off the coast of Saudi Arabia. Concentrations of DOC and living particulate organic carbon (LPOC) were significantly higher in incurrent (ambient) seawater on inshore reefs than mid-shelf and offshore reefs. Consistent with studies of *X. muta* in the Caribbean, the diet of *X. testudinaria* is comprised primarily of DOC; mean values of the nutritional components across all sites were 60.5% DOC, 35.7% detritus, and 3.8% LPOC. Taking into account the specific filtration rates of nutritional components and oxygen consumption of sponges across the inshore-offshore gradient, there is evidence (1) of a threshold concentration of DOC below which sponges cease to be net consumers of DOC, and (2) that sponges on offshore reefs are food-limited. Contrary to the sponge loop hypothesis, there was no evidence that *X. testudinaria*, returned DOC to the benthos in the form of detritus, but was, instead, a net consumer of detritus from the water column. Unlike the cryptic, interstitial sponges that were studied to advance the sponge-loop hypothesis, emergent sponges may have an alternate pathway for returning DOC to the benthos by converting it to sponge biomass rather than sponge detritus.
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### LIST OF ABBREVIATIONS

<table>
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>DO</td>
<td>dissolved oxygen</td>
</tr>
<tr>
<td>DOC</td>
<td>dissolved organic carbon</td>
</tr>
<tr>
<td>DOM</td>
<td>dissolved organic matter</td>
</tr>
<tr>
<td>HMA</td>
<td>high microbial abundance</td>
</tr>
<tr>
<td>HNA</td>
<td>high nucleic acid</td>
</tr>
<tr>
<td>LMA</td>
<td>low microbial abundance</td>
</tr>
<tr>
<td>LNA</td>
<td>low nucleic acid</td>
</tr>
<tr>
<td>LPOC</td>
<td>living particulate organic carbon</td>
</tr>
<tr>
<td>LPOM</td>
<td>live particulate organic mater</td>
</tr>
<tr>
<td>Euk</td>
<td>nano and pico-eukaryotes</td>
</tr>
<tr>
<td>POC</td>
<td>particulate organic carbon</td>
</tr>
<tr>
<td>Pro</td>
<td>Prochlorococcus</td>
</tr>
<tr>
<td>Syn</td>
<td>Synechococcus</td>
</tr>
<tr>
<td>TOC</td>
<td>total organic carbon</td>
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1. Introduction

On coral reefs, sponge biomass and diversity can exceed that of reef-building corals (Diaz & Rutzler 2001; Richter et al. 2001; Lesser et al. 2009; McMurray et al. 2010), with sponge abundance increasing on many coral reefs worldwide (McMurray et al. 2010; Bell et al. 2013). Sponge biomass is generally far lower on reefs in the Pacific relative to those in the Caribbean; however, many surveys of sponge abundance have not considered coral cavities, which have extensive surface areas (Richter et al. 2001; Scheffers et al. 2004) and support cryptic benthic communities that are largely dominated by encrusting sponges (Richter et al. 2001; Wunsch et al. 2003; Van Duyl et al. 2006). For example, in surveys of reefs in the Red Sea, Richter et al. (2001) found surface areas of internal cavities were 2.5-7.4 m² per projected 1 m² of the reef surface, where encrusting sponge cover could reach 60%. In a study comparing various benthic diversity assessments, Pearman et al. (2016) found estimates of sponge cover to be ~1% using visual surveys, ~25% using photo analysis of Autonomous Reef Monitoring Structure (ARMS) plates, and 30% using metabarcoding suggesting that coral cavities support vast communities of cryptic sponges. Another possible reason for the lower sponge biomass in the Pacific versus Caribbean reefs is the more oligotrophic nature of the waters in the Pacific (Karl and Lukas 1996). There are currently debates about the food limitations of sponge communities in the Caribbean (Pawlik et al. 2015a, b; Slattery & Lesser 2015), but sponges can be food limited as shown by Wilkinson and Cheshire (1990). They looked at sponge communities on oceanic and inshore sites off the Great
Barrier Reef (GBR) along with Belize reefs in the Caribbean. The sponge communities on the food limited oceanic Pacific reefs were characterized by lower percent cover, low biomass, and the dominance of phototrophic sponge species. The Red Sea is known to be an oligotrophic environment (Raitos et al. 2013), and with the low biomass and percent coverage of sponges compared to the Caribbean (Pawlik et al. 2015a) there might be cases of food limitations on more oligotrophic reefs.

Given the high sponge biomass within the reef framework, and their capacity to turn over large volumes of seawater in the process of feeding, sponges may play a greater role in reef trophodynamics than previously assumed. The ability of some sponges to process huge volumes of water (Reiswig 1974; McMurray et al. 2014) allows them to ingest a large amount of carbon ranging from 29 –1970 mg C m² sponge⁻¹ day⁻¹ (Gili and Coma 1998 and references therein) and 0.04–18.5 mmol C cm³ sponge⁻¹ h⁻¹ (de Goeij et al. 2008a and references therein) and therefore play an important role in benthic-pelagic coupling of organic matter and nutrients (Gili and Coma 1998; Okamura 1990).

Sponges are known to consume both particulate organic matter (POM) and dissolved organic matter (DOM) via suspension feeding (Ribes et al. 2003). The earliest studies of sponge feeding considered only POM, which includes living (LPOM) and nonliving (detritus) particles (Ribes et al. 1999; Yahel et al. 2003; Hadas et al. 2009). The LPOM that is consumed consists mostly of picoplankton (0.2-2 µm) (Pile et al. 1997; Ribes et al. 1999; Kötter & Pernthaler 2002) and includes eukaryotic phytoplankton (picoeukaryotes) as well as photoautotrophic and heterotrophic bacterioplankton.
Ferrier-Pagès & Gattuso 1998; Charpy & Blanchot 1999; Ferrier-Pagès & Furla 2001; Gast et al. 1998). More recently, investigations have determined the importance of DOC and detritus to the sponge diet (Yahel et al. 2003; Hadas et al. 2009; McMurray et al. 2016). The ability of sponges to take-up DOM has been known for quite some time (Stephens and Schinske 1961; Schmidt 1970; Weissenfels 1976; Wilkinson and Garrone 1979; Jaeckle 1995). However, it was not until recently that it was experimentally shown that sponges could absorb large amounts of naturally-occurring DOC (e.g., Yahel et al. 2003; De Goeij et al. 2008a; Gibson 2011; McMurray et al. 2016; Archer et al. 2017; Hoer et al. 2017; Morganti et al. 2017). DOC is operationally defined as the fraction of organic carbon that passes through a fine filter with a pore size of 0.2-0.7 µm (Benner 2002; Carlson 2002). The composition of natural DOM is largely unknown but includes small particles such as viruses or ultramicrobacteria (<0.2 mm diameter), small colloidal compounds, and true dissolved material (Reiswig 1981; Yahel et al. 2003; Hadas et al. 2006; de Goeij et al. 2008b; Nebbioso & Piccolo 2013; Carlson & Hansell 2015). In the first quantitative assessment of in situ ambient bulk DOC retention, Yahel et al. (2003) found mean DOC uptake of 1.56 mmol C cm$^{-3}$ h$^{-1}$ for the tropical sponge *Theonella swinhoei*, a rate that was an order of magnitude greater than the rate of LPOM uptake. Similarly, de Goeij et al. (2008a) reported DOC removal from ambient water at even higher rates by the encrusting coral cavity sponges *Halisarca caerulea*, *Mycale microsigmatosa*, and *Merlia normani*, which ranged from 11 to 21 mmol C cm$^{-3}$ h$^{-1}$. It is now well established that sponges can take up large amounts of DOC, which can make up to 90% of their diet (Yahel et al. 2003; de Goeij et al. 2008a; Van Duly et al. 2008;
Ribes et al. 2012; Mueller et al. 2014). Detritus consumption is not well understood in sponges and seems to vary with some species utilizing it while others do not (Ribes et al. 1999; Yahel et al. 2003; Hadas et al. 2009; McMurray et al. 2016). Detritus is a structurally and chemically diverse fraction deriving from a range of different sources that often exceeds LPOM biomass (Lenz 1977; Wilson et al. 2003; Hadas et al. 2009). Hadas et al. (2009) found that detritus uptake constituted about 1/3 of the POC consumed by the reef sponge Negombata magnifica and McMurray et al. (2016) found that Xestospongia muta retained detritus at 79.2% and made up ~20% of its diet. These studies show evidence that detritus can be an important part of the sponge diets. With a better understanding of sponges diet, we can get a better picture of sponge energetics. In the early sponge feeding studies, it was realized that something was missing from our knowledge of the diet of sponges because dissolved oxygen (DO) removal was higher than food consumption (Reiswig 1974, 1981). Recently, with the knowledge of DOC consumption, the metabolic gap between DO removal and food consumption has been filled (Yahel et al. 2003; Hoer et al. 2017).

Recent work on sponge mediated DOC uptake led to development of the sponge loop hypothesis (de Goeij et al. 2013), which proposes that sponges take up large amounts of DOC largely exuded by primary producers on reefs and return it to the benthos as shed cellular detritus that can be consumed by detritivores and brought back up the food web (de Goeij et al. 2013; Alexander et al. 2014; Maldonado 2016). If the sponge-loop is indeed functioning as proposed, it would represent a major undocumented source of carbon, and may partly address Darwin’s paradox of how reefs
exist in such oligotrophic waters (Darwin 1842). In the Caribbean, DOM turnover through sponges is hypothesized to be the same order of magnitude as the total gross primary production rates of the entire reef ecosystem (de Goeij et al. 2013). Rix et al. (2016) further advanced the sponge loop hypothesis by demonstrating that it occurs in the warm water reefs in the Red Sea and deep North Atlantic cold-water reefs. Using stable isotope tracer experiments in a laboratory setting, they were the first to demonstrate that these sponges take up coral mucus and release a portion of this assimilated carbon as detritus. It is important to note, however, that all of these studies used small and encrusting sponges which constitute a small fraction of overall sponge biomass on reefs (at least in the Caribbean). Results from Xestospongia muta, a large emergent sponge, support the sponge loop theory in that this species consumed large amounts of DOC (McMurray et al. 2016), although it was also a net sink for detritus. Combined with the proposed sponge loop-driven DOM cycling, another hypothesis called the vicious cycle could play a role in shifting reef communities (Pawlik et al. 2016). The vicious cycle hypothesis provides an explanation for the reduced resilience observed for Caribbean coral reefs versus reefs in other tropical areas (Pawlik et al. 2016). It proposes that sponges and macroalgae are involved in a feedback loop in which sponges provide nutrients to macroalgae, and macroalgae provide labile DOC to sponges, both to the detriment of reef-building corals. Rix et al. (2017) demonstrated that algal DOM was more labile and taken up more readily by sponges. This supports the premise of the vicious cycle hypothesis where sponges would uptake algal DOM then release nutrients which further enhance algae growth while both are competing for
space with coral. The shifts in reef communities to algal dominance is more readily seen on Caribbean reefs (Bellwood et al. 2004; Bruno et al. 2009) where there is a larger input of DOC and nutrients to the system. The feedback loop, in addition to a larger sponge community, could explain why coral communities are slow to recover in the Caribbean.

The three objectives of this study were to: 1) characterize the diet of Xestospongia testudinaria of the Red Sea, 2) examine how food availability (DOC, live particulate organic carbon (LPOC), detritus), feeding, and respiratory demands vary with distance from shore, and 3) test the sponge loop hypothesis for an emergent Red Sea sponge species. In-situ “In-Ex” sampling methods (i.e., taking incumbent and excurrent water samples) were employed. This is the preferred technique for carbon flux studies because they do not disturb the sponge and assess its natural feeding behavior. *Xestospongia testudinaria* was chosen because it is among the few massive, conspicuous sponges on the reefs of the Red Sea, and giant barrel sponges are known to play important functional roles as benthic suspension feeders elsewhere (e.g., McMurray et al. 2017). Recent molecular studies have suggested that the currently recognized three species of giant barrel sponges, in the genus *Xestospongia*, may be incorrect and there may be multiple sympatric species among them (Swierts et al. 2017). Samples from around the world have resulted in 17 haplotypes making up possibly nine separate species. The Red Sea samples have a unique haplotype, suggesting there may be a distinct species in the region (Swierts et al. 2017). This study was designed to determine whether *X. testudinaria*, an emergent and large biomass sponge, participates in the
cycling of carbon in the Red Sea as predicted by the sponge loop hypothesis.

*Xestospongia testudinaria* is considered a high microbial abundance (HMA) sponge and it is uncertain whether the symbiotic bacteria it contains are mutualistic or commensal. As has been shown in other sponge species, we predicted that a large proportion of the sponges’ diet would consist of DOC, with a smaller fraction of POC. However, it is probable that there will be shifts in diet percentages as a function of distance from shore, as the local abundance of POC and DOC may differ. The metabolic activity was further examined by comparing dissolved oxygen (DO) consumption to food consumption. Similar to *X. muta* in the Caribbean, we also hypothesize that *X. testudinaria* will be a net sink of detritus, resulting in sponge growth rather than the production of shed cellular debris, due to the more prominent oligotrophic environment the Red Sea.
2. Methods

Carbon flux was quantified for *Xestospongia testudinaria* on reefs south of Al-Lith in the Farasan Banks and on reefs near Thuwal in the Saudi Arabian Red Sea (Supporting Information Fig. S1) in May 2017. Reefs were classified primarily by distance from shore as one of three types: 1) “offshore” reefs were located off the continental shelf and surrounded by deep water, 2) “mid-shelf” reefs were located on the shelf edge or the continental shelf, and 3) “inshore” reefs were on the continental shelf and close to shore. Sponges on twenty different reefs were sampled, including eight inshore, eight mid-shelf, and four offshore reefs. A total of forty sponges were haphazardly selected for study primarily between 10 and 21 m depths and six individuals were additionally sampled at night. The number of sponges sampled on the different reef types and number of day and night pairs in parentheses is 18(3), 15(2), and 7(1) for inshore, mid-shelf, and offshore reefs respectively.
Suspension feeding by *X. testudinaria* was investigated following the methods of McMurray et al. (2016). For each sponge, 1 L of incident seawater was collected adjacent to the external sponge surface and 1.5-1.6 L of excurrent seawater water was collected below the osculum within the inner empty space of the sponge. Excurrent seawater samples were collected with 100 mL syringes at a rate slower than that of excurrent flow to minimize any potential contamination of samples with ambient
seawater that had not passed through the sponge. Thus, samples represent approximately 10-20 minutes of sponge feeding. To quantify total POC (LPOC + detritus), each sample was filtered through a 100 µm mesh and a precombusted (500°C for 4 h) 0.7 µm GF/F glass fiber filter. Filters were individually wrapped in aluminum foil and frozen until analysis. To quantify DOC, 20 mL of the filtrate from each sample was transferred to an EPA precleaned glass vial, acidified in the field with 100 µL of 50% phosphoric acid, and stored at 4°C until analysis. DOC concentrations were measured using high-temperature catalytic oxidation with a Shimadzu TOC-L TN analyzer. POC was measured using a Flash 2000 CHNS/O Elemental analyzer after filters were dried at 50°C and subsequently exposed to hydrochloric acid fumes for 24 h. All glassware and aluminum foil used to process samples were combusted before use and all plastic used for sample collection was acid washed before use (Tupas et al. 1994).

To quantify LPOC, 5 mL samples of incurrent and excurrent seawater were collected as described above using 5 mL syringes. Samples were preserved in electron microscopy grade glutaraldehyde at a final concentration of 0.1% in cryovials and, after 10 min in the dark, quickly frozen in liquid nitrogen and stored at -80°C until analysis. Phytoplankton (*Prochlorococcus* (Pro), *Synechococcus* (Syn), and photosynthetic pico and nanoeukaryotes (Euk)) in seawater samples were enumerated using a BD FACSCanto II Flow Cytometer using a syringe pump. Population geometric mean properties (scatter and fluorescence) were normalized to 1.0 µm yellow-green polystyrene beads. Phytoplankton were classified based on their characteristic flow cytometric signatures relative to standard fluorescent microspheres following standard
population gating schemes (Cavender-Bares et al. 1998; Lindstrom et al. 2002).

Bacterioplankton (high nucleic acid bacteria (HNA) and low nucleic acid bacteria (LNA)) were similarly quantified by staining samples with Sybr Green-I as previously described (Marie et al. 1997) and relative cellular DNA quantified assuming stoichiometric dye binding. Each sample was run until either 10,000 events (cells counted) were reached or 5 minutes had elapsed. Flow cytometer flow rates were quantified by measuring the changes in mass of 1 mL water samples after 5-minute runs. Carbon content was determined using semi-empirically determined conversion factors based on changes in SSC signals, which were converted into biovolume using known values found in the literature (Calvo-Díaz and Morán 2006 for picophytoplankton; Gurdensen et al. 1988 for heterotrophic bacteria). Due to difficulties in quantifying detritus via flow cytometry, the detrital carbon in each seawater sample was estimated as the difference between total POC and LPOC. A caveat to this approach is that detritus estimates may include non-detrital material < 100µm that were not within the range of detection of the flow cytometer.

Following seawater collection, the velocity of excurrent seawater at the centerline of each sponge was determined by videotaping the movement of dye fronts in excurrent flow (Savarese et al. 1997; Weisz et al. 2008). A ruler was held parallel to the central axis of each sponge and a video camera was used to record the vertical movements of small volumes of fluorescein dye that were injected into the osculum of the sponge using a syringe. Videos were later analyzed frame by frame using Tracker (version 4.97; Open Source Physics) video analysis software to quantify the vertical
velocity of excurrent seawater at the centerline. Studies of the congener *X. muta* suggest that barrel sponge excurrent velocity profiles are typically non-uniform (parabolic); therefore, the mean excurrent velocity across the planar area of the osculum of each sponge was estimated as 0.5 times the excurrent velocity measured at the osculum centerline. The pumping rate for each sponge was then calculated as the product of the mean excurrent velocity and the area of the sponge osculum.

Seawater dissolved oxygen (DO) concentrations were measured with a PME MiniDOT logger which recorded concentrations every minute. Incurrent DO concentrations were measured over an approximately 3-minute interval with the logger sensor positioned adjacent to the external sponge surface; excurrent DO concentrations were measured by positioning the sensor inside of the osculum of each sponge for approximately 4 minutes. Subsequently, the dimensions of each sponge were measured with a flexible plastic measuring tape and sponge biomass estimates were obtained by approximating the morphology of *X. testudinaria* as a frustum of a cone (McMurray et al. 2008).

Due to constraints in the field, videos of sponge pumping were not obtained for 5 sponges; therefore, pumping rates for these individuals were estimated indirectly via the direct relationship between sponge pumping rates (*Q*) and sponge volume (*V_{sponge}*),

\[ Q = 41.189V + 126.812 \quad (R^2 = 0.513, p < 0.0001; \text{ Supporting Information Fig. S2}) \]

Sponge-specific filtration rates, or carbon flux (\(\mu \text{mol C s}^{-1} \text{L}^{-1}\) sponge), of DOC, LPOC, and detritus were calculated as:
\[ C \text{ flux} = \frac{(C_{in} - C_{ex}) \times Q}{V_{sponge}} \]

where \( C_{in} \) and \( C_{ex} \) are the incurrent and excurrent concentrations of each carbon pool (\( \mu \text{mol C L}^{-1} \) seawater), \( V_{sponge} \) is sponge tissue volume (L), and \( Q \) is the volume flow or pumping rate for each sponge (L s\(^{-1}\)).

Figure 2. The relationship between pumping rate and sponge volume for *Xestospongia testudinaria* on coral reefs in the Red Sea.

For all statistical comparisons, Levene’s test was used to test for homogeneity of variances and the goodness of fit test was used to test for normality. Differences in the concentration of ambient carbon between carbon pools (i.e., DOC, LPOC, detritus) were tested with a one-way Kruskal-Wallis test. Ordinary least square regressions were used to examine the relationship between loge-transformed incurrent food concentrations and distance from shore, which was determined using Google Earth with the GPS...
coordinates of each site. Paired t-tests were used to compare the concentrations of carbon in incurrent and excurrent seawater to test whether sponges were net consumers (or producers) of each food type. Daytime specific filtration rates were compared between reef types and carbon pools using the two-way Scheirer-Ray-Hare test. Ordinary least squares regression was used to assess the relationship between specific filtration rates and log$_e$-transformed incurrent carbon concentrations for each food type. Statistical analyses were performed using JMP Pro 13 (SAS Institute) and SPSS Statistics (version 22 for Windows; IBM) statistical software.
3. Results

Sponge size did not significantly differ across reef types and mean ± SE sponge volumes were 3.57 ± 0.57, 6.46 ± 1.06, and 8.60 ± 2.98 L on inshore, mid-shelf, and offshore reefs, respectively. The mean ± SE volumetric pumping rate for *X. testudinaria* was 0.0791 ± 0.049 L seawater s⁻¹ L⁻¹ sponge. There was a significant difference in the concentrations of carbon available in the form of the three food types (i.e., DOC, LPOC, and detritus) in ambient incurrent seawater (*H*=105.78, *p*<0.0001). Pairwise comparisons revealed that there was more carbon available in the form of DOC relative to detritus and LPOC (*p*<0.0001), and more detritus relative to LPOC (*p*<0.0001) (Fig. 3, Table 1). Ambient total organic carbon (TOC) available to sponges was significantly higher on inshore reefs relative to mid-shelf and offshore reefs (*F*=19.61, df= 2, *p*<0.0001) (Fig. 3). Furthermore, there was a significant inverse relationship between ambient food concentration and distance from shore for each food resource (Fig. 4).
Figure 3. Mean ± SE concentrations of total organic carbon (TOC), dissolved organic carbon (DOC), particulate organic carbon (POC), live particulate organic carbon (LPOC), and detritus in incurrent seawater on inshore, mid-shelf, and offshore reefs in the Red Sea.
Table 1. Mean (± SE) sponge volumes, pumping rates, incurrent carbon, and carbon fluxes for *Xestospongia testudinaria* on inshore, mid-shelf, and offshore reefs in the Red Sea. Food types considered were dissolved organic carbon (DOC), live particulate organic carbon (LPOC), and detritus (DET). % total carbon uptake’ is the percent contribution of each food type to the specific filtration rate for total organic carbon. n: number of samples. An asterisk indicates significant differences between incurrent and excurrent concentrations of the different food types: *p < 0.05, paired t-test.

<table>
<thead>
<tr>
<th>Reef type</th>
<th>Sponge volume (l)</th>
<th>Pumping rate (l s⁻¹ l⁻¹ sponge)</th>
<th>Food type</th>
<th>n</th>
<th>Incurrent Carbon (µmol l⁻¹ seawater)</th>
<th>Carbon consumed (µmol l⁻¹ seawater)</th>
<th>Specific filtration rate (µmol C s⁻¹ l⁻¹ sponge)</th>
<th>% total carbon uptake</th>
<th>% total carbon uptake for net consumers</th>
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<tr>
<td>Inshore</td>
<td>3.57 ± 0.57</td>
<td>0.068 ± 0.007</td>
<td>DOC</td>
<td>18</td>
<td>134.75 ± 9.22</td>
<td>14.89 ± 6.42*</td>
<td>0.917 ± 0.396</td>
<td>67.2</td>
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<td>LPOC</td>
<td>18</td>
<td>0.71 ± 0.05</td>
<td>0.60 ± 0.05*</td>
<td>0.040 ± 0.005</td>
<td>3.0</td>
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<td></td>
<td></td>
<td></td>
<td>DET</td>
<td>18</td>
<td>10.21 ± 0.89</td>
<td>6.73 ± 0.83*</td>
<td>0.413 ± 0.046</td>
<td>29.8</td>
<td>33.1</td>
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<td>Mid-shelf</td>
<td>6.46 ± 1.06</td>
<td>0.092 ± 0.015</td>
<td>DOC</td>
<td>15</td>
<td>82.52 ± 4.26</td>
<td>4.11 ± 2.64</td>
<td>0.314 ± 0.256</td>
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<td>LPOC</td>
<td>15</td>
<td>0.39 ± 0.06</td>
<td>0.32 ± 0.06*</td>
<td>0.038 ± 0.015</td>
<td>5.8</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DET</td>
<td>15</td>
<td>6.32 ± 0.35</td>
<td>3.16 ± 0.38*</td>
<td>0.301 ± 0.057</td>
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<td>23.9</td>
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<td>DOC</td>
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<td>1.76 ± 7.38</td>
<td>0.368 ± 0.656</td>
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<td>84.6</td>
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<td>LPOC</td>
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<td>0.25 ± 0.05*</td>
<td>0.027 ± 0.009</td>
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</tr>
<tr>
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<td>0.315 ± 0.041</td>
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Figure 4. Relationships between the distance from shore and incident carbon availability for A) total organic carbon (TOC), B) dissolved organic carbon (DOC), C) live particulate organic carbon (LPOC), and D) detritus.

Mean ± SE total number of picoplankton cells were similar among reef types ($F=1.28$, df=2, $p=0.29$); however, the composition of the picoplankton community...
significantly varied between inshore, mid-shelf, and offshore reefs (Fig. 5). The abundance of Syn and Euk was significantly higher on inshore relative to mid-shelf and offshore reefs ($H=22.65$, df=2, $p<0.0001$; $H=25.02$, df=2, $p<0.0001$ respectively), while the abundance of Pro was lower on inshore reefs compared to mid-shelf and offshore ($H=14.13$, df=2, $p=0.0009$). The abundance of LNA and HNA bacteria did not differ between reef types ($F=0.212$, df=2, $p=0.749$; $F=1.926$, df=2, $p=0.16$ respectively).

Consequently, the percentage contribution of carbon (μmol L$^{-1}$) by the different picoplankton types fractions to the LPOC carbon pool significantly varied among reef types (Fig. 6). On all reef types, the majority of carbon was consistently contributed by Syn, and the percent contribution of Syn to LPOC was significantly higher on inshore reefs compared to mid-shelf and offshore reefs ($F=29.58$, df=2, $p<0.0001$). The proportion of LPOC contributed by Euk also significantly varied between reef types ($F=4.07$, df=2, $p=0.025$), and was higher on inshore relative to mid-shelf reefs ($p=0.048$) and marginally higher on mid-reef compared to offshore reefs ($p=0.078$). In contrast, the proportion of Pro in LPOC was lower on inshore reefs compared to mid-shelf and offshore reefs ($H=20.87$, df=2, $p<0.0001$). Similarly, the percent of LNA and HNA bacteria was significantly lower on inshore reefs compared to mid-shelf and offshore reefs ($F=30.85$, df=2, $p<0.0001$; $F=18.09$, df=2, $p<0.0001$ respectively)(Fig. 6).
Figure 5. Mean ± SE abundance of picoplankton in incurrent (ambient) seawater at inshore, mid-shelf, and offshore reefs. Euk = pico and nanoeukaryotes, Pro = Prochlorococcus, Syn = Synechococcus, LNA = low nucleic acid bacteria, HNA = high nucleic acid bacteria, Total = total cells.
Figure 6. Percent ± SE of live particulate organic carbon (LPOC) in the form of each picoplankton type on inshore, mid-shelf, and offshore reefs. Pro = Prochlorococcus, Syn = Synechococcus, Euk = pico and nanoeukaryote, LNA = low nucleic acid bacteria, HNA = high nucleic acid bacteria

Sponges on all reef types were found to consume LPOC and detritus significantly, whereas consumption of DOC was only significant for sponges on inshore reefs (Table 1, Fig. 7). Of the 40 sponges investigated, 25 were net consumers of DOC, with 72.2%, 60%, and 42.9% of the individuals found to consume DOC on inshore, mid-shelf, and offshore reefs, respectively. In contrast, all sponges were net consumers of LPOC, and 39 of the individuals investigated were net consumers of detritus. The one individual sponge that had a negative flux for detritus was on a mid-shelf reef and produced only 0.0005 μmol detrital C s⁻¹ L⁻¹ sponge. The mean proportion of DOC, LPOC, and detritus in the diet of X. testudinaria for the different reefs was: 67.2, 3, and 29.8% for inshore reefs; 48.1, 5.8, and 46.1% for mid-shelf reefs; and 51.9, 3.8, 44.3% for offshore reefs respectively (Table 1). However, with the exclusion of sponges that were not net consumers of all three food types, the mean proportion of DOC, LPOC, and detritus in the diet of X. testudinaria for the different reefs changed to: 64.0, 2.9, and 33.1% for inshore reefs; 74.1, 2.0, and 23.9% for mid-shelf; and 84.6, 1.4, and 13.9% for offshore reefs respectively (Table 1).
Figure 7. Mean ± SE carbon consumed in the form of dissolved organic carbon (DOC), live particulate organic carbon (LPOC), and detritus on inshore, mid-shelf, and offshore reefs.

Specific filtration rates were significantly different between food types ($H=12.42$, df=2, $p=0.002$), and tended to decrease with increasing distance from shore, but were not significantly different between reef types ($H=1.61$, df=2, $p=0.45$) (Fig. 8). Pairwise comparisons for food types indicated that the mean specific filtration rate for LPOC was significantly lower than that of detritus ($p<0.0001$) and DOC ($p=0.0528$). There was a significant direct relationship between specific filtration rates and incident food concentrations for TOC as well as the three different food types (Fig. 9), which explained between 22 and 28% of the rates variance.
Figure 8. Mean ± SE specific filtration rates for dissolved organic carbon (DOC), live particulate organic carbon (LPOC), and detritus food types on the different reef types on inshore, mid-shelf, and offshore reefs in the Red Sea.
Figure 9. Relationship between specific filtration and the concentration of incurrent carbon for on offshore (grey circles), mid-shelf (white circle) and inshore (black circles) for A) total organic carbon (TOC), B) dissolved organic carbon (DOC), C) live particulate organic carbon (LPOC), and D) detritus.

Ambient DO concentrations (overall range, units) significantly differed between sites ($F= 6.7$, $df= 2$, $p=0.0071$). Pairwise comparison revealed that offshore DO concentrations (mean ± SE = 197.38 ± 0.99 µmol L^{-1}) were significantly higher than those measured on inshore reefs (mean ± SE 177.85 ± 2.6 µmol L^{-1}) ($p=0.0052$) and similar to those found on mid-shelf reefs (mean ± SE 184.14 ± 1.61 µmol L^{-1}) ($p=0.072$). All sponges were found to consume DO at the time of measurements, and excurrent DO was significantly reduced relative to ambient DO ($t= 15.59$, $df=35$, $p<0.0001$). The flux of DO was similar between reef types ($F=1.23$, $df=2$, $p=0.305$) and there was no significant difference between the flux of TOC and DO (paired t-test, inshore $p=0.19$, mid-shelf...
$p=0.36$, offshore $p=0.52$; Fig. 10). There was a significant direct relationship between DO demand and the specific filtration rate of total organic carbon (TOC) (Fig. 11); DO demand was also found to increase with increasing incident DO available to sponges (Fig. 12). Incident concentrations of DO and the three food types considered were similar over daylight and nighttime sampling periods (Fig. 13). Similarly, with the exception of the flux of detritus, which was lower at night relative to daylight ($p=0.0011$), rates of uptake for DO, DOC, and LPOC did not differ between daylight and nighttime periods (Fig. 14).

Figure 10. Mean ± SE specific filtration rates for total organic carbon (TOC) and dissolved oxygen (DO) on inshore, mid-shelf, and offshore reefs in the Red Sea.
Figure 11. The relationship between the specific filtration rate of total organic carbon (TOC) and dissolved oxygen (DO) demand.

Figure 12. The relationship between dissolved oxygen (DO) demand and ambient dissolved oxygen.
Figure 13. Mean ± SE daytime and nighttime incident concentrations of dissolved organic carbon (DOC), live particulate organic carbon (LPOC), detritus, and dissolved oxygen (DO).

Figure 14. Mean ± SE day and nighttime specific filtration rates (µmol s⁻¹ L⁻¹ sponge⁻¹) of dissolved organic carbon (DOC), living particulate organic carbon (LPOC), detritus, and dissolved oxygen (DO).
4. Discussion

The diet of *Xestospongia testudinaria* examined in this study consisted of mostly DOC followed by detritus and then LPOC, with the mean across all three reef types being 60.5% DOC, 35.7% detritus, and 3.8% LPOC. However, on mid-shelf and offshore reefs, the percentage of DOC and detritus became more even (Table 1). The mean percentage of the three carbon pools in the ambient seawater was 92.13% DOC, 7.42% detritus, and 0.45% LPOC. The proportions of the three food types to the diet of *X. testudinaria* are consistent with the proportions of the food types in the ambient carbon pools and the efficiency at which a close relative, *Xestospongia muta*, retained each of the carbon pools in the Caribbean (McMurray et al. 2016). The high percentage of DOC in the diet is consistent with previous studies of HMA sponges (Yahel et al. 2003; de Goeij et al. 2008b; Mueller et al. 2014; McMurray et al. 2016; Hoer et al. 2107), specifically with the cited *X. muta*, which consisted of ~70% DOC, ~10 LPOC, and ~20% detritus (McMurray et al. 2016).

Even though the diet of *X. testudinaria* consisted mostly of DOC for the sponges that were net consumers of DOC, 15 out the 40 sponges were found to be net producers of DOC. The type and available concentration of DOC are believed to affect the uptake rates of DOC (McMurray et al. 2016; Rix et al. 2017). It is thought that sponges primarily consume the labile fraction of DOC rather than refractory forms (Yahel et al. 2003; de Goeij et al. 2008b). Higher rates of uptake of algal- versus coral-derived DOC were found in incubation experiments (Rix et al. 2017). Consistent with several studies, this study
shows evidence that there may be a lower limit to which DOC can be taken up by sponges and that uptake varies directly with ambient DOC availability (Fig. 3; e.g. Mueller et al. 2014; McMurray et al. 2016, 2017; Archer et al. 2017; Morganti et al. 2017; McMurray et al. 2018). From inshore to offshore as the mean incurrent concentration of DOC became lower so did the consumption of DOC, consistent with the hypothesis that there is a threshold DOC concentration for DOC uptake (Table 1, Figure 4). For the 15 sponges that had negative flux, the average ambient DOC levels (±SE) was 83.36 ± 7.41 µmol L\(^{-1}\). The ambient carbon concentration for sponges that had a positive flux for DOC was 118.59 ± 7.89 µmol L\(^{-1}\). Although the amount and type of refractory DOC that can be consumed by sponges is unknown, it has been suggested that the lower limit of DOC that sponges can consume may reflect the refractory DOC values when the labile DOC available in seawater has been consumed (Morganti et al. 2017). Lower DOC concentrations in the same area were clearly labile for heterotrophic prokaryotes (Silva et al. in prep./ pers. Comm.), so the >100 µmol L\(^{-1}\) value might be reflecting the different nature of labile products for prokaryotes and sponges. The value is however comparable /greater than at other sites.

Although the paired t-test between the flux of TOC and DO were not significant, it seems that sponges on inshore reefs have an excess of respiratory DO demand meaning that they are largely heterotrophic and on mid-shelf and offshore reefs the sponges do not appear to be consuming enough carbon to meet the DO demand (Figure 4). This could indicate that for offshore sponges there might be a higher
contribution of phototrophic symbiont which could be partially supplying their carbon need. This could mean that the sponges may be food limited as you go to some offshore reefs. It also seems like ambient DO concentrations have a better relationship to DO demand values than the specific filtration rate of total organic carbon so DO demand might be influenced more by the ambient oxygen concentration rather than the flux of carbon (Supporting Information Fig. S7 and S8).

Similar to the microbial loop hypothesis (Azam et al. 1983), the sponge loop hypothesis (de Goeji et al. 2013) has the potential to further our understanding of resource allocation on reefs. Recent studies support the idea that some sponges take up large amounts of DOM and recycle it back into the food web as sponge-generated detritus (de Goeji et al. 2013; Rix et al. 2016, 2017). However, there is still much that needs to be tested concerning the sponge loop hypothesis. Much of the work on the sponge loop has been on encrusting species that are found in the reef interstices, with comparatively little work on the role of emergent sponge species. Our data for the emergent sponge *Xestospongia testudinaria* on Red Sea reefs indicates that this species does not fulfill all criteria defined in the sponge loop hypothesis. While our data may initially support the sponge loop hypothesis in that most of the sponges’ diets consisted of DOC, they were inconsistent with the hypothesis regarding the production of detritus as a major export of carbon back to the benthos. This conclusion was also the case for *X. muta* and other recently studied emergent sponges from the Caribbean, which had diets consisting mostly of DOC, but which were not net producers of detritus (McMurray et al. 2015).
2016; McMurray et al. 2018). These results may be explained by the fact that emergent sponges may use any extra energy for growth instead of for the production of sponge detritus (through fast turnover rates of sponge cells). In the present study, one sponge individual had a negative detrital flux, but the concentration of detritus generated was relatively small compared to the ambient detrital concentrations. It should be noted that the data for detritus was calculated for this study in an indirect way, making it impossible to distinguish sponge-generated detritus from incumbent detritus that was not consumed by the sponge. Sponges could be producing detritus that may be more readily taken up by reef organisms, just at a slower rate than the consumption of ambient detritus. However, some believe that the detritus produced by emergent sponges is mostly likely waste rather than cellular detritus (Kahn & Leys 2016; McMurray et al. 2018). McMurray et al. (2018) is one of the few studies focused on emergent sponges, and it seems to show that the emergent sponges may be storing the assimilated DOC as sponge biomass. Sponge biomass would then be reintroduced to the food web via spongivorous fishes, turtles, and sponge-eating invertebrates, rather than through the production of sponge detritus for detritivores to consume (McMurray et al. 2018).

Distance from shore appears to affect sponge feeding by having a relationship with food availability where the three food types decrease from inshore waters to more oligotrophic offshore waters (Supporting Information Fig. S3). The inshore reefs had significantly more carbon than the mid-shelf and offshore reefs. The different
components of the LPOC fractions also differed with distance from shore. The percent contribution of Pro and heterotrophic bacteria increased as the distance from shore increased, while Syn and Euk decreased (Supporting Information Fig. S5). The specific filtration rate of the sponges was higher on inshore reefs and decreased on mid-shelf and offshore reefs, which correlates with food availability on the reefs (Figure 2). Regressions of the relationship between the specific filtration rates and the incurrent carbon concentration showed that sponges had higher filtration rates for the different food types when there was more food available, as shown in previous studies (Figure 3) (McMurray et al. 2016, 2017).

In conclusion, this study with Red Sea X. testudinaria supports previous studies conducted in the Caribbean with a congeneric species, X. muta, in demonstrating that the diet, and the carbon flux through the sponge, is affected by the availability of food in the surrounding seawater, and that most of the sponge diet consists of DOC. Also, like X. muta, X. testudinaria in the Red Sea does not appear to return DOC to the benthic ecosystem in the form of detritus, contrary to the sponge loop hypothesis. Our data for X. testudinaria are congruent with a recent study conducted on emergent sponges that suggests that the sponge loop hypothesis, as originally proposed, may not be correct for emergent sponges. Emergent sponges appear to have an alternate pathway for returning DOC to the benthos by converting it to sponge biomass rather than sponge detritus (McMurray et al. 2018).
5. Conclusion

In conclusion, this study with Red Sea *X. testudinaria* supports previous studies conducted in the Caribbean with a congeneric species, *X. muta*, in demonstrating that the diet, and the carbon flux through the sponge, is affected by the availability of food in the surrounding seawater, and that most of the sponge diet consists of DOC. Also, like *X. muta*, *X. testudinaria* in the Red Sea does not appear to return DOC to the benthic ecosystem in the form of detritus, contrary to the sponge loop hypothesis. Our data for *X. testudinaria* are congruent with a recent study conducted on emergent sponges that suggests that the sponge loop hypothesis, as originally proposed, may not be correct for emergent sponges. Emergent sponges appear to have an alternate pathway for returning DOC to the benthos by converting it to sponge biomass rather than sponge detritus (McMurray et al. 2018).
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