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Molecular composition and biodegradation of loggerhead sponge Spheciospongia vesparium exhalent dissolved organic matter

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ABSTRACT

Sponges are critical components of marine reefs due to their high filtering capacity, wide abundance, and alteration of biogeochemical cycling. Here, we characterized dissolved organic matter (DOM) composition in the sponge holobiont exhalent seawater of a loggerhead sponge (*Spheciospongia vesparium*) and in ambient seawater in Florida Bay (USA), as well as the microbial responses to each DOM pool through dark incubations. The sponge holobiont removed 6% of the seawater dissolved organic carbon (DOC), utilizing compounds that were low in carbon and oxygen, yet high in nitrogen content relative to the ambient seawater. The microbial community accessed 7% of DOC from the ambient seawater during a 5-day incubation but only 1% of DOC from the sponge exhalent seawater, suggesting a decrease in lability possibly due to holobiont removal of nitrogen-rich compounds. If this holds true for other sponges, it may have important implications for DOM lability and cycling in coastal environments.

1. Introduction

Sponge holobionts, the complex ecosystem consisting of the sponge host, the microbiota and the interactions among them (Pita et al., 2018), are key components of coral reef communities due to their high filtering capacity and ability to influence biogeochemical cycling on the reef (Ribes et al., 2005; Rix et al., 2017). Corals and macroalgae release up to 50% of their fixed carbon (Tanaka et al., 2008; Haas et al., 2010) of which up to 80% is in the form of dissolved organic matter (DOM) (Wild et al., 2004), which can be uptaken by various sponge species on the reef community (Yahel et al., 2003; de Goeij et al., 2013; Mueller et al., 2014; Pawlik et al., 2015). Dissolved organic carbon (DOC) has been shown to make up a large portion of sponge diet and sponges have the ability to transform it into particulate organic carbon (POC), which is then accessible to higher trophic levels through what is termed the sponge loop (de Goeij et al., 2013). At least some of the DOM is processed by pinocytosis of the sponge without mediation of resident bacteria (Achlatis et al., 2019). Through the sponge loop, sponges participate in reef biogeochemical cycling as a sink and modifier of DOM (de Goeij et al., 2013).

Different species of sponges in various reef environments have shown the ability to uptake and utilize DOC. Previous studies have shown that several species of encrusting sponges (de Goeij et al., 2008; Mueller et al., 2014; Rix et al., 2017; McMurray et al., 2018) and several species of massive sponges (Yahel et al., 2003; McMurray et al., 2016, 2018; Hoer et al., 2018a) all uptake and rely on DOC to meet the majority (up to 60–90%) of their carbon demand (McMurray et al., 2016; Wooster et al., 2019), indicating that DOM turnover by sponges is thus important ecologically.

Sponge microbiomes have also been shown to remove not just DOC, but also inorganic and organic nitrogen from ambient seawater. Sponge holobionts are able to simultaneously perform competing nitrogen cycling pathways (e.g., nitrification and denitrification; Hoffmann et al., 2009; Schläppy et al., 2010; Fiore et al., 2015) and many species are net producers of inorganic nitrogen (Southwell et al., 2008; Hoer et al., 2018b), playing critical roles in biogeochemical cycling of benthic ecosystems. Although there have been fewer studies on dissolved organic nitrogen, it appears that several sponge species actively uptake nitrogen in its organic form as well (de Goeij et al., 2013). These studies indicated that nitrogen, in various forms, is an important aspect of sponge holobiont metabolism and can be removed from or added to seawater by sponge holobionts.

While it has been demonstrated that DOC is a major component of the metabolism of some species of sponges and their respective

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microbial holobionts, there have been few studies attempting to characterize sponge exhalent DOM (de Goeij et al., 2008; Fiore et al., 2017). Several metabolites, including 4-hydroxybenzoic acid, glycerol-3-phosphate, 5-methylthioadenosine, and pantothenic acid can be removed by sponge holobionts, whereas several nucleosides and riboflavin have been shown to be significantly increased in exhalent water, suggesting a release by the sponge holobiont (Fiore et al., 2017). These studies have shown that the sponge holobiont has the ability to take up and release specific DOM compounds. Thus, sponge holobionts can affect not only the quantity but also the quality and composition of the DOM pool, highlighting its potential to influence a variety of processes (e.g., carbon processing by microbes) that depend on that composition (Moran et al., 2016).

The research presented here aims to build upon previous studies by using an untargeted approach to characterize how the sponge holobiont can modify the DOM pool in the Florida Bay ecosystem, where sponges are dominant contributors to the benthic community biomass (Wall et al., 2012). We specifically focused on *Spheciospongia vesparium*, the most abundant sponge species in the Florida Keys reef ecosystem, which has a round, squat morphology and distinct oscula for expelling filtered water (Weisz et al., 2008). Using Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS), the chemical composition of DOM in ambient seawater in Florida Bay was compared to the exhalent seawater from *Spheciospongia vesparium* to characterize the resulting changes in DOM composition. We also quantified changes in DOC lability associated with seawater filtration by the sponge holobiont, an important step to better constrain and quantify the influence of sponges on carbon cycling in these ecosystems.

2. Methods

2.1. Study site

Sampling was conducted in Buttonwood Sound in Florida Bay, USA, during July 2017. Florida Bay is a sub-tropical lagoon between mainland Florida and the Florida Keys. It is the largest estuary in Florida, valuable for recreation and fisheries, and adjacent to the sensitive habitats of the Florida Keys National Marine Sanctuary and Everglades National Park (Wall et al., 2012). The Bay is made up of many shallow basins with depths ranging from 1 to 3 m surrounded by mangrove islands and coastal lagoons (Melo and Lee, 2012). Florida Bay was chosen over an oceanside offshore sampling site due to the conducive low flow environment in the Bay, which could potentially allow for a larger accumulation of DOM components associated with sponge holobionts. The dominant benthic suspension feeders in Florida Bay are sponges, especially the loggerhead sponge *Spheciospongia vesparium* (Butler et al., 1995; Lynch and Phlips, 2000).

2.2. Water sampling and filtration

A total of 10 L of exhalent seawater was collected directly above the osculum of a single, large (~40 cm diameter), healthy, actively pumping loggerhead sponge (S. vesparium; Fig. 1). Exhalent seawater was collected using a peristaltic pump at a slower rate (\sim 0.003 L s⁻¹) than the average pumping rate of the sponge (0.17 $\mathrm{L}~\mathrm{s}^{-1}~\mathrm{L}^{-1}$ sponge, Fiore et al., 2017). At approximately the same time (<10 min), an additional sample of 10 L of ambient seawater was collected into an acid-washed carboy (high density polyethylene, Nalgene) in the vicinity of but away (\sim 10 m) from the influence of the sponge exhalent seawater. The rationale for collecting the ambient seawater sample far enough away from the sponge is that ambient seawater in the immediate vicinity of the sponge has likely already been partially modified by the sponge holobiont, as exhalent seawater is mixed and entrained with ambient seawater. Variability in DOM composition on spatial scales of ~ 10 m (e. g., between replicates collected within a few minutes of each other in a background flow of $\sim 0.1 \text{ m s}^{-1}$) is generally indistinguishable from



Fig. 1. Collection of exhalent seawater directly above the osculum of a *Spheciospongia vesparium*. The yellow tubing in the center of the figure is the site of water collection (note that the tubing is not touching the sponge and is positioned directly above the osculum). The three feet of a holder with red ends can also be seen, positioned away from the osculum. The sponge exhalent seawater travels out of the sponge osculum through the yellow tubing connected to a holder (at the water surface) using a peristaltic pump and is collected into a 10 L carboy placed on a nearby dock. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

instrument variability in FT-ICR MS analysis. Immediately after collection, sponge exhalent and ambient seawater samples were taken to the Key Largo Marine Research Laboratory and processed as shown in Fig. S1. Samples were first filtered sequentially through Whatman GF/D filters (pre-combusted at 450 °C for 5 h; nominal 2.7 μm pore size; to remove photosynthetic material and/or particles) and 0.2 μm Pall Supor membrane filters (142 mm in diameter) into triplicate acid-washed 1 L polycarbonate bottles.

Fifty mL aliquots of the filtrate from each sampling site were set aside in separated beakers for the preparation of microbial inocula (Fig. S1). For that, the $0.2~\mu m$ filters from both sites (exhalent and ambient seawater) were aseptically cut into pieces and equal areas of the filters were pooled into 50 mL aliquots of $0.2 \mu m$ filtrate from each of the two sites and stirred for 30 min. The filter fragments were removed and the filtrates, now containing microbes from both sites, were added back to the respective triplicate set of bottles. This ensured that functional capabilities of the microbes were similar during all incubations and changes in DOM composition could therefore be attributed predominantly to the different initial composition of the DOM pools. A triplicate set from each site was immediately filtered (0.2 μm) to remove the added microbial inoculum and used to characterize the initial condition for each incubation. Aliquots (~50 mL) were stored frozen (-20 °C) and refrigerated (4 °C) for DOC/TDN (total dissolved organic nitrogen) and chromophoric DOM (CDOM) measurements, respectively. The remaining filtrates (900 mL for each replicate) were acidified to pH 2 (using HCl) and DOM was extracted using solid phase extraction (SPE) cartridges (Agilent Bond Elut PPL) as in Dittmar et al. (2008) for FT-ICR MS analysis. We refer to those samples as T₀. The remaining triplicate sets were incubated in the dark at the temperature measured at the time of collection (29 °C) for 5 days. At day 5, samples were filtered (0.2 μm) and processed for DOC, TDN, and FT-ICR MS analyses as described above. These samples are referred to as T₅.

2.3. Cell counts

Triplicate 1 mL samples, preserved in 0.1% glutaraldehyde solution (Hopwood, 1969), were prepared for flow cytometry to measure cell counts before and after the incubations of ambient and sponge exhalent seawater. Bacterial counts were obtained using a CytoFLEX S (Beckman

Coulter, Hialeah, Florida) flow cytometer at the Cytometry Shared Resource Laboratory at the University of Georgia. Replicate samples were analyzed with Milli-Q water between each sample in order to keep flowlines clean. Cell counts were quantified by staining cells with SYBR Green-I, as described by Marie et al. (1997).

2.4. Bulk DOC, TDN, and chromophoric DOM

Concentrations of DOC from initial and post-incubation samples of ambient and sponge exhalent seawater were measured with a Shimadzu TOC- $L_{\rm CPH}$ analyzer with potassium hydrogen phthalate as a standard. Concentrations of TDN were measured alongside DOC measurements with potassium nitrate as a standard. Milli-Q water blanks were tested before sample analysis and interspersed between sample runs on the instrument. Accuracy and precision were tested against deep-sea reference material (Hansell, 2005) and were better than 5%. Biodegradation of DOC (%) was determined as

$$\frac{DOC_{70} - DOC_{75}}{DOC_{70}} \times 100$$
 (1)

where DOC_{T0} is the concentration of DOC in the samples before incubations, and DOC_{T5} is the concentration of DOC in samples after five-day incubations.

UV–visible absorbance scans for chromophoric DOM (CDOM) were made on a single-beam spectrophotometer (Agilent UV-VIS 8453) using a 1 cm quartz cuvette, and absorption coefficients were computed as in D'Sa et al. (1999). Milli-Q water was used prior to sample measurement to complete blank calibrations to achieve a baseline background level. In order to track CDOM compositional changes, the ratio of absorptivity at 250 nmto 365 nm (a_{250} : a_{365}) was calculated; higher values can be used as indicators of lower aromaticity and higher proportion of small molecules (Peuravuori and Pihlaja, 2007).

2.5. FT-ICR MS

Molecular composition of the DOM of triplicate To and T5 ambient seawater and sponge exhalant samples were analyzed with a 9.4 T Fourier transform-ion cyclotron resonance mass spectrometer (FT-ICR MS) at the National High Magnetic Field Laboratory in Tallahassee, FL following Letourneau and Medeiros (2019). Samples were injected at concentrations of 50 mg $\rm C~L^{-1}$ in methanol with negative electrospray ionization mode (direct infusion) and 150 scans were accumulated. The spray voltage was 2.5 kV and the mass range for acquiring data was 150-1800 Da. Each mass spectrum was internally calibrated based on a "walking" calibration of highly abundant homologous alkylation series that differed in mass by multiples of 14.01565 Da confirmed by isotopic fine structure (Savory et al., 2011), achieving a mass error of <0.5 ppm. The restrictions $^{12}C_{1-130}$ $^{1}H_{1-200}$ O_{1-150} $^{14}N_{0-4}$ S_{0-2} P_{0-1} were used to calculate masses from the mass range of 150-750 Da. Molecular formulae assignments were performed by Kendrick mass defect analysis (Wu et al., 2004) with PetroOrg software (Corilo, 2014) and the criteria described by Rossel et al. (2013). The peak intensity of each formula was normalized to the sum peak intensities of the total identified peaks in each sample and compounds with a signal-to-noise ratio of 6 or higher were used in the analysis.

2.6. Statistical analyses

The variability of DOM molecular composition for triplicate sponge exhalent and ambient seawater samples was analyzed using principal component (PC) analysis of the FT-ICR MS data. All peaks with molecular formulae assigned were used in the PC analysis. All principal components shown here are significantly different (95% confidence level) from results obtained by pursuing a PC analysis of random processes that are spatially and temporally uncorrelated, following the

significance test described in Overland and Preisendorfer (1982). This indicates that the signals in the principal components described here are significantly greater than the level of noise. Loadings from the PCA were plotted on van Krevelen diagrams according to their molecular hydrogen-to-carbon (H/C) and oxygen-to-carbon ratios (O/C) for each molecular formula. We note that the sponge exhalent sample was collected from a single organism, and thus our replicate cannot capture ecological variability between individual organisms. Nevertheless, the replicates can capture variability due to differences in DOM biodegradation during incubations and instrument variability.

3. Results and discussion

3.1. Initial DOC concentration and DOM composition

The sponge holobiont actively removed dissolved organic carbon (DOC) as compared to the ambient seawater. The DOC concentration for the ambient seawater was $564 \pm 2~\mu\text{M}$, whereas the concentration for the exhalent seawater was $529 \pm 3~\mu\text{M}$ (Table 1), showing a $6.2 \pm 0.7\%$ reduction in DOC concentration associated with removal by the sponge holobiont. Observed removal values were consistent with DOC removal previously reported for several species of sponges, which ranges from 0% to 24% of ambient seawater (Yahel et al., 2003; Mueller et al., 2014; Hoer et al., 2018a). However, DOC removal for *S. vesparium* was not statistically different from zero in a previous study (Hoer et al., 2018a).

Analysis at the molecular level revealed that the removal of DOC by the sponge holobiont transformed the composition of the DOM pool, with compounds relatively enriched in sponge exhalent DOM occupying a different region of the van Krevelen diagram compared to compounds relatively enriched in ambient seawater (Fig. 2). This shows that the compounds that were more prevalent in the sponge exhalent samples had different characteristics (in terms of their molecular oxygen-tocarbon and hydrogen-to-carbon ratios) than the compounds that were more prevalent in the ambient seawater samples. In particular, compounds with relative abundance enriched in ambient seawater (and thus depleted in sponge exhalent DOM; shown in blue in Fig. 2) were characterized by a low number of carbon and oxygen atoms and by a higher number of nitrogen atoms (Fig. 3). This suggests that small compounds with low carbon and oxygen content, and nitrogen-rich compounds may have been preferentially removed from the ambient seawater by this sponge holobiont, resulting in the exhalent seawater being enriched in compounds with a comparatively larger number of carbon and oxygen atoms and no nitrogen. This is supported by the optical analysis (CDOM), which revealed lower values of the ratio a₂₅₀:a₃₆₅ (indicating higher aromaticity and higher proportion of large molecules; Peuravuori and Pihlaja, 2007) for the sponge exhalent compared to the ambient seawater samples (Table 1). This is consistent with the sponge holobiont removing small, less aromatic compounds, resulting in DOM in the exhalent seawater being characterized by a comparatively higher proportion of large molecules and higher aromatic content. The molecular and optical analyses suggest active transformation of the DOM pool by this individual sponge holobiont. Removal of low molecular weight DOM compounds has been previously reported for two sponge species in Florida Bay, one of which was S. vesparium (Fiore et al., 2017). Interestingly, compounds associated with 49 molecular formulae that were not present in the ambient seawater samples were detected in the exhalent water, indicating that they must have been released by the sponge holobiont. This is consistent with previous studies that have shown that the sponge holobiont also releases metabolites to the environment (Fiore et al., 2017), including dissolved waste products from metabolized particulate organic matter.

The preferential depletion in relative abundance of nitrogencontaining organic compounds in exhalent seawater is particularly interesting. While only 20% of the molecular formulae with abundance enriched in sponge exhalent DOM contained at least one nitrogen atom, about 50% of the formulae enriched in ambient seawater had at least

Table 1Chemical and biological variables for ambient seawater and loggerhead sponge exhalent seawater samples.

Sample Name	Time Point (days)	DOC* (μM)	DOC biodegradation (after 5-day incubation; %)	TDN (μM)	a250:a365	Microbial Abundance (Bacteria mL ⁻¹)
Ambient Seawater	0	564.3 ± 2.1	_	47.6 ± 0.1	8.8 ± 0.4	$6.7 \times 10^4 \pm 9.8 \times 10^3$
Ambient Seawater	5	526.3 ± 1.5	6.7 ± 0.5	44.3 ± 0.2	_	$2.4 \times 10^5 \pm 3.2 \times 10^4$
Sponge Exhalent	0	529.3 ± 3.4	_	46.5 ± 0.2	8.0 ± 0.2	$6.1 \times 10^4 \pm 1.8 \times 10^4$
Sponge Exhalent	5	524.2 ± 1.7	1.0 ± 0.7	45.1 ± 0.1	-	$1.3 \ x \ 10^5 \pm 3.0 \ x \ 10^4$

^{*}Values reported are the average of three replicates \pm one standard deviation.

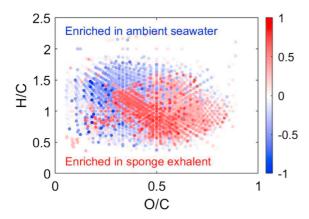


Fig. 2. Van Krevelen diagram showing loadings of dominant principal component of ambient seawater and sponge exhalent seawater DOM composition. Each dot represents a molecular formula, plotted according to their oxygen-to-carbon (O/C) and hydrogen-to-carbon (H/C) ratios. Molecular formulae shown in red had their abundances relatively enriched in sponge exhalent seawater (in comparison to ambient seawater, where they were relatively depleted), while formulae shown in blue were enriched in ambient seawater (and thus depleted in sponge exhalent seawater). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

one nitrogen (Fig. 3c). In addition, molecular-level analysis also revealed that ~60% of the compounds associated with molecular formulae that were present in ambient seawater samples and that were completely removed after filtration by *S. vesparium* contained at least one nitrogen atom in the molecular formula. These results showed that the majority of the molecular formulae that were actively removed by the sponge holobiont from the ambient seawater contained at least one nitrogen atom. The uptake of nitrogen-containing compounds by the sponge holobiont was also revealed by total dissolved organic nitrogen (TDN) concentrations (Table 1), with sponge exhalent TDN being less concentrated than ambient seawater. While preferential uptake of nitrogen-containing organic compounds was observed, *S. vesparium* has been shown to excrete inorganic nitrogen (Hoer et al., 2018b).

The preferential uptake of nitrogen-containing organic compounds could have ecological significance in the Florida Bay hard-bottom environment. Spheciospongia vesparium is the most dominant member of the sponge community in Florida Bay and is estimated to make up 58% of the community biomass (Stevely et al., 2010). In the early 1990s, widespread sponge mortality events in the Florida Keys caused sponge biomass to decline by up to 90% in some locations (Butler et al., 1995). The direct cause of these mortality events was not determined, but the loss of these sponges, the dominant suspension feeders in the system, was accompanied by widespread phytoplankton and cyanobacteria blooms (Peterson et al., 2006). The large phytoplankton blooms are thought to have been caused by the reduction in sponge grazing following the large-scale mortality event, since the five most common species of sponges in Florida Bay (including S. vesparium) have been shown to graze upon multiple plankton species, including the cyanobacteria Synechococcus elongatus, the diatom Cyclotella choctawhatcheeana, and the dinoflagellate Prorocentrum hoffmanianum (Peterson et al., 2006). In addition to decreasing grazing pressure, it is possible that the mortality of these sponges in the 1990s could have resulted in an increase of nitrogen-containing organic compounds in the ambient seawater due to the lack of sponge holobiont preferential removal of these compounds. If true, to the extent that photochemical reactions can release bioavailable nitrogen from organic nitrogen (e.g., Bushaw et al., 1996; Vähatalo and Zepp, 2005), sponge mortality/abundance may also influence nutrient concentrations in these systems.

An important limitation of this analysis is that sponge exhalent seawater was collected from a single organism. The difference in DOC concentration and in DOM composition between ambient and exhalent seawater is larger than variability expected from instrument noise, and it is encouraging that many of the patterns reported here are consistent with previous studies (e.g., DOC removal on the order of a few percent, preferential removal of low-molecular weight compounds; Fiore et al., 2017). However, our analyses are unable to capture ecological variability between individual organisms. It will be interesting to see if future studies focusing on multiple organisms will reveal a pattern of transformation consistent with the one reported here. It is also important that future studies address DOM transformations associated with other species of sponges, in particular those that can remove larger

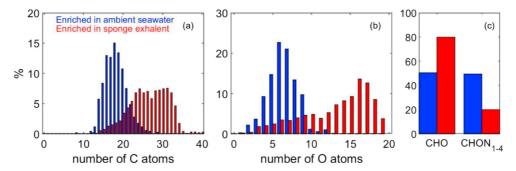


Fig. 3. Relative frequency of occurrence of molecular formulae with different numbers of (a) C-atoms, (b) O-atoms and (c) formulae with and without N that had relative abundance enriched in ambient seawater (blue) and in sponge exhalent seawater (red). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

amounts of DOC from ambient seawater (Hoer et al., 2018a).

3.2. Microbial responses through dark incubations

Many previous studies have shown that DOM composition plays a key role controlling microbial degradation of DOC (e.g. Moran et al., 2016). Given the changes in DOM composition observed in the sponge exhalent sample associated with the depletion in relative abundance of small organic compounds with a low number of carbon and oxygen atoms and rich in nitrogen, it is possible that the interaction of the sponge with the DOM pool (either directly or via interactions with the POM pool releasing dissolved waste products) may also alter the lability of that carbon pool. To test this, we analyzed the microbial degradation of each DOM pool through 5-day dark incubations.

At first glance, the general patterns of DOM composition transformations observed during the incubations were somewhat similar regardless of starting material (ambient or exhalent DOM), with molecular formulae that had their relative abundance depleted or enriched during the incubations occupying approximately the same location in van Krevelen space (Fig. 4). During the incubations of both ambient and exhalent seawater samples, there was a general tendency for preferential depletion of compounds characterized by high O/C and low H/C ratios, and preferential enrichment of compounds with low O/C and high H/C ratios. This is consistent with changes in DOM composition associated with microbial degradation observed in other coastal systems (e.g., Medeiros et al., 2015, 2017).

However, analysis of changes in DOC concentrations between the two sets of incubations suggested differences in DOC consumption. For the dark incubations of ambient seawater, 6.7 \pm 0.5% of the DOC contained in the pre-incubation sample was consumed during the 5-day long experiment (Table 1). For the incubations using sponge exhalent seawater, on the other hand, DOC consumption was lower at $1.0 \pm 0.7\%$. This suggests a difference in DOC lability between the two samples, with ambient seawater DOC being more labile than sponge exhalent DOC. Similarly, TDN also had a larger reduction during incubation of ambient seawater compared to incubation of sponge exhalent seawater (Table 1). To investigate if this is consistent with the observed changes in DOM composition at the molecular level, we compared the molecular formulae associated with compounds with relative abundance depleted during the incubations (blue dots in Fig. 4) with the compounds with relative abundance depleted after filtration by the sponge holobiont (blue dots in Fig. 2). We note that although visual inspection of van Krevelen diagrams can be useful, they can also be challenging since each dot in a van Krevelen diagram can represent different molecular formulae with identical O/C and H/C ratios (dots in Figs. 2 and 4 were plotted in increasing order of absolute value, so that only the largest positive or negative principal component loading for each O/C and H/C ratio can be seen). A total of 735 molecular formulae had relative

abundance significantly depleted after filtration by the sponge holobiont (blue dots in Fig. 2). Approximately 63% of those 735 molecular formulae also had their relative abundance depleted during incubation of ambient seawater (blue dots in Fig. 4a). This suggests that as the sponge filtered ambient seawater, the holobiont may have preferentially removed compounds that were microbially labile. This is also consistent with microbial incubations pursued in other coastal environments, which have revealed that compounds preferentially targeted by bacteria often had a higher number of nitrogen heteroatoms compared to the average DOM pool (Vorobev et al., 2018). Since about half of the molecular formulae associated with compounds with relative abundance depleted during filtration by the sponge contained nitrogen (Fig. 3), it is possible that many microbially labile compounds were removed in the process. On the other hand, the fraction of the 735 molecular formulae with relative abundance significantly depleted during filtration by the sponge holobiont (blue dots in Fig. 2) that also had abundance depleted during the incubation with sponge exhalent DOM (blue dots in Fig. 4b) was much smaller at 4%. Since the abundance of these compounds was reduced to begin with, other compounds were likely targeted for degradation during the dark incubations, indicating that the microbial communities interacted differently with the DOM after it had been filtered by the sponge holobiont. It is therefore possible that the sponge holobiont removed a large fraction of the compounds that were labile to bacteria during filtration, and the DOM pool left behind in the sponge exhalent samples was more recalcitrant, resulting in reduced DOC degradation for incubations of sponge exhalent seawater (Table 1). Changing composition of exudates of other benthic organisms, such as corals, have been reported to influence microbial activity in the adjacent water column (Wild et al., 2008, 2009). For example, Nakajima et al. (2018) observed that soft coral-derived dissolved and particulate organic matter fostered a lower microbial growth rate with a lower growth efficiency compared to DOC and POC of hard corals, suggesting that soft coral exudates are relatively refractory compared to the mucus of hard corals.

Differences in DOC consumption may have been related to differences in DOM composition as discussed above, but they may also have been influenced by changes in microbial community. The initial samples for incubations of both ambient seawater and sponge exhalent seawater had bacterial abundances of around 6×10^4 bacteria mL⁻¹ (Table 1). The average bacterial abundance for the final ambient seawater samples was 2.4×10^5 bacteria mL⁻¹, whereas the average bacterial abundance for the final sponge exhalent samples was 1.3×10^5 bacteria mL⁻¹ (Table 1). This indicated that the bacterial community in the ambient seawater increased in density by about 3.4×10^4 cells mL⁻¹ day⁻¹, whereas the bacterial community in the sponge exhalent samples only increased by about 1.4×10^4 cells mL⁻¹ day⁻¹, a difference of almost two and a half times. Thus, it is possible that components of the DOM pool that are important to sustain the microbial community were removed during

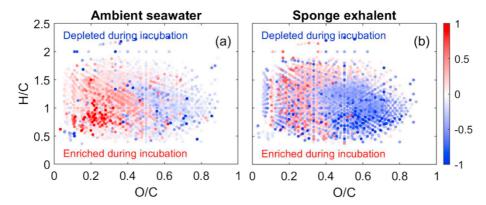


Fig. 4. Van Krevelen diagram showing loadings of dominant principal component of DOM transformation during dark incubations as compared to initial compositions of (a) ambient seawater and (b) sponge exhalent seawater.

filtration by the sponge holobiont, resulting in lower bacterial growth rates in sponge exhalent seawater. The resulting increased bacterial abundance during the incubation of ambient seawater could at least partially explain the larger DOC consumption in that case compared to the incubation of sponge exhalent seawater.

4. Conclusions

We found that the S. vesparium holobiont actively uptook approximately 6% of the DOC from the surrounding ambient seawater, resulting in a decrease in relative abundance of compounds with low carbon numbers, low oxygen content, and with high nitrogen content in exhalent seawater as compared to the ambient seawater. The microbial communities interacted differently with sponge holobiont exhalent and ambient seawater over 5-day dark incubations. While the microbial community was only able to utilize 1% of the DOC in the sponge exhalent seawater, there was an almost 7% DOC biodegradation in the ambient seawater, revealing a decrease in lability of DOC after being exhaled by the sponge holobiont. Analyses at the molecular level confirmed that several of the molecular formulae whose relative abundance decreased during filtration by the sponge holobiont were preferentially degraded by bacteria during dark incubations, suggesting that the S. vesparium holobiont may have removed labile compounds leaving behind the more recalcitrant fraction of the DOM pool. The comparison between ambient and exhalent seawater was based on a single organism, however, and thus cannot capture ecological differences between different organisms. Additional studies are needed to confirm if the patterns reported here are observed widely for S. vesparium or if they represented anomalous conditions. If these patterns of DOM transformation are representative of multiple S. vesparium individuals, this may have far-reaching implications in carbon and nitrogen cycling beyond the Florida Bay hard bottom since the same species of sponge studied here is also found in the Caribbean Sea coral reefs (Weisz et al., 2008). This study is one of the first to examine the changes in DOM composition before and after interaction with S. vesparium, as well as the microbial community's interaction with the DOM in ambient seawater samples as compared to sponge holobiont exhalent DOM. Further studies are also necessary to determine how these findings vary for other species of marine sponges, especially those known for removing larger amounts of DOC from seawater.

CRediT authorship contribution statement

Maria L. Letourneau: Formal analysis, Investigation, Writing - original draft. Brian M. Hopkinson: Investigation, Writing - review & editing. William K. Fitt: Investigation, Writing - review & editing. Patricia M. Medeiros: Conceptualization, Methodology, Validation, Resources, Writing - review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2020.105130.

References

- Achlatis, M., Pernice, M., Green, K., de Goeij, J.M., Guagliardo, P., Kilburn, M.R., Hoegh-Guldberg, O., Dove, S., 2019. Single-cell visualization indicates direct role of sponge host in uptake of dissolved organic matter. Proc R Soc B 286. https://doi.org/10.1098/rspb.20192153.
- Bushaw, K., Zepp, R., Tarr, M., Schulz-Jander, D., Bourbonniere, R.A., Hodson, R.E., Miller, W.L., Bronk, D.A., Moran, M.A., 1996. Photochemical release of biologically available nitrogen from aquatic dissolved organic matter. Nature 381, 404–407.
- Butler, M.J.I.V., Hunt, J.H., Hernkind, W.F., Childress, M.J., Bertelsen, R., Sharp, W., Matthews, T., Field, J.M., Marshall, H.G., 1995. Cascading disturbances in Florida Bay, USA: cyanobacteria blooms, sponge mortality, and implications for juvenile spiny lobsters *Panulirus argus*. Mar. Ecol. Prog. Ser. 129, 119–125.
- Corilo, Y.E., 2014. PetroOrg Software. Omics. Florida State University, Omics LLC, Tallahassee, Fl.
- de Goeij, J.M., Moodley, L., Houtekamer, M., Carballeira, N.M., van Duyl, F.G., 2008. Tracing ¹³C-enriched dissolved and particulate organic carbon in the bacteria-containing coral reef sponge *Halisarca caerulea*: evidence for DOM feeding. Limnol Oceanogr. 53, 1376–1386.
- de Goeij, J.M., van Oevelen, D., Vermeij, M.J.A., Osinga, R., Middelburg, J.J., de Goeij, A.F.P.M., Admiraal, W., 2013. Surviving in a marine desert: the sponge loop retains resources within coral reefs. Science 342, 108–110.
- Dittmar, T., Koch, B., Hertkorn, N., Kattner, G., 2008. A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater. Limnol Oceanogr. Methods 6, 230–235.
- D'Sa, E.J., Steward, R.G., Vodacek, A., Blough, N.V., Phinney, D., 1999. Determining optical absorption of colored dissolved organic matter in seawater with a liquid capillary waveguide. Limnol. Oceanogr. 44, 1142–1148.
- Fiore, C.L., Freeman, C.J., Kujawinski, E.B., 2017. Sponge exhalent seawater contains a unique chemical profile of dissolved organic matter. PeerJ 5, e2870.
- Fiore, C.L., Labrie, M., Jarett, J.K., Lesser, M.P., 2015. Transcriptional activity of the giant barrel sponge, *Xestospongia muta* Holobiont: molecular evidence for metabolic interchange. Front. Microbiol. 6, 364.
- Haas, A.F., Naumann, M.S., Struck, U., Mayr, C., el-Zibdah, M., Wild, C., 2010. Organic matter release by coral reef associated benthic algae in the Northern Red Sea. J. Exp. Mar. Biol. Ecol. 389, 53–60.
- Hansell, D.A., 2005. Dissolved organic carbon reference material program. Eos. Trans. Am. Geophys. Union 86, 318-318.
- Hoer, D.R., Gibson, P.J., Tommerdahl, J.P., Lindquist, N.L., Martens, C., 2018a. Consumption of dissolved organic carbon by Caribbean reef sponges. Limnol. Oceanogr. 63, 337–351.
- Hoer, D.R., Tommerdahl, J.P., Lindquist, N.L., Martens, C.S., 2018b. Dissolved inorganic nitrogen fluxes from common Florida Bay (U.S.A.) sponges. Limnol. Oceanogr. 63, 2563–2578.
- Hoffmann, F., Radax, R., Woebken, D., Holtappels, M., Lavik, G., Rapp, H.T., Schläppy, M.L., Schleper, C., Kuypers, M.M.M., 2009. Complex nitrogen cycling in the sponge *Geodia barretti*. Environ. Microbiol. 11, 2228–2243.
- Hopwood, D., 1969. A comparison of the crosslinking abilities of glutaraldehyde, formaldehyde and α -hydroxyadipaldehyde with bovine serum albumin and casein. Histochemie 17, 151–161.
- Letourneau, M.L., Medeiros, P.M., 2019. Dissolved organic matter composition in a marsh-dominated estuary: Response to seasonal forcing and to the passage of a hurricane. J Geophys Res-Biogeo 124, 1545–1559.
- Lynch, T.G., Phlips, E.J., 2000. Filtration of the bloom-forming cyanobacteria Synechococcus by three sponge species from Florida Bay, USA. Bull. Mar. Sci. 67, 923-936.
- Marie, D., Partensky, F., Jacquet, S., Vaulot, D., 1997. Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. Appl. Environ. Microbiol. 63, 186–193.
- McMurray, S.E., Johnson, Z.I., Hun, t D.E., Pawlik, J.R., Finelli, C.M., 2016. Selective feeding by the giant barrel sponge enhances foraging efficiency. Limnol. Oceanogr. 61, 1271–1286.
- McMurray, S.E., Stubler, A.D., Erwin, P.M., Finelli, G.M., Pawlik, J.R., 2018. A test of the sponge-loop hypothesis for emergent Caribbean reef sponges. Mar. Ecol. Prog. Ser. 588, 1–14.
- Medeiros, P.M., Seidel, M., Ward, N.D., Carpenter, E.J., Gomes, H.R., Niggemann, J., Krusche, A.V., Richey, J.E., Yager, P.L., Dittmar, T., 2015. Fate of the Amazon River dissolved organic matter in the tropical Atlantic Ocean. Global Biogeochem. Cycles 29, 677–690.

- Medeiros, P.M., Seidel, M., Gifford, S.M., Ballantyne, F., Dittmar, T., Whitman, W.B., Moran, M.A., 2017. Microbially-mediated transformations of estuarine dissolved organic matter. Front. Mar. Sci. 4 https://doi.org/10.3389/fmars.2017.00069.
- Melo, N., Lee, T.N., 2012. Water circulation and renewal in Florida Bay is influenced by flows from the Southwest Florida Shelf and tidal passes. In: Kruczynski, W.L., Fletcher, P.J. (Eds.), Tropical Connections, pp. 80–82.
- Moran, M.A., Kujawinski, E.B., Stubbins, A., Fatland, R., Aluwihare, L.I., Buchan, A., Crump, B.C., Dorrestein, P.C., Dyhrman, S.T., Hess, N.J., Howe, B., Longnecker, K., Medeiros, P.M., Niggemann, J., Obernosterer, I., Repeta, D.J., Waldbauer, J.R., 2016. Deciphering ocean carbon in a changing world. Proc. Natl. Acad. Sci. U.S.A. 113, 3143–3151.
- Mueller, B., de Goeij, J.M., Vermeij, M.J.A., Mulders, Y., van der Ent, E., Ribes, M., van Duyl, F.C., 2014. Natural diet of coral-excavating sponges consists mainly of dissolved organic carbon (DOC). PloS One 9, e90152.
- Nakajima, R., Haas, A.F., Silveira, C.B., Kelly, E.L.A., Smith, J.E., Sandin, S., Kelly, L.W., Rohwer, F., Nakatomi, N., Kurihara, H., 2018. Release of dissolved and particulate organic matter by the soft coral *Lobophytum* and subsequent microbial degradation. J. Exp. Mar. Biol. Ecol. 504, 53–60. https://doi.org/10.1016/j.jembe.2018.02.008.
- Overland, J., Preisendorfer, R., 1982. A significance test for principal components applied to a cyclone climatology. Mon. Weather Rev. 110, 1–4.
- Pawlik, J.R., McMurray, S.E., Erwin, P., Zea, S., 2015. A review of evidence for food limitation of sponges on Caribbean reefs. Mar. Ecol. Prog. Ser. 51, 265–283.
- Peterson, B.J., Chester, C.M., Jochem, F.J., Fourqurean, J.W., 2006. Potential role of sponge communities in controlling phytoplankton blooms in Florida Bay. Mar. Ecol. Prog. Ser. 328, 93–103.
- Peuravuori, J., Pihlaja, K., 2007. Characterization of freshwater humic matter. In: Nollet, L.M.L. (Ed.), Handbook of Water Analysis. CRC Press, Boca Raton, London, New York, pp. 435–448.
- Pita, L., Rix, L., Slaby, B.M., Franke, A., Hentschel, U., 2018. The sponge holobiont in a changing ocean: from microbes to ecosystems. Microbiome 6, 46. https://doi.org/ 10.1186/s40168-018-0428-1.
- Ribes, M., Coma, R., Atkinson, M.J., Kinzie III, R.A., 2005. Sponges and ascidians control removal of particulate organic nitrogen from coral reef water. Limnol. Oceanogr. 50, 1480–1489.
- Rix, L., de Goeij, J.M., van Oevelen, D., Struck, U., Al-Horani, F.A., Wild, C., Naumann, M.S., 2017. Differential recycling of coral and algal dissolved organic matter via the sponge loop. Funct. Ecol. 31, 778–789.
- Rossel, P.E., Vähätalo, A.V., Witt, M., Dittmar, T., 2013. Molecular composition of dissolved organic matter from a wetland plant (*Juncus effusus*) after photochemical and microbial decomposition (1.25 yr): Common features with deep sea dissolved organic matter. Org. Geochem. 6, 62-71.
- Savory, J.J., Kaiser, N.K., McKenna, A.M., Xian, F., Blakney, G.T., Rodgers, R.P., Hendrickson, C.L., Marshall, A.G., 2011. Parts-per-billion Fourier transform ion cyclotron resonance mass measurement accuracy with a "walking" calibration equation. Anal. Chem. 83, 1732–1736.

- Schläppy, M.L., Schöttner, S.I., Lavik, G., Kuypers, M.M.M., de Beer, D., Hoffmann, F., 2010. Evidence of nitrification and denitrification in high and low microbial abundance sponges. Mar. Biol. 157, 593–602.
- Southwell, M.W., Weisz, J.B., Martens, C.S., Lindquist, N., 2008. In situ fluxes of dissolved inorganic nitrogen from the sponge community on Conch Reef, Key Largo, Florida. Limnol. Oceanogr. 53, 986–996.
- Stevely, J.M., Sweat, D.E., Bert, T.M., Sim-Smith, C., Kelly, M., 2010. Commercial bath sponge (Spongia and Hippospongia) and total sponge community abundance and biomass estimates in the Florida middle and upper Keys, USA. Proc. Gulf Caribb. Fish. Inst. 62, 394–403.
- Tanaka, Y., Miyajima, T., Koike, I., Hayashibara, T., Ogawa, H., 2008. Production of dissolved and particulate organic matter by the reef-building corals *Porites cylindrica* and *Acropora pulchra*. Bull. Mar. Sci. 82, 237–245.
- Vähätalo, A.V., Zepp, R.G., 2005. Photochemical mineralization of dissolved organic nitrogen to ammonium in the Baltic Sea. Environ. Sci. Technol. 39, 6985–6992.
- Vorobev, A., Sharma, S., Yu, M., Lee, J., Washington, B.J., Whitman, W.B., Ballantyne, F. I.V., Medeiros, P.M., Moran, M.A., 2018. Identifying labile DOM components in a coastal ocean through depleted bacterial transcripts and chemical signals. Environ. Microbiol. 20, 3012–3030.
- Wall, C.C., Rodgers, B.S., Gobler, C.J., Peterson, B.J., 2012. Responses of loggerhead sponges Spheciospongia vesparium during harmful cyanobacterial blooms in a subtropical lagoon. Mar. Ecol. Prog. Ser. 451, 31–43.
- Weisz, J.B., Lindquist, N., Martens, C.S., 2008. Do associated microbial abundances impact marine demosponge pumping rates and tissue densities? Oecologia 155, 367-376.
- Wild, G., Huettel, M., Klueter, A., Kremb, S.G., Rasheed, M.Y.M., Jørgensen, B.B., 2004.
 Coral mucus functions as an energy carrier and particle trap in the reef ecosystem.
 Nature 428, 66–70.
- Wild, C., Mayr, C., Wehrmann, L., Schöttner, S., Naumann, M., Hoffmann, F., Rapp, H.T., 2008. Organic matter release by cold water corals and its implication for fauna-microbe interaction. Mar. Ecol.: Prog. Ser. 372, 67-75.
- Wild, C., Wehrmann, L.M., Mayr, C., Schöttner, S.I., Allers, E., Lundälv, T., 2009. Microbial degradation of cold-water coral-derived organic matter: potential implication for organic C cycling in the water column above Tisler Reef. Aquat. Biol. 7, 71–80. https://doi.org/10.3354/ab00185.
- Wooster, M.K., McMurray, S.E., Pawlik, J.R., Morán, X.A.G., Berumen, M.L., 2019. Feeding and respiration by giant barrel sponges across a gradient of food abundance in the Red Sea. Limnol. Oceanogr. 64, 1790–1801.
- Wu, Z., Rodgers, R.P., Marshall, A.G., 2004. Two- and three-dimensional van Krevelen diagrams: A graphical analysis complementary to the Kendrick mass plot for sorting elemental compositions of complex organic mixtures based on ultrahigh-resolution broadband Fourier transform ion cyclotron resonance mass measurements. Anal. Chem. 76. 2511–2516.
- Yahel, G., Sharp, J.H., Marie, D., Häse, C., Genin, A., 2003. In situ feeding and element removal in the symbiont-bearing sponge *Theonella swinhoei*: Bulk DOC is the major source for carbon. Limnol. Oceanogr. 48, 141–149.

Supplementary Information

Molecular composition and biodegradation of loggerhead sponge Spheciospongia vesparium exhalent dissolved organic matter

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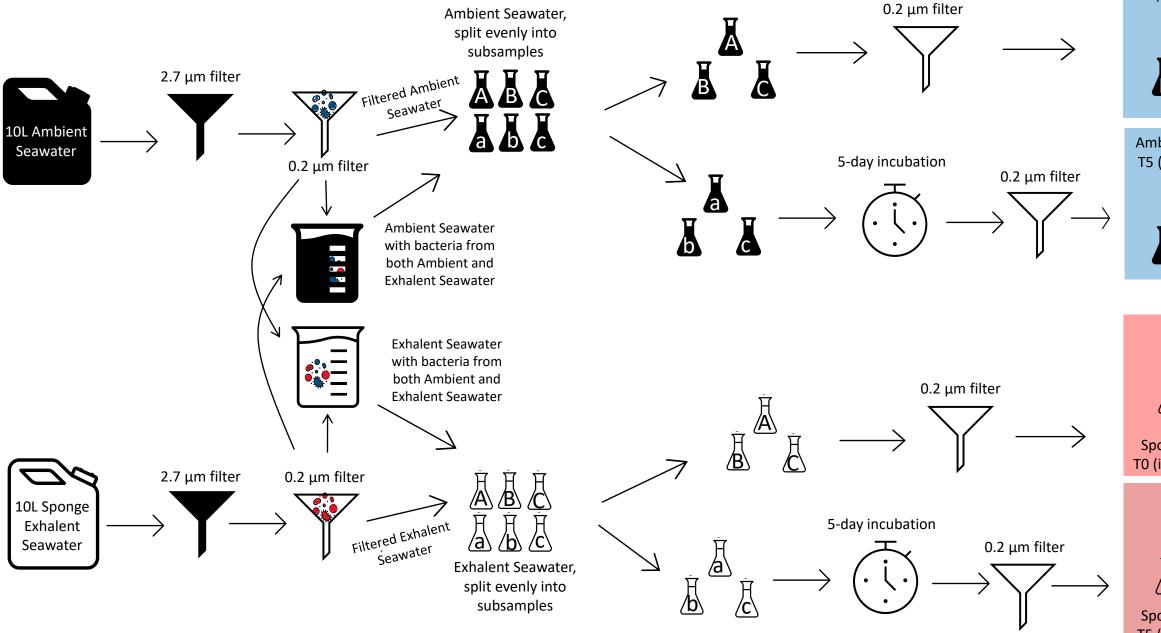
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This Supplementary Information contains:

- Figure S1

Figure S1: Flow chart showing the manipulation of ambient and sponge exhalent seawater samples after collection and before flow cytometry, DOC, CDOM, and FT-ICR MS analyses.



Ambient Seawater
T0 (initial) Samples

Ambient Seawater T5 (final) Samples





