SUPPLEMENTARY MATERIAL

Sponge exhalent metabolites influence coral reef picoplankton dynamics

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SUPPLEMENTAL METHODS

Estimates of sponge pumping rate

The flow rate (FR) of each sampled sponge was calculated using the equation provided in Morganti et al.[1] All sponges sampled for this project (n=4 each of *Xestospongia muta* and *Niphates digitalis*) had a single osculum. Following the completion of exhalent water collection for this experiment, the osculum diameter of each sponge was measured and used to acquire an estimated osculum cross-sectional area (OSA). The OSA was input into the flow rate equation to obtain an estimated FR for each sponge (Table S5).

Flow cytometry

Paraformaldehyde-preserved and frozen samples were sent to Center for Aquatic Cytometery at Bigelow Laboratory for Ocean Sciences for flow cytometry analysis using a Bio-Rad ZE5 (Hercules, California, USA) with a 488 nm laser activated. Samples were thawed and pre-screened through 70 µm mesh and diluted 1:10 with Tris EDTA (TE) Buffer pH 8.0. Cells were then stained using 10x working stock DNA stain SYBRGreen I (Thermo Fisher Scientific, Waltham, MA, USA) following the protocol of Marie et al. (2005). A total of 180 µl of diluted sample was run at a flow rate of 0.5 µl sec -1. Particles were excited with the 488 nm blue later and data acquisition was triggered by green fluorescence. Data signals were recorded by detectors with three bandpass filters including forward scatter (FSC), right angle light scatter (SSC), and fluorescence emission in green (525/35nm). To minimize noise, samples were subgated in FSC-405. Data files logarithmic scatter plots of fluorescent and light scattering properties were analyzed and total bacterial counts were identified based on size and presence of the green fluorescence. All counts were converted to cell abundance based on sample volume (including adjustments for preservation, dilution, and staining).

Biogeochemical Analyses

Inorganic macronutrients

Approximately 25 µl of filtered (0.22 µm) and frozen seawater from each sample was shipped to College of Earth, Ocean, and Atmospheric Sciences at Oregon State University for inorganic macronutrient analyses. Phosphate (PO_4^{3-}) and ammonium (NH_4^+) were measured using a Technicon Auto Analyzer II (SEAL Analytical Inc., Mequon, Wisconsin, USA) and silicic acid (i.e., silicate), nitrate (NO_3^-) and nitrate+nitrite ($NO_3^- + NO_2^-$) were measured using an Alpkem RFA 300 colorimetric autoanalyzer (Alpkem, Kranj, Slovenia). Analytical methods and data processing were completed as described in Gordon et al.[2], but a brief summary of each analysis is provided. The PO_4^{3-} method was modified from the molybdenum blue procedure[3], in which PO_4^{3-} is determined as reduced phosphomolybdic acid employing hydrazine as the reductant. The indophenol blue method, modified from ALPKEM RFA methodology, was used for the measurement of NH_4^+. Silicic acid was measured using the methodology of Atlas et al.[4] in which the addition of an acidic molybdate reagent forms silicomolybdic acid that is then reduced by stannous chloride. Lastly, NO_3^- and $NO_3^- + NO_2^-$ were measured based on the methods of Atlas et al.[4] with modifications to improve precision. Sulfanilamide

and N-(1-Napthyl)ethylenediamine dihydrochloride react with NO_2^- to form a diazo compound. For the NO_3^- + NO_2^- analysis, NO_3^- is first reduced to NO_2^- using an OTCR and imidazole buffer as described by Patton[5]. The NO_2^- analysis is performed on a separate channel, omitting the cadmium reductor and the buffer.

Dissolved combined neutral sugars (DCNS)

Dissolved combined neutral sugars (DCNS) were measured from frozen 20 µl aliquots of 0.22 µm filtered seawater from each sample at the Complex Carbohydrate Research Center, University of Georgia. Each sample was first desalted and hydrolyzed. For desalting, each sample was loaded onto a gravity column, containing forty milliliters of mixed ion exchange resins (AG 501-X8, 20-50 mesh, Bio-Rad), that was packed and prewashed with 200 ml (5x bed volume) of nano-pure water. Samples were then eluted with 120 ml (3x bed volume) of nano-pure water. The resulting flow-through and wash solution were lyophilized. Following lyopholization, the recovered materials were hydrolyzed wth 2 ml of 2 N TFA at 100ºC until high-performance anion exchange chromatography (HPAEC) analysis. By employing a specific HPAEC program, as detailed below, the neutral monosaccharides can be separated allowing the measurement of carbohydrates in each sample.

Monosaccharide standards, including fucose (Fuc), rhamnose (Rha), arabinose (Ara), glucose (Glc), galactose (Gal), xylose (Xyl), mannose (Man), and fructose (Frc), were hydrolyzed in the same manner and at the same time as the samples. Three concentrations of the standard mixture were prepared serially to establish a calibration equation. The quantity of each residue in the sample was calculated by linear interpolation of respective residue area units into the calibration equation.

Monosaccharides from each sample were analyzed by HPAEC with pulsed amperometric detection (HPAEC-PAD) using a DIONEX ICS3000 system (Thermo Fisher Scientific) equipped with a gradient pump and an electrochemical detector. The carbohydrates were separated by a Dionex CarboPac PA20 (3x150mm) analytical column with an amino trap column and eluted with degassed 12 mM NaOH. Injections were made every 40 min. Under the HPAEC conditons, Xyl and Man cannot be separated[6] and are presented as combined results. Samples were analyzed in triplicate and mean values were reported as ng ml^{-1} based on the volume analyzed.

Fluorescent dissolved organic matter (fDOM)

Flourescent dissolved organic matter (fDOM) was measured from cool (4ºC) aliquots of 0.22 µm filtered seawater from each sample following the methods detailed in Nelson et al.[7]. Samples were analyzed using a Horiba Aqualog scanning fluorometer with 150 W Xe excitation lamp, Peltier-cooled CCD emission dector, and a simultaneous absorbance spectrometer (Horiba Scientific, Piscataway, New Jersey, USA). Samples were brought to room temperature and loaded into DIW-leached and rinsed quartz cuvettes (1 cm diameter). Excitation-emission matrices (EEMs), 3D contour plots of excitation and emission fluorescence, were measured for each sample. Analysis began and ended with 4 DIW-filled cuvettes as blanks. EEMs were processed with a MATLAB script (https://github.com/zquinlan/fDOMmatlab/script.md) that employs parallel factor analysis (PARAFAC) to identify peaks that correspond to previously characterized fDOM components[7,8].

Targeted metabolomics sample preparation

To concentrate and extract the metabolites from the acidified filtrate we performed solid phase extraction (SPE) using a vacuum manifold (Waters Corporation, Milford, MA, USA) following the protocols from Kido Soul et al.[9] and Fiore et al.[10]. Briefly, the acidified filtrate from each sample is passed through acid-washed FEP tubing and a pre-conditioned (with 100% HPLC-grade methanol) 1g/6cc BondElut PPL cartridges (Agilent, Santa Clara, CA, USA) following the protocol in Kido Soul et al.[9]. Following filtration, each cartridge is wrapped in combusted aluminum foil, placed in a labeled, sterile Whirl-Pak bag and frozen at -80ºC. Frozen cartridges were shipped to Appalachian State University where the extraction process was completed by rinsing each thawed cartridge with 4 volumes of 0.1 M HCL followed by a gentle 5 min drying cycle using vacuum pressure and finally eluted into acid-washed, combusted 8-ml glass vials with 6 ml of 100% methanol. Using combusted glass pipettes, the methanol extracts were transferred to acid-washed, combusted 8-ml amber EMP vials and dried down to a single droplet using vacuum centrifuged then stored at -20ºC. All further preparation of samples for LC-MS analysis was completed at Woods Hole Oceanographic Institution using UPLC (Accela Open Autosampler and Accela 1250 Pump, Thermo Scientific) coupled with a heated electrospray ionization source (H-ESI) and a triple stage quadrupole mass spectrometer (TSQ Vantage, Thermo Fisher Scientific) in selective reaction monitoring (SRM) mode per methods detailed in Kido Soule et al.[9]. Additionally, a subsample of the metabolite extract from each individual experimental sample were pooled, and the pooled sample was divided into 11 equimolar samples to serve as positive control for the analysis. The raw XCalibur files were converted to mzML files using msConvert[11] and processed with MAVEN[12] to obtain calibration curves based on the integrated peak area generated for each metabolite. To calculate environmental concentrations the concentration of the metabolite was divided by the volume of the original acidified filtrate passed through the PPL cartridge. Lastly, metabolites that met the threshold detection and quantification limits for the targeted analysis were corrected for extraction efficiency by dividing their environmental concentrations by their published extraction efficiency in seawater[13].

Quality control and processing for targeted metabolite data

Following correction for extraction efficiency, the 31 identified metabolites went through a series of quality control steps. First, only metabolites that were present in at least 3 of the 11 pooled samples were retained resulting in retention of 19 metabolites. Of these 19 metabolites, one was not present in any of the experimental samples and was removed from further analyses. The concentrations of the remaining 18 metabolites in each experimental sample were corrected for volume (based on the original sample volume run through the PPL cartridge) and then converted from ng ml^{-1} to pg ml^{-1}.

16S rRNA Gene amplification and sequencing of picoplankton community

Picoplankton DNA was extracted from the entire 25 mm Supor filter using the DNeasy PowerBiofilm kit (Qiagen, Hilden, DE, USA) according to the manufacturer's instructions. Following extraction, DNA was quantified using the Qubit High Sensitivity dsDNA Assay (Life Technologies, Carlsbad, CA, USA). Extracted DNA was amplified

for the 16S ribosomal RNA gene (V4 region) using barcoded primers (515FY-806RB[14,15]. Each 50 µl PCR reaction contained 10 µl GoTaq 5x Flexi buffer (Promega, Madison, WI, USA), 5 µl 25 mM MgCl2 (Promega), 1 µl 10 mM deoxynucleotide triphosphates (dNTPS; Promega), 0.5 µl GoTaq Flexi DNA polymerase (Promega), 29.5 µl sterile water, 1 µl each of 10 µM forward and reverse primers, and 2 µl of extracted DNA. PCR conditions included one cycle of 2 min at 95ºC, followed by 28-30 cycles of 20 sec at 95ºC, 15 sec at 55ºC and 5 min at 72ºC, and ended with 10 min at 72ºC. Amplified products were quantified and size verified by gel electrophoresis using a 1% agarose gel and then purified using either MinElute Gel Extraction Kit (Qiagen) on bands excised form the agarose gel. A 2 µl sterile water (instead of DNA) negative PCR control was run with each back of PCR reactions and no amplification was detected in any of the controls. Purified PCR products were quantified using the Qubit High Sensitivity dsDNA Assay. Amplicons were combined equimolar ratios and sequenced using a 2x250 bp MiSeq platform (Illumina, Inc., San Diego, CA, USA) at Middle Tennessee State University. A mock community was also amplified and sequenced (Microbial Mock Community B; HM-782D; BEI Resources, NIAID, NIH, Manassas, VA, USA).

Calculating Bacterial Growth Efficiency (BGE)

BGE is the ratio of bacterioplankton carbon production (i.e., rate of increase in bacterioplankton carbon) to the rate of carbon removal. BGE was calculated following the method in Haas et al.[16]. First, the TOC concentration (µM) in each sample is multiplied by total volume of the pooled sample (T_0) or the incubation bottle (T_{48}).

EQ1.

$$\mu\text{moles TOC}_{sample} = \mu\text{M TOC}_{sample} \times \text{Total Volume (L)}_{sample}$$

Next, to obtain the bacterioplankton carbon (BC) for each sample the total picoplankton cell count (PCC) is multiplied by a standard measure of carbon units (i.e., per-cell carbon biomass of 20 fg C per cell[16,17]). First, picoplankton cell concentration (cells/ml) is multiplied by the total volume (ml) of the pooled sample or incubation bottle to obtain the PCC, then PCC is multiplied by 20 fg C to get bacterioplankton carbon per sample (BC_{sample}).

EQ2.

$$\text{PCC}_{sample} = \text{Cell Concentration (cells/ml)}_{sample} \times \text{Total Volume (ml)}_{sample}$$

EQ3.

$$\text{BC}_{sample} = \text{PCC}_{sample} \times 20 \text{ fg C}$$

The BC_{sample} (cells fg C) is then converted to total moles of C (cells µmols of C) by first converting from fg C to grams of C, then converting g of C to total moles of C, and finally, converting total moles of C to µmols of C for a final BC_{sample} that is in units of cells µmols of C.

EQ4.

$$\text{BC}_{sample}(\text{cells µmols C}) = \left(\frac{(\text{BC}_{sample}\ (\text{cells fg C})/10^{15})}{12}\right) \times 10^{6}$$

TOC is converted to dissolved organic carbon (DOC) by subtracting the bacterioplankton carbon (BC) from the total TOC in each sample.

EQ5.

$$\mu\text{moles DOC}_{sample} = \mu\text{M TOC}_{sample} - \text{BC}_{sample}$$

The total μmoles DOC_{sample} is then converted to removal of DOC (i.e., ΔDOC) by calculating the difference in μmoles DOC_{sample} for each T_{48} sample and the average $DOC_{Treatment}$ at T_0.

EQ6.

$$\Delta\text{DOC}_{sample} = \text{DOC}_{sample} - \text{DOC}_{Average\ per\ Treatment\ at\ T0}$$

This value is then converted to a rate of change in DOC (DOC umol hr^{-1}) by dividing the $\Delta\text{DOC}_{sample}$ by the total time of the incubation experiment (48 hours).

EQ7.

$$\text{DOC}_{sample}\ \text{umol hr}^{-1} = \frac{\Delta\text{DOC}_{sample}}{48}$$

Then, $\text{BC}_{sample}(\text{cells } \mu\text{mols } C)$ is converted to the rate of increase in bacterioplankton carbon (ΔBC_{sample}) by obtaining the difference between BC_{sample} for each T_{48} sample and the average $BC_{Treatment}$ at T_0,

EQ8.

$$\Delta\text{BC}_{sample} = \text{BC}_{\ sample} - \text{BC}_{Average\ per\ Treatment\ at\ T0}$$

and divided by the total time of incubation to get a rate of change in cell biomass ($\text{CB}_{sample}\ \text{hr}^{-1}$).

EQ9.

$$\text{CB}_{sample}\ \text{hr}^{-1} = \frac{\Delta\text{BC}_{sample}}{48\ \text{hrs}}$$

Finally, BGE_{sample} for each T_{48} sample is calculated as the ratio of the rate of change in cell biomass ($\text{CB}_{sample}\ \text{hr}^{-1}$) and the rate of change in DOC ($\text{DOC}_{sample}\ \text{umol hr}^{-1}$

EQ10.

$$\text{BGE}_{sample} = \frac{\text{CB}_{sample}\ \text{hr}^{-1}}{\text{DOC}_{sample}\ \text{umol hr}^{-1}}$$

Table S1. Sample counts per treatment (Reef Water and Sponge Exhalent) and time point (T_0, T_{24}, T_{48}) for each analysis. Analyses included total organic carbon (TOC) and total nitrogen (TN), inorganic nutrients, dissolved combined neutral sugars (DCNS), fluorescent dissolved organic matter (fDOM), targeted metabolomics and picoplankton amplicon sequencing.

Analysis	T_0 Reef Water	T_0 Sponge Exhalent	T_{24} Reef Water	T_{24} Sponge Exhalent	T_{48} Reef Water	T_{48} Sponge Exhalent
TOC/TN	3	3	0	0	4	4
Inorganic Nutrients	1	2	0	0	4	4
DCNS	1	2	0	0	4	4
fDOM	1	2	0	0	4	4
Targeted Metabolomics	1	1	0	0	4	4
Picoplankton Amplicon Sequencing	3	3	4	4	4	4

Table S2. Analysis of Similarity (ANOISM) results for picoplankton community composition across time points and treatments in the incubation experiment. Because each treatment only had one replicate at T_0 there is no individual statistical data shown comparing the picoplankton community from T_0 to the community at T_{24} or T_{48}. The only test that includes T_0 data is listed as "All Time Points." All results based on Bray-Curtis dissimilarity.

	Sponge vs Reef (all time points)	Sponge vs Reef (T_{24} & T_{48})	T_{24} vs T_{48}	T_{24} Sponge vs Reef	T_{48} Sponge vs. Reef	T_{24} vs T_{48} Sponge	T_{24} vs T_{48} Reef
ANOSIM R Statistic	0.5864	0.9832	0.1353	1	1	1	0.875
Significance (*p*-value)	0.0002	0.0002	0.1198	0.0267	0.0290	0.0285	0.0251

Table S3. Average net change (T_{48}-T_0) and results of statistical comparisons of incubation bottle water chemistry variables for reef water and sponge exhalent treatments. Kruskal-Wallis non-parametric t-tests were used for all comparisons and chi-squared values (χ^2), degrees of freedom (df), and significance (p) are reported for comparisons between treatments for each variable. Asterisks denote significant results.

Variable	Net Change (T_{48}-T_0) Per Treatment	χ^2	df	p
Picoplankton Concentration (cells/ml)	Reef: 267699.67 Sponge: 1179134.33	5.3333	1	0.0209*
Bulk Organic Nutrient Variables				
Total Organic Carbon (µM)	Reef: -2.3667 Sponge: -2.700	0	1	1
Total Nitrogen (µM)	Reef: -0.0667 Sponge: 0.0500	0.0833	1	0.7728
Inorganic Nutrient Variables				
Phosphate (PO_4^{3-}; µl)	Reef: 2.0817E-17 Sponge: 0	1.4737	1	0.2248
Nitrate (NO_3^-; um/l)	Reef: 0 Sponge: 8.3267E-17	0.0833	1	0.7728
Nitrite (NO_2^-; µm/l)	Reef: 5.2042E-18 Sponge: 1.7347E-18	0.0886	1	0.7660
Ammonium (NH_4^+; µm/l)	Reef: 0 Sponge: -2.7756E-17	0.0843	1	0.7715
Silicate (µm/l)	Reef: 0 Sponge: -4.1633E-17	0.3544	1	0.5516
Fluorescent Dissolved Organic Matter (fDOM) Variables				
Ultraviolet Humic-like (R.U.)	Reef: 0.0021 Sponge: 0.0010	2.0833		0.1489
Marine Humic-like (R.U.)	Reef: 0.0022 Sponge: 0.0020	0	1	1
Visible Humic-like (R.U.)	Reef: 0.0019 Sponge: 0.0013	0.0833	1	0.7728
Tryptophan-like (R.U.)	Reef: 0.0058 Sponge: 0.0022	0.3333	1	0.5637
Tyrosine-like (R.U.)	Reef: 0.0026 Sponge: -0.0004	0.75	1	0.5637
Phenylalanine-like (R.U.)	Reef: 0.0043 Sponge: 0.0082	0.75	1	0.3865
Fulvic Acid-like (R.U.)	Reef: 0.0016 Sponge: 0.0018	0.3333	1	0.5637
Targeted Metabolite Variables				
Adenosine (pg/l)	Reef: 0 Sponge: -0.0309	7	1	0.0082*
Inosine (pg/l)	Reef: -0.0014 Sponge: -0.004	7	1	0.0082*
Tryptophan (pg/l)	Reef: -0.0024 Sponge: -0.0031	7	1	0.0082*

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S-Adenosyl-L-homocysteine (pg/l)	Reef: 0 Sponge: -0.0020	7	1	0.0082*
4-Methyl-2-oxopentanoic Acid (pg/l)	Reef: -0.0224 Sponge: 0.0063	6.4	1	0.0114*
Anthranilate (pg/l)	Reef: 0.0020 Sponge: 0.1605	5.3333	1	0.0209*
Chorismate (pg/l)	Reef: 0.0034 Sponge: 0.0543	5.6	1	0.0180*
Riboflavin (pg/l)	Reef: 0.0003 Sponge: 0.0018	5.4634	1	0.0194*
4-Aminobenzoic Acid (pg/l)	Reef: -0.0002 Sponge: 0.0062	5.3333	1	0.0209*
Tyrosine (pg/l)	Reef: -0.0063 Sponge: -0.0021	5.4634	1	0.0194*
Phenylalanine (pg/l)	Reef: -0.0453 Sponge: -0.0288	7	1	0.0085*
Desthiobiotin (pg/l)	Reef: -0.0006 Sponge: 0.0007	5.4634	1	0.0194*
N-Acetyl Muramic Acid (pg/l)	Reef: 0 Sponge: 0.0050	6.0541	1	0.0139*
5-hydroxy-L-tryptophan (pg/l)	Reef: -0.0011 Sponge: 0.0016	6.137	1	0.0132*
Pantothenic Acid (pg/l)	Reef: 0.0001 Sponge: 0.0003	0.7975	1	0.3719
Glutathione Oxidized (pg/l)	Reef: -0.0011 Sponge: -0.0005	1.5342	1	0.2155
4-Hydroxybenzoic Acid (pg/l)	Reef: 0 Sponge: 0.0036	1.5135	1	0.2186
3,3-Dimethyl-2-Oxobutanoic Acid (pg/l)	Reef: -0.0073 Sponge: -0.0063	2.3026	1	0.1292
Dissolved Combine Neutral Sugars (Monosaccharides)				
Fucose (ng/ml)	Reef: 0.7720 Sponge: 0.0548	2.0833	1	0.1489
Rhamnose	Reef: 0.8320 Sponge: 0.4070	0.7500	1	0.3865
Arabinose (ng/ml)	Reef: -1.6918 Sponge: 0.4023	5.3333	1	0.0209*
Galactose (ng/ml)	Reef: 0.2405 Sponge: 0.4203	0	1	1
Glucose (ng/ml)	Reef: -2.0268 Sponge: 1.5258	5.3333	1	0.0209*
Xylose + Mannose (ng/ml)	Reef: -22.8518 Sponge: -2.5365	5.3333	1	0.0209*
Fructose (ng/ml)	Reef: -7.2503 Sponge: 0.0530	4.0833	1	0.0433*

Table S4. Average relative abundance (%) of the 55 most abundant ASVs identified in the rarefied picoplankton community at three time points (T_0, T_{24}, and T_{48}). The abundance of all remaining taxa is also included. ASVs are taxonomically identified by phylum, order and genera (or their lowest taxonomic designation). The data in this table is also visually displayed in Fig. 4 and Supplemental Fig. S1.

ASV Taxonomic Designation	T_0 Reef Water	T_0 Sponge Exhalent	T_{24} Reef Water	T_{24} Sponge Exhalent	T_{48} Reef Water	T_{48} Sponge Exhalent
Proteobacteria; Alteromondales; *Alteromonas*	1	0.8	49.1	66.1	40.8	56.4
Proteobacteria; Alteromondales; *Pseudoalteromonas*	0.4	0.6	4.1	20.4	2.9	14.3
Proteobacteria; Alteromondales; *Alteromonas*	0.2	0.1	3.4	8.1	2.7	6.9
Proteobacteria; Rhodobacterales; HIMB11	0.2	0.1	5.2	0.3	10.4	0.8
Proteobacteria; Rhodobacterales; HIMB11	0.3	0.4	4.5	0.2	9.9	0.4
Cyanobacteria; Synechococcales; *Prochlorococcus*	11.9	11.4	3.1	0.4	2.6	0.3
Proteobacteria; Pelagibacterales; SAR11 Clade I	6.4	6.1	2.9	0.3	3.5	0.4
Cyanobacteria; Synechococcales; *Synechococcus*	6.7	7	1.9	0.2	1.5	0.2
Proteobacteria; Pelagibacterales; SAR11 Clade I	5.4	4.9	2	0.3	2.1	0.2
Proteobacteria; Pelagibacterales; SAR11 Clade I	2.8	2.9	1.2	0.3	1.3	0.2
Proteobacteria; Pelagibacterales; SAR11 Clade I	1.8	1.9	1	0.1	1.2	0.2
Proteobacteria; Pelagibacterales; SAR11 Clade II	2.3	2.1	0.9	0.2	0.9	0.1
Proteobacteria; Flavobacteriales; *Mesoflavibacter*	0	0	0	0.1	0	2.9
Proteobacteria; Flavobacteriales; *Mesoflavibacter*	0	0	0	0.2	0	2.8
Proteobacteria; Pelagibacterales; SAR11 Clade I	1.8	2.1	0.8	0.1	0.9	0
Proteobacteria; Alteromondales; *Alteromonas*	0	0	0.6	0.7	0.5	0.6
Actinobacteria; Actinomarinales; *Candidatus* Actinomarina	1.9	2	0.5	0.1	0.5	0.1
Cyanobacteria; Synechococcales; *Prochlorococcus*	2.2	1.9	0.6	0.1	0.5	0
Proteobacteria; SAR86	1	1.1	0.7	0.1	0.7	0.1
Bacteroidetes; Flavobacteriales; NS5 Marine Group	1.7	1.9	0.6	0.1	0.5	0
Proteobacteria; Pelagibacterales; SAR11 Clade IV	1.4	1.5	0.5	0	0.5	0
Proteobacteria; Pseudomonadales; *Pontibacterium*	0	0	0	0	0	1.6
Proteobacteria; Rhodospirillales; AEGEAN-169 Marine Group	0.6	0.6	0.4	0	0.7	0.1
Proteobacteria; Rhodospirillales; AEGEAN-169 Marine Group	1.1	1.1	0.4	0	0.5	0
Proteobacteria; SAR86	0.6	0.6	0.4	0	0.6	0

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Proteobacteria; Rhodobacterales; *Aliiroseovarius*	0	0	0	0	0	1.4
Proteobacteria; Oceanospirillales; *Marinomonas*	0	0	0	0	0	1.4
Proteobacteria; SAR86	1.4	1.4	0.5	0	0.2	0
Proteobacteria; SAR86	0.7	0.9	0.4	0	0.3	0
Proteobacteria; Pelagibacterales; SAR11 Clade II	0.8	0.8	0.4	0	0.3	0
Proteobacteria; Unidentified Rhodobacterales	0.6	0.6	0.3	0	0.4	0
Proteobacteria; Cellvibrionales; OM60 (NOR5)	0	0	0.4	0	0.6	0
Proteobacteria; Pelagibacterales; SAR11 Clade I	0.8	0.8	0.3	0	0.3	0
Proteobacteria; Puniceispirillales; SAR116	0.3	0.4	0.3	0	0.4	0.1
Proteobacteria; SAR86	0.7	0.9	0.2	0.1	0.2	0.1
Proteobacteria; Oceanospirillales; *Litoricola*	0.1	0.1	0.2	0	0.4	0.3
Proteobacteria; SAR86	0.5	0.6	0.3	0	0.3	0
Proteobacteria; Unidentified Rhodobacterales	0	0	0	0	0	0.9
Proteobacteria; SAR324; Marine Group B	1.2	1.4	0.1	0	0.1	0
Proteobacteria; Cellvibrionales; OM60 (NOR5)	0.4	0.4	0.3	0	0.3	0
Proteobacteria; SAR86	0.5	0.5	0.3	0	0.3	0
Proteobacteria; Pelagibacterales; SAR11 Clade I	0.5	0.6	0.2	0	0.3	0
Actinobacteria; Actinomarinales; *Candidatus* Actinomarina	0.7	0.9	0.2	0	0.2	0
Proteobacteria; SAR86	0.5	0.5	0.3	0	0.3	0
Proteobacteria; Unidentified Rhodobacterales	0.9	0.9	0.2	0	0.1	0
Thermoplasmata; unknown *Candidatus* Poseidoniia	1.1	1	0.1	0	0	0
Proteobacteria; Flavobacteriales; *Mesoflavibacter*	0	0	0	0	0	0.7
Proteobacteria; Oceanospirillales; Amphritea	0	0	0	0	0	0.7
Unknown *Candidatus* Marinimicrobia	0.6	0.6	0.2	0	0.1	0
Proteobacteria; SAR86	0.4	0.4	0.2	0	0.2	0
Proteobacteria; Pelagibacterales; SAR11 Clade I	0.6	0.5	0.2	0	0.2	0
Proteobacteria; SAR86	0.4	0.3	0.2	0	0.3	0
Proteobacteria; *Pseudohongiella*	0.7	0.7	0.2	0	0.1	0
Proteobacteria; Rhodospirillales; AEGEAN-169 Marine Group	0.6	0.5	0.2	0	0.2	0
Bacteroidetes; Cytophagales; *Marinoscillum*	0.6	0.6	0.2	0	0.1	0
Remaining taxa (1127 ASVs)	33	32.4	8.5	1	8	5.6

Table S5. Estimated flow rates (FR) for all sampled sponges. FR (mL min^{-1}) was calculated for each sponge using the equation provided in Morganti et al.[1]

Sponge Species	Flow Rate (mL min^{-1})
Niphates digitalis	2529.60
Niphates digitalis	9437.63
Niphates digitalis	50323.13
Niphates digitalis	9437.63
Xestospongia muta	661330.21
Xestospongia muta	479962.22
Xestospongia muta	1481276.36
Xestospongia muta	589548.10

Table S6. Average total nitrogen (TN) and dissolved organic nitrogen (DON) in reef water and sponge exhalent treatments at T_0. DON is calculated by subtracting the dissolved organic nitrogen (DIN) from TN for each sample. At T_0, the reef treatment has a single DIN value that was subtracted from each TN value to acquire DON. The sponge exhalent treatment has two DIN values that were averaged, and the averaged DIN was subtracted from each TN value to acquire DON. Paired t-tests were used to determine significant differences in TN and DON between treatments (TN: p=0.119; DON: p=0.113).

Treatment	TN (uM)	DON (uM)
T_0 Reef Water	5.77	4.63
T_0 Sponge Exhalent	8.1	7.03

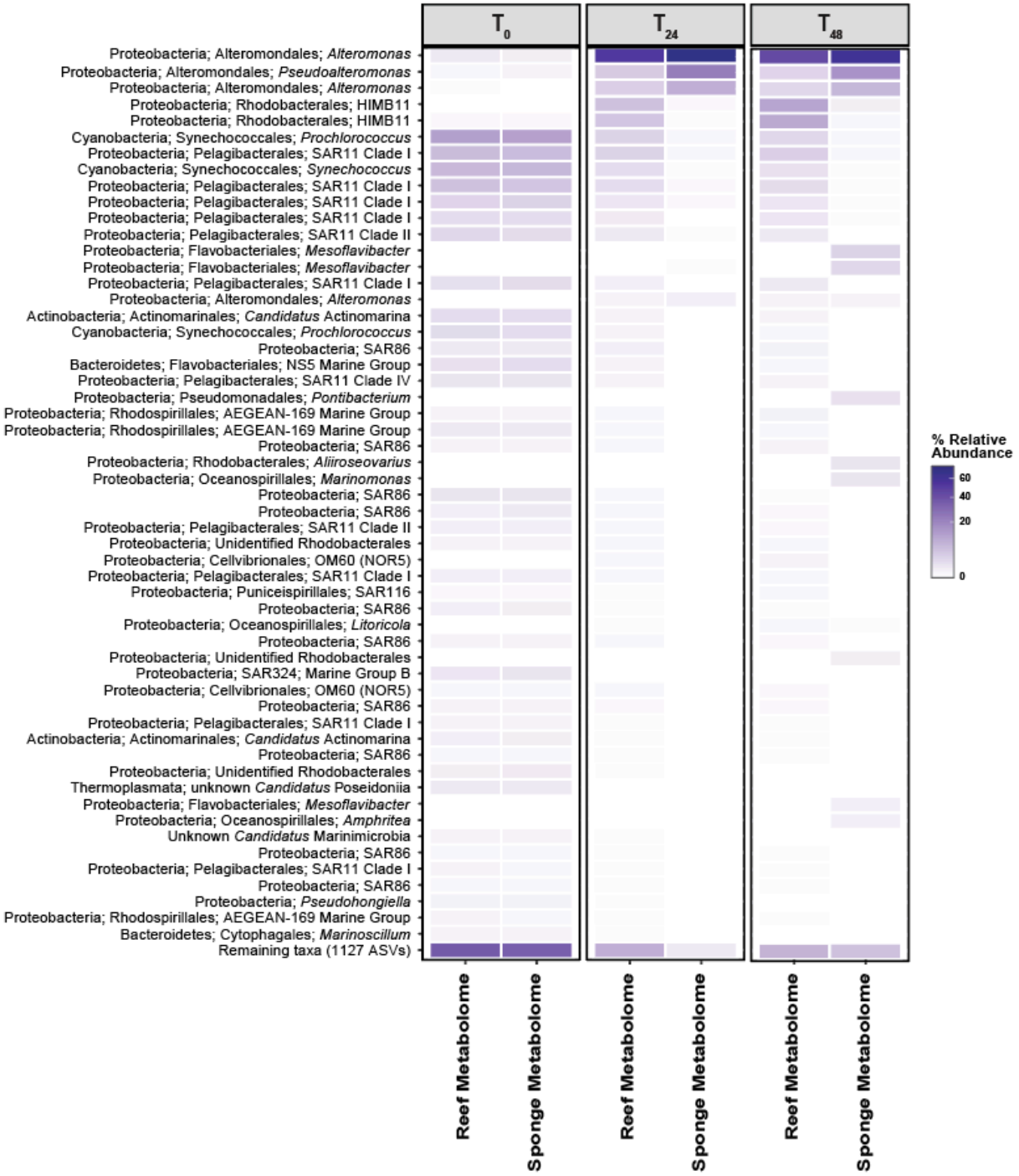

Figure S1. Heat map of relative abundance (%) for the 55 most abundant microbial ASVs (and 'Remaining taxa') in the rarefied picoplankton community at three time points (T_0, T_{24}, and T_{48}) for both the reef water and sponge exhalent treatments. ASVs are taxonomically identified by phylum, order and genera (or their lowest taxonomic designation). Less abundant taxa are combined into the 'Remaining taxa' category. Darker colors denote a higher relative abundance.

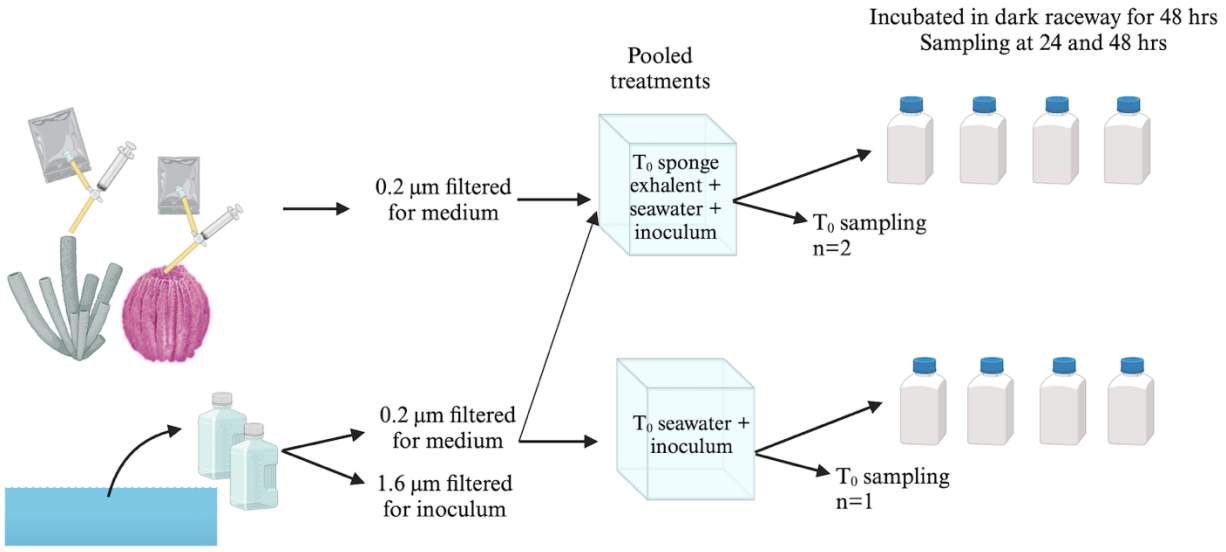

Figure S2. Simplified visualization of experimental setup. To create the sponge exhalent media, exhalent water was collected from *Niphates digitalis* and *Xestospongia muta*, 0.22 µm filtered, and evenly mixed. The sponge exhalent mixture was then diluted 3:1 with 0.22 µm filtered surface reef water (i.e., seawater; not shown in the image). Reef water control was made using 0.22 µm filtered surface reef water. Both sponge exhalent and reef water media were inoculated with 1.6 µm filtered picoplankton collected from the surface reef water. T0 sampling was completed on the pooled media + inoculum (n=2 for sponge exhalent, n=1 for reef water, except for TOC/TN, n=3 per treatment). Pooled treatments were then divided into 2L polycarbonate bottles and incubated in the dark. Sampling was completed at 24 and 48 hrs. Picoplankton were sampled at T_0, T_{24}, and T_{48} while water chemistry was sampled only at T_0 and T_{48}.

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