Effects of sponge excurrent seawater on coral reef picoplankton composition in the Florida Keys January 2022

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Project
» Collaborative Research: The Influence of Sponge Holobiont Metabolism on Coral Reef Dissolved Organic Matter and Reef Microorganisms (Sponge Holobiont DOM)

Contributors	Affiliation	Role
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Abstract
Sponge exhalent water was collected from Looe Key reef, coral exudates water was collected from a coral incubation at Mote Marine lab, and ambient reef water was collected from Looe Key Reef surface water. All seawater was 0.2 um filtered to use as media in 2L bottles for an incubation experiment. Inoculum from reef surface water (1.6 um filtered) was used for the incubation. Bottles were incubated in the dark for 48 hours and samples were taken at the start (T0) and end (T48) for nutrient and microbiome analysis.

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Coverage

Location: Looe Key Reef, Florida Keys National Marine Sanctuary
Spatial Extent: **Lat**:24.562002777778 **Lon**:-81.40871111
Temporal Extent: 2022-01-06 - 2022-01-08

Methods & Sampling

Incubation experiments were set up with sponge excurrent water, coral exudates, sponge excurrent and coral exudates, or surface reef water as media and with reef surface picoplankton as inoculum to compare microbial growth over 48 hours. Excurrent seawater samples of three sponge species (Verongula rigida, Xestospongia muta, and Niphates digitalis) were collected at Looe Key Reef in the Florida Keys, U.S.A. at approximately 10m depth (24.54605, -81.40610). The exhalent seawater was collected using acid-rinsed glass syringes with a three-way valve and PharMed BPT tubing (Cole-Parmer, USA) connected to 1L FlexFoil Plus sampling bags (SKC, Pittsburgh, PA, USA). Water was drawn in at about 2 mL per minute, which is slower than the pumping rate of the sponges. The exhalent seawater was collected the day before the experiment and filtered (0.2 µm, Omnipore, Millipore Sigma, USA) prior to storage at 4°C. Coral exudates were collected by incubating four fragments each of Acropora cervicornis and Orbicella faveolata were incubated in aquaria containing 0.2

µm filtered (Omnipore) seawater from Mote Marine laboratory seawater intake which pulls from the nearby seawater canal. The aquaria was placed in flowthrough seawater raceway in the sunlight under one layer of shade cloth (~23 – 262 µE s-1 with a mix of sun and clouds) for 6.5 hrs. The coral exudate seawater was then filtered 0.2 µm filtered and stored at 4°C.

Surface seawater over Looe Key Reef was collected the morning of the experimental setup and was used for both the inoculum of bacterioplankton and for background seawater control medium (0.2 µm filtered to create the ambient seawater medium). The sponge excurrent seawater was combined in a 1:1:1 ratio from the three sponge species. Then four different mediums were created: coral exudate water, sponge exhalant water, sponge exhalant water plus coral exudates (1:1 ratio of each medium), and ambient seawater. Coral reef surface picoplankton, served as inoculum and was added to the media in a 1:3 ratio of inoculum to medium. An initial 'soup' was made for each treatment in 5L acid-cleaned polycarbonate food grade containers and the total volume of the initial soup was 3900 mL for treatments with microbial inoculum and 2900 mL for control treatments without inoculum. From the initial soup, three T0 samples of seawater (1400 mL) were taken for chemical and microbial analyses. The rest of the experimental seawater with inoculum was then distributed into 2L acid-cleaned polycarbonate bottles (1L per bottle) and incubated in a flow through seawater table in the dark for 48 hours. There were five replicate bottles for each treatment (coral, sponge, sponge + coral, ambient seawater) and three replicate bottles for each no-inoculum control corresponding to each treatment. Nutrients and the bacterioplankton community composition and abundance were assessed at the start of the experiment (T0) and after 48hrs (T48).

Seawater sampled from the incubation 'soup' or bottles was used for multiple nutrient analyses and DNA extraction. Prior to filtration, 1 mL of seawater was preserved in 500 µL of paraformaldehyde (0.5% final concentration), which were stored in the refrigerator for 1-2 hours and then stored at -80°C until shipped to the University of Hawai'i for flow cytometry analysis. Also prior to filtration, approximately 30 mL of sample was poured into acid and milliQ water rinsed and combusted glass amber EPA vials with acid cleaned septa and lids. These 30 mL samples were acidified to pH ~2 with concentrated HCl and stored at room temperature until analysis for total organic carbon (TOC) and total nitrogen (TN) at Woods Hole Oceanographic Institution. The rest of the seawater was filtered through an Omnipore 0.2 um filter using a peristaltic pump and acid and milliQ water rinsed Pharmed BP tubing and Teflon filter holders. The filter was stored at -80C until DNA extraction, while the filtrate was poured into acid and milliQ water rinsed: 1) HDPE bottles (~20 mL) for inorganic nutrient analysis at the University of Oregon, 2) polycarbonate bottles (~500 mL) for extraction of dissolved organic matter (DOM). Filtered seawater was processed using PPL solid phase extraction following the protocol by Dittmar et al. 2008. Extracts were dried to nearly completeness, leaving a small viscous drop in the vial. These extracts were then shipped to WHOI for metabolomics analysis.

DNA from the Omnipore filters was extracted using a commercial kit and the 16S rRNA gene was amplified using the modified earth microbiome primer set for the V4 region (515F and 806R, Apprill et al. 2015). PCRs were sent to Middle Tennessee State University for library construction and sequencing on an Illumina MiSeq, producing FASTQ files as output.

Targeted Metabolite Analysis by UPLC-MS:

DOM extracts were reconstituted in 200 µl MilliQ water with 50 ng/ml isotopically-labeled injection standards d2 biotin, d6 succinic acid, d4 cholic acid, and d7 indole 3 acetic acid. We used ultra-performance liquid chromatography (Accela Open Autosampler and Accela 1250 Pump, Thermo Scientific) coupled to a heated electrospray ionization source (H-ESI) and a triple quadrupole mass spectrometer (TSQ Vantage, Thermo Scientific) operated under selected reaction monitoring (SRM) mode. We performed chromatographic separation with a Waters Acquity HSS T3 column (2.1 × 100 mm, 1.8 µm) equipped with a Vanguard pre-column and maintained at 40°C. We eluted the metabolites from the column with (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile at a flow rate of 0.5 mL min-1, according to the gradient: 0 min, 1% B; 1 min, 1%B; 3 min, 15%B; 6 min, 50%B; 9 min, 95%B; 10 min, 95%B; 10.2 min, 1%B; 12 min, 1%B (total run time = 12 min). Settings for source gases were 55 (sheath), 20 (auxiliary) and 0 (sweep), and these settings are presented in arbitrary units. The heated capillary temperature was 375 °C and the vaporizer temperature was 400 °C. For positive and negative modes, we performed separate autosampler injections of 5 µL each.

Inorganic nutrients:

Inorganic nutrients included phosphate, nitrate+nitrite, nitrite, ammonia, and silicic acid. The phosphate method is a modification of the molybdenum blue procedure of

Bernhardt and Wilhelms (1967), in which phosphate is determined as reduced phosphomolybdic acid employing hydrazine as the reductant. The nitrate + nitrite analysis uses the basic method of Armstrong et al. (1967), with modifications to improve the precision and ease of operation. Sulfanilamide and N-(1-Napthyl)ethylenediamine dihydrochloride react with nitrite to form a colored diazo compound. For the nitrate + nitrite analysis, nitrate is first reduced to nitrite using an OTCR and imidazole buffer as described by Patton (1983). Nitrite analysis is performed on a separate channel, omitting the cadmium reductor and the buffer. The method is based on that of Armstrong et al. (1967) as adapted by Atlas et al. (1971). Addition of an acidic molybdate reagent forms silicomolybdic acid which is then reduced by stannous chloride. This indophenol blue method is modified from ALPKEM RFA methodology which references Methods for Chemical Analysis of Water and Wastes, March 1984, EPA-600/4-79-020, "Nitrogen Ammonia", Method 350.1 (Colorimetric, Automated Phenate) A detailed description of the continuous segmented flow procedures used can be found in Gordon et. al. (1994).

Flow cytometry:

Samples were preserved and stored at -80°C until later batch analysis at the University of Hawaii SOEST Flow Cytometry Facility (www.soest.hawaii.edu/sfcf). Microbial cells were enumerated using flow cytometry (Selph, 2021). In brief, samples

(0.1 mL) were thawed in batches, stained with the DNA dye Hoechst 34580 (1 µg/mL final), then run at 30 µL min-1 on a Beckman-Coulter CytoFlex S flow cytometer, using lasers emitting at 375 nm (to detect Hoechst), 488 nm (for scatter and chlorophyll parameters), and 561 nm (for phycoerythrin). Resulting listmode files (FCS 3.0) were analyzed using FlowJo software (Becton Dickinson, v. 10.8.2) to distinguish microbial populations based on their fluorescence signals (chlorophyll, phycoerythrin, DNA), as well as forward and right-angle light scatter. Heterotrophic bacteria were distinguished from phytoplankton by their DNA signature and absence of pigment. Prochlorococcus and Synechococcus were separated from larger eukaryotic phytoplankton by their light scatter signatures, as well as their characteristic pigment and DNA signatures. Other phytoplankton (eukaryotes, mostly 2-20 µm pico- and nano-sized cells given the small volume analyzed) had higher light scatter and more chlorophyll fluorescence per cell. >

Data Processing Description

Targeted Metabolomics:

Samples were analyzed in random order and injected pooled samples at regular intervals (every 8 samples). We monitored two SRM transitions per compound for quantification and confirmation; these transitions were optimized previously using authentic standards. We generated 8-point external calibration curves based on peak area for each compound. We converted raw data files from proprietary Thermo (.RAW) format to mzML using the msConvert tool (Chambers et al., 2012) prior to processing with El-MAVEN (Agrawal et al., 2019). Metabolite concentrations were provided from WHOI as raw data in ng per ml in the 200 µl extract. Extraction efficiency values in seawater were used from Johnson et al. 2017. For any metabolites with extraction efficiency below 1%, these were removed from the analysis. For any metabolites with extraction efficiency of 30% or higher, the values were corrected based on the extraction efficiency (e.g., if the extraction efficiency was 50% then the value was multiplied by 2). We then converted the ng/ml values into total nanograms by multiplying by 0.2 then divided by the sample volume in liters to produce ng/L concentrations. These concentrations were converted to picomolar concentrations using the formula weight for each metabolite.

BCO-DMO Processing Description

* adjusted names to comply with database requirements
* Added sampling lat/lon and dates where missing
*

Problem Description

Several samples for inorganic nutrient analysis were mixed up (labels fell off) during shipping and are therefore missing these data: FswM_T0_5_3, SpongeCtl_T48_2_2, SpongeCoralCtl_T48_4_2, FswM_T48_5_2, FswCtl_T48_6_2, CoralCtl_T48_8_3.

For targeted metabolomics, two samples were lost during processing: one sample from the sponge control (no inoculum added, sample SpongeCtl_T0_2_1) and one sample from the reef seawater medium with inoculum (FswM_T0_5_3).

The T6, T12, T18, T24, T30, T36 and T42 samples were collected for flow cytometry only (cell population data of Syn, Pro, picoeukaryote, and heterotrophic bacteria). All other data were collected at T0 and T48.

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Data Files

File
963872_v1_experiment.csv (Comma Separated Values (.csv), 118.42 KB) MD5:6e913ea4d755245af71d4a1a47a84b6c Primary data file for dataset ID 963872, version 1

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Related Datasets

IsRelatedTo

Fiore, C. L., Easson, C. G., Apprill, A., Reigel, A. M., Fortier, K., Moore, D. (2025) **Microbiome profiling of**

bacterioplankton communities on sponge excurrent water, coral exudate water, and surface reef water.
Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2025-06-17
doi:10.26008/1912/bco-dmo.964182.1 [view at BCO-DMO]
Relationship Description: The NCBI dataset is the microbiome profiling data of the incubation experiment.

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Parameters

Parameter	Description	Units
SampleID	Unique Sample Identity as Treatment_Time_TreatmentNumber_RreplicateNumber_	unitless
latitude	Latitude of sampling location, south is negative	decimal degrees
longitude	Longitude of sampling location, west is negative	decimal degrees
sampling_date	Sampling date	
Trtmt	Treatment of sponge exhalent media, coral exhalent media, reef ambient seawater media, or sponge exhalant + coral exhalent media, each with and without picoplankton inoculum. SpongeM: Treatment 1, Sponge exhalant (0.2µm filtered) inoculated with picoplankton (1.6µm filtered surface water); SpongeCtl: Treatment 2, Sponge exhalant (0.2µm filtered) without picoplankton inoculum; SpongeCoralM: Treatment 3, Sponge exhalant + Coral exhalant media (0.2 µm filtered) inoculated with picoplankton (1.6 µm filtered surface water); SpongeCoralCtl: Treatment 4, Sponge exhalant + Coral exhalant media (0.2 µm filtered) without picoplankton inoculum; FswM: Treatment 5, reef ambient seawater media (0.2 µm filtered) inoculated with picoplankton (1.6 µm filtered surface water); FswCtl: Treatment 6, reef ambient seawater media (0.2 µm filtered) without picoplankton inoculum; CoralM: Treatment 7, Coral exhalant media (0.2 µm filtered) inoculated with picoplankton (1.6 µm filtered surface water); CoralCtl: Treatment 8, Coral exhalant media (0.2 µm filtered) without picolanton inoculum	unitless
Time	Time of sampling in hours with the start of the experiment as T0	hours
PO4	Concentration of phosphate per liter of surface water sample	micromolar per liter (µM/L)
NOx	Concentration of nitrite + nitrate	micromolar per liter (µM/L)
Silicate	Concentration of dissolved silicate in micromoles per liter of sample water	micromoles per liter (µM/L)

NH4	Concentration of ammonium per liter of surface water sample	micromolar per liter (µM/L)
TOC	Concentration of dissolved organic carbon	micromolar per liter (µM)/L
TON	Concentration of total organic nitrogen (derived from TOC - dissolved inorganic nitrogen)	micromolar per liter (µM/L)
metabolite_2_3_dihydroxypropane_1_sulfonate	2,3-dihydroxypropane-1-sulfonate metabolite concentration	picomolar (pM)
metabolite_2_3_dihydroxybenzoic_acid	2,3-dihydroxybenzoic acid metabolite concentration	picomolar (pM)
metabolite_2_deoxycytidine	2'-deoxycytidine metabolite concentration	picomolar (pM)
metabolite_2_methyl_4_oxopentanoic	2-methyl-4-oxopentanoic metabolite concentration	picomolar (pM)
metabolite_2_Keto_3_deoxy_6_phosphogluconate	2-Keto-3-deoxy-6-phosphogluconate metabolite concentration	picomolar (pM)
metabolite_3_methyl_2_oxobutanoic_acid	3-methyl-2-oxobutanoic acid metabolite concentration	picomolar (pM)
metabolite_4_aminobenzoic_acid	4-aminobenzoic acid metabolite concentration	picomolar (pM)
metabolite_4_methyl_2_oxopentanoic_acid	4-methyl-2-oxopentanoic acid metabolite concentration	picomolar (pM)
metabolite_6_hydroxymelatonin	6-hydroxymelatonin metabolite concentration	picomolar (pM)
metabolite_adenine	Adenine metabolite concentration	picomolar (pM)
metabolite_adenosine_5_monophosphate	Adenosine 5'-monophosphate metabolite concentration	picomolar (pM)
metabolite_adenosine	Adenosine metabolite concentration	picomolar (pM)
metabolite_alanine	Alanine metabolite concentration	picomolar (pM)
metabolite_4_amino_5_aminomethyl_2_methylpyrimidine	4-amino-5-aminomethyl-2-methylpyrimidine metabolite concentration	picomolar (pM)
metabolite_anthranilate	Anthranilate metabolite concentration	picomolar (pM)
metabolite_arginine	Arginine metabolite concentration	picomolar (pM)

metabolite_argininosuccinic_acid	Argininosuccinic acid metabolite concentration	picomolar (pM)
metabolite_caffeine	Caffeine metabolite concentration	picomolar (pM)
metabolite_carnitine	Carnitine metabolite concentration	picomolar (pM)
metabolite_choline	Choline metabolite concentration	picomolar (pM)
metabolite_citric_acid	Citric acid metabolite concentration	picomolar (pM)
metabolite_cyanocobalamin	Cyanocobalamin metabolite concentration	picomolar (pM)
metabolite_cysteine	Cysteine metabolite concentration	picomolar (pM)
metabolite_cytidine	Cytidine metabolite concentration	picomolar (pM)
metabolite_cytosine	Cytosine metabolite concentration	picomolar (pM)
metabolite_desthiobiotin	Desthiobiotin metabolite concentration	picomolar (pM)
metabolite_chitobiose	Chitobiose metabolite concentration	picomolar (pM)
metabolite_dimethylsulfoniopropionate	Dimethylsulfoniopropionate metabolite concentration	picomolar (pM)
metabolite_D_fructose_1_6_bisphosphate	D-fructose 1,6-bisphosphate metabolite concentration	picomolar (pM)
metabolite_fructose_6_phosphate	Fructose 6-phosphate metabolite concentration	picomolar (pM)
metabolite_fumaric_acid	Fumaric acid metabolite concentration	picomolar (pM)
metabolite_x001a_aminobutyric_acid	-aminobutyric acid metabolite concentration	picomolar (pM)
metabolite_D_glucosamine	D-glucosamine metabolite concentration	picomolar (pM)
metabolite_glucose_6_phosphate	Glucose 6-phosphate metabolite concentration	picomolar (pM)
metabolite_glutamic_acid	Glutamic acid metabolite concentration	picomolar (pM)
metabolite_glutamine	Glutamine metabolite concentration	picomolar (pM)
metabolite_glutathione	Glutathione metabolite concentration	picomolar (pM)

metabolite_sn_glycerol_3_phosphate	sn-glycerol 3-phosphate metabolite concentration	picomolar (pM)
metabolite_glycine	Glycine metabolite concentration	picomolar (pM)
metabolite_glycine_betaine	Glycine betaine metabolite concentration	picomolar (pM)
metabolite_guanine	Guanine metabolite concentration	picomolar (pM)
metabolite_guanosine	Guanosine metabolite concentration	picomolar (pM)
metabolite_4_methyl_5_thiazoleethanol	4-methyl-5-thiazoleethanol metabolite concentration	picomolar (pM)
metabolite_histadine	Histadine metabolite concentration	picomolar (pM)
metabolite_histidinol	Histidinol metabolite concentration	picomolar (pM)
metabolite_homocysteine	Homocysteine metabolite concentration	picomolar (pM)
metabolite_hydroxocobalamin	Hydroxocobalamin metabolite concentration	picomolar (pM)
metabolite_hydroxyproline	Hydroxyproline metabolite concentration	picomolar (pM)
metabolite_hypoxanthine	Hypoxanthine metabolite concentration	picomolar (pM)
metabolite_inosine	Inosine metabolite concentration	picomolar (pM)
metabolite_inosine_5_monophosphate	Inosine 5'-monophosphate metabolite concentration	picomolar (pM)
metabolite_isoleucine	Isoleucine metabolite concentration	picomolar (pM)
metabolite_kynurenine	Kynurenine metabolite concentration	picomolar (pM)
metabolite_leucine	Leucine metabolite concentration	picomolar (pM)
metabolite_lumichrome	Lumichrome metabolite concentration	picomolar (pM)
metabolite_malic_acid	Malic acid metabolite concentration	picomolar (pM)
metabolite_methionine	Methionine metabolite concentration	picomolar (pM)
metabolite_methylmalonic_acid	Methylmalonic acid metabolite concentration	picomolar (pM)

metabolite_5_methylthioadenosine	5'-methylthioadenosine metabolite concentration	picomolar (pM)
metabolite_n_acetyl_glucosamine	n-acetyl glucosamine metabolite concentration	picomolar (pM)
metabolite_N_acetyl_D_glucosamine_6_phosphate	N-acetyl-D-glucosamine 6-phosphate metabolite concentration	picomolar (pM)
metabolite_n_acetyl_glutamic_acid	n-acetyl glutamic acid metabolite concentration	picomolar (pM)
metabolite_n_acetyl_muramic_acid	n-acetyl muramic acid metabolite concentration	picomolar (pM)
metabolite_phosphoserine	Phosphoserine metabolite concentration	picomolar (pM)
metabolite_ornithine	Ornithine metabolite concentration	picomolar (pM)
metabolite_pantothenic_acid	Pantothenic acid metabolite concentration	picomolar (pM)
metabolite_phenylalanine	Phenylalanine metabolite concentration	picomolar (pM)
metabolite_proline	Proline metabolite concentration	picomolar (pM)
metabolite_riboflavin	Riboflavin metabolite concentration	picomolar (pM)
metabolite_D_Ribose_5_phosphate	D-Ribose 5-phosphate metabolite concentration	picomolar (pM)
metabolite_serine	Serine metabolite concentration	picomolar (pM)
metabolite_taurocholic_acid	Taurocholic acid metabolite concentration	picomolar (pM)
metabolite_spermidine	Spermidine metabolite concentration	picomolar (pM)
metabolite_succinic_acid	Succinic acid metabolite concentration	picomolar (pM)
metabolite_sucrose	Sucrose metabolite concentration	picomolar (pM)
metabolite_syringic_acid	Syringic acid metabolite concentration	picomolar (pM)
metabolite_thiamine	Thiamine metabolite concentration	picomolar (pM)
metabolite_threonine_homoserine	Threonine / Homoserine metabolite concentration	picomolar (pM)
metabolite_thymidine	Thymidine metabolite concentration	picomolar (pM)

metabolite_chitotriose	Chitotriosemetabolite concentration	picomolar (pM)
metabolite_tryptophan	Tryptophan metabolite concentration	picomolar (pM)
metabolite_tyrosine	Tyrosine metabolite concentration	picomolar (pM)
metabolite_uric_acid	Uric acid metabolite concentration	picomolar (pM)
metabolite_uridine	Uridine metabolite concentration	picomolar (pM)
metabolite_uridine_5_monophosphate	Uridine 5'-monophosphate metabolite concentration	picomolar (pM)
metabolite_valine	Valine metabolic concentration	picomolar (pM)
metabolite_xanthine	Xanthine metabolite concentration	picomolar (pM)
metabolite_xanthosine	Xanthosine metabolite concentration	picomolar (pM)
Proch_conc	Prochlorococcus concentration (mL-1), very low abndances (< 100 cells/mL) may represent noise	cells per millliliter (mL-1)
Syn_conc	Synechococcus concentration (mL-1), very low abundances (< 100 cells/mL) may represent noise	cells per millliliter (mL-1)
Picoeuk_conc	Picoeukaryote (photosynthetic) concentration (mL-1)	cells per millliliter (mL-1)
HetBac_conc	Heterotrophic (nonpigmented) bacteria concentration (mL-1)	cells per millliliter (mL-1)

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Instruments

Dataset-specific Instrument Name	Beckman-Coulter CytoFLEX S
Generic Instrument Name	Flow Cytometer
Dataset-specific Description	Flow Cytometry: Preserved samples were analyzed using a Beckman-Coulter CytoFLEX S flow cytometer with 375 nm, 405 nm, 488 nm, and 561 nm lasers activated.
Generic Instrument Description	Flow cytometers (FC or FCM) are automated instruments that quantitate properties of single cells, one cell at a time. They can measure cell size, cell granularity, the amounts of cell components such as total DNA, newly synthesized DNA, gene expression as the amount messenger RNA for a particular gene, amounts of specific surface receptors, amounts of intracellular proteins, or transient signalling events in living cells. (from: http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm)

Dataset-specific Instrument Name	Horiba Aqualog scanning fluorometer
Generic Instrument Name	Fluorometer
Dataset-specific Description	Fluorometer was used to measure fDOM
Generic Instrument Description	A fluorometer or fluorimeter is a device used to measure parameters of fluorescence: its intensity and wavelength distribution of emission spectrum after excitation by a certain spectrum of light. The instrument is designed to measure the amount of stimulated electromagnetic radiation produced by pulses of electromagnetic radiation emitted into a water sample or in situ.

Dataset-specific Instrument Name	TSQ Vantage, Thermo Scientific
Generic Instrument Name	Quadrupole Mass Spectrometer
Dataset-specific Description	Targeted metabolomics were measured using the ultra-performance liquid chromatography (Accela Open Autosampler and Accela 1250 Pump, Thermo Scientific) coupled to a heated electrospray ionization source (H-ESI) and a triple quadrupole mass spectrometer (TSQ Vantage, Thermo Scientific) operated under selected reaction monitoring (SRM) mode at the Woods Hole Oceanographic Institution (WHOI).
Generic Instrument Description	A piece of apparatus that consists of an ion source, a mass-to-charge analyser, a detector and a vacuum system and is used to measure mass spectra. The detector is a quadrupole mass-to-charge analyser, which holds the ions in a stable orbit by an electric field generated by four parallel electrodes.

Dataset-specific Instrument Name	Technicon AutoAnalyzer II™ and Alpkem RFA 300™
Generic Instrument Name	Technicon AutoAnalyzer II
Dataset-specific Description	Inorganic nutrients measured using Technicon AutoAnalyzer II™ components were used to measure phosphate and ammonium; and Alpkem RFA 300™ components were used for silicic acid, nitrate plus nitrite, and nitrite.>
Generic Instrument Description	A rapid flow analyzer that may be used to measure nutrient concentrations in seawater. It is a continuous segmented flow instrument consisting of a sampler, peristaltic pump, analytical cartridge, heating bath, and colorimeter. See more information about this instrument from the manufacturer.

Dataset-specific Instrument Name	Accela Open Autosampler and Accela 1250 Pump, Thermo Scientific
Generic Instrument Name	Ultra high-performance liquid chromatography
Dataset-specific Description	Targeted metabolomics were measured using the ultra-performance liquid chromatography (Accela Open Autosampler and Accela 1250 Pump, Thermo Scientific) coupled to a heated electrospray ionization source (H-ESI) and a triple quadrupole mass spectrometer (TSQ Vantage, Thermo Scientific) operated under selected reaction monitoring (SRM) mode at the Woods Hole Oceanographic Institution (WHOI).
Generic Instrument Description	Ultra high-performance liquid chromatography: Column chromatography where the mobile phase is a liquid, the stationary phase consists of very small (< 2 microm) particles and the inlet pressure is relatively high.

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Project Information

Collaborative Research: The Influence of Sponge Holobiont Metabolism on Coral Reef Dissolved Organic Matter and Reef Microorganisms (Sponge Holobiont DOM)

Coverage: Caribbean Sea

NSF Award Abstract:
The seawater around coral reefs is typically low in nutrients, yet coral reefs are teeming with life and are often compared to oases in a desert. Life exists in these 'marine deserts' in large part, due to symbiotic associations between single-celled microbes and invertebrates such as corals and sponges. The concentration and type of dissolved organic matter (DOM), a complex pool of organic nutrients such as amino acids, vitamins, and other diverse compounds, also affects the health of coral reefs. The composition of DOM on coral reefs is linked to both the composition of free-living microbes in the seawater and to the nutrition of filter-feeding organisms, such as corals and sponges. However, the factors that influence the composition of DOM on coral reefs and the consequences of how it changes are not well understood. Recent work suggests that sponges could have a significant impact on the composition of reef dissolved organic nutrients, depending on sponge species due to differences in filtration capacity and in their symbiotic microbial communities. This project characterizes how diverse sponge species process DOM on coral reefs and determines the impacts of this processing on the free-living microbial community. Seawater is collected from sponges (pre- and post- sponge filtration) on coral reefs in the relatively pristine region of Curacao, and incubation experiments measure the impact of sponge filtration on the growth of the free-living microbial community. The organic nutrients of seawater samples are analyzed using cutting-edge techniques to distinguish the types of nutrients that are processed by sponges. The incubation experiments, using free-living microbes collected from the coral reef, quantify the impact of sponge filtration on the growth and composition of this community. This project provides fundamental understanding of how sponges contribute to the base of the coral reef food web. As the human-driven impacts continue to alter the composition of organisms on reefs, this understanding is necessary to predict changes to reef microbial food webs and is thus essential for scientists, reef managers, and policy decision makers. This project trains undergraduate students and a postdoctoral scholar and contributes to undergraduate and K-12 education through development of sponge-centric lessons that focus on local U.S. east coast aquatic environments as well as coral reef ecosystems.

Sponges vary in their capacity to filter seawater and in their associated microbial communities, leading to diverse metabolic strategies that often coexist in one habitat. While it is well-established that sponges are important in processing dissolved organic matter (DOM), an important reservoir of reduced carbon compounds, and transferring this energy to benthic food webs, there has been limited work to understand the consequences of sponge processing on the composition of coral reef DOM and on pelagic food webs. Specifically, while studies have shown that exudates of corals and algae select for specific groups of picoplankton (autotrophic and heterotrophic, respectively), similar data for sponges are required to understand the multiple factors that shape the composition of DOM and of the picoplankton community on coral reefs. Thus, this project is aimed at addressing a major knowledge gap of the role of sponge-derived DOM (sponge exometabolome) in coral reef biogeochemistry. An in situ sampling design targeting prominent Caribbean sponges and picoplankton incubation experiments is coupled to address both the composition of sponge exometabolomes and delineate shifts in the picoplankton community derived from sponge exometabolomes. Molecular-level changes to seawater DOM by sponge processing and the impact of these changes on the overall coral reef DOM profile is assessed with two DOM analysis techniques: a commonly used fluorometry technique (fDOM analysis) and with high-resolution mass spectrometry (LC-MS/MS). Additionally, microbiome and functional gene profiling, growth metrics, and nutrient analyses are employed to assess changes in the picoplankton community in response to sponge exometabolomes. Advanced data analysis techniques then synthesize data generated by each approach to provide novel insight on a poorly uncharacterized biogeochemical pathway on coral reefs. The work outlined here represents entirely novel information on the impact of sponge metabolism on the composition of DOM, sheds light on biologically important molecules involved in benthic-pelagic coupling, and importantly, generates data using standardized methods, thus facilitating comparison to previous and future DOM datasets.

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

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Funding

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