Seawater nutrient/metabolite and flow cytometry metadata - Picoplankton incubation experiment 2020

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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)

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Abstract
Sponges are known for their efficient uptake of organic matter, both particulate (POM) and dissolved (DOM), from the water column, but there is also evidence that sponges, and their associated symbionts, produce DOM rich in nucleosides and other unidentified metabolites that is transferred to the surrounding reef via their exhalent seawater. However, little is known about the effect of this sponge metabolome on another vital component of reef ecosystems, picoplankton. If the metabolome impacts the structure of the picoplankton community, then there may be a subsequent alteration to the reef dissolved nutrient pool. To address these questions, we exposed Caribbean reef picoplankton communities to either filtered reef sea water (control) or a sponge exhalent metabolome mixture from two Caribbean sponges, Niphates digitalis and Xestospongia muta, in closed, dark incubations for 24 - 48 hours. Sponge exhalent and reef sea water, for use as media in the incubation experiment, was collected on 13-December-2020 from Looe Key Reef Sanctuary Preservation Area (24.54605, -81.40610) in the Florida Keys National Marine Sanctuary (FKNMS) as permitted via FKNMS-2020-149. We used flow cytometry to identify any changes in picoplankton cell concentrations following exposure to sponge-derived nutrients. We also used a cutting-edge suite of analyses to identify nutrient components contributed by the sponge holobiont to reef water and to monitor changes in the incubation bottles, including: targeted and untargeted metabolomics, fluorescent DOM (fDOM), dissolved combined neutral sugars (DCNS), total organic carbon (TOC), total nitrogen (TN), and inorganic nutrients.

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Coverage

Spatial Extent: **Lat**:24.54605 **Lon**:-81.4061
Temporal Extent: 2020-12-13 - 2020-12-15

Methods & Sampling

Collection Media and inoculum:

Sponge exhalent water and reef sea water, for use as media in the incubation experiment, were collected on 9-December-2020 from Looe Key Reef Sanctuary Preservation Area (24.54605, -81.40610) in the Florida Keys National Marine Sanctuary (FKNMS). Sponge exhalent water was collected from each of 4 individuals of two sponge species, *Niphates digitatalis* and *Xestospongia muta*. Exhalent water collection was completed by scuba divers using a three-way valve with one valve attached to a ~8cm piece of PharMed L/S 25 tubing (Masterflex® L/S® Precision Pump Tubing, Radnor, PA, USA) that was placed into the excurrent osculum of the sponge to collect exhalent water, a second valve attached to a syringe to create suction and pull in exhalent water, and the final valve attached to ~8cm piece of Teflon tubing that further attaches to the stainless-steel fitting on a 1 L FlexFoil PLUS bag (SKC, Eighty Four, PA, USA) for final exhalent water storage. Exhalent water was pulled into the syringe at a rate of ~2ml min-1, below the estimated pumping rate of both sponge species. Two bags (~1L) were filled per sponge and all sponges were located within 4 meters of one another at ~8m depth. Upon return to the boat all bags were sealed and placed in an ice-filled cooler until further processing in the lab (~2 hrs).

Reef surface water, for use as both reef metabolome media and picoplankton inoculum during the incubation experiment, was collected in 6 acid-washed 2 L Nalgene© HDPE bottles (Thermo Fisher Scientific, Waltham, MA, USA) from ~1 m below the surface just above the reef at Looe Key. Metabolome bottles were immediately placed in a cooler filled with ice until return to the lab (~1 hr). The reef picoplankton inoculum was kept in the dark at ambient seawater temperature.

Picoplankton incubation experimental setup:

The 48-hour experiment included two treatments, reef (i.e., control) and sponge metabolome, that were sampled at 0 hours, 24 hours, and 48 hours (T0, T24, T48, respectively). Surface seawater (6 L) for picoplankton inoculum was filtered through three 1.6 µm, 47 mm pre-combusted GF/A filter to remove larger eukaryotic plankton and particular matter. Filters were housed in a 47 mm acid-washed in-line PFA filter holder (Advantec, Cole-Parmer, Vernon Hills, IL, USA). Filtering was completed via peristalsis (MasterFlex L/S pump and pump heads, Cole-Parmer, Vernon Hills, IL, USA). Samples were filtered slowly (50 ml min-1) to avoid bursting of microbial cells on the filter membrane and filters were changed every 2 L. PharMed L/S 25 (Masterflex® L/S® Precision Pump Tubing, Radnor, PA, USA) acid-washed tubing was used for all filtration. The sponge and reef metabolome media (6 L each) were both passed through 0.22 µm, 47 mm polytetrafluoroethylene (PTFE) filters (Omnipore, EMD Millipore Corporation, Billerica, MA, USA) using the same process detailed above. Sponge metabolome media consisted of a 3:1 ratio of filtered sponge exhalent water (1:1 exhalent water from *X. muta* and *N. digitalis*) to 0.22 µm filtered sea water.

Following filtering, two 20 L food grade containers were used, one per treatment, to pool the filtered inoculum with the filtered media. Pooling was done to ensure consistent starting mixtures for each incubation bottle. Following initial (T0) sampling as detailed below, pooled treatments were evenly distributed into four 2 L Nalgene© bottles per treatment for an approximate ratio of 1:2 filtered picoplankton inoculum to filtered media. All incubation bottles were kept in a dark incubator at 26ºC for the duration of the 48-hr experiment and only removed for picoplankton community sampling at T24. Water chemistry and cell abundance was assessed for both the sponge and reef metabolome treatments at the start (T0) and end (T48) of the incubation experiment. Samples were also taken at T24, but they were only used to obtain cell abundance.

Data Processing Description

Flow cytometry

To quantify picoplankton abundances, 1 ml samples of water from each pooled treatment (T0; n=3 per treatment) and from each incubation bottle (n=4 per treatment) at both T24 and T48 was collected in a sterile cryovial and fixed with paraformaldehyde (1% final volume), incubated in the dark at 4ºC for 1-hr, and then frozen at -80ºC. The fixed samples were used for enumeration of total bacterial cells (*Prochlorococcus*, *Synechococcus*, picoeukaryotic cells, and unpigmented cells (heterotrophic bacteria and archaea)) via flow cytometry at Bigelow Laboratory for Ocean Sciences. Flow cytometry analysis was completed 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).

Biochemical Analyses

Total Organic Carbon (TOC) and Total Nitrogen (TN)

For TOC and TN analyses, 20 ml of water was collected from the pooled treatments prior to their distribution into individual incubation bottles (T0: reef metabolome, n=3; sponge metabolome, n=3) and for each bottle at the end of the experiment (T48: n=4 per treatment) in acid-washed, combusted 40-ml amber EPA vials. Samples were immediately acidified to pH 3 using 12M trace-metal grade hydrochloroic acid (HCL, OptimaTM, Fisher Chemical, Fisher Scientific, Hampton, NH, USA) and stored in the dark at 4ºC. The acidified samples were processed at Woods Hole Oceanographic Institute using a Shimadzu TOC-L analyzer (Longnecker et al. 2015). Additionally,

Filtering for Biochemical Analyses

All other biochemical analyses were completed only on filtered water samples from the pooled treatments at T0 (reef metabolome, n=1 and sponge metabolome, n=2) and T48 incubation bottles (n=4 per treatment). Approximately 680 ml of water from each incubation bottle was individually passed through a 0.22 µm, 47 mm PTFE filter using peristalsis as previous detailed except for a slightly slower filtering rate (30 ml/min) to avoid bursting the microbial cells. Following filtration, the PTFE filters were placed in individual sterile cryovials and stored at -80ºC.

Inorganic macronutrients

For inorganic macronutrient analyses (PO43-, NO3-, Silicate, NO2, NH4+), ~25 ml of the 0.22 µm filtrate from each sample was collected in an acid-washed 30 ml break-proof Nalgene© HDPE bottle (Thermo Fisher Scientific, Waltham, MA, USA), frozen and stored at -20ºC before being shipped to College of Earth, Ocean, and Atmospheric Sciences at Oregon State University for inorganic macronutrient analyses. Phosphate (PO43-) and ammonium (NH4+) were measured using a Technicon Auto Analyzer II (SEAL Analytical Inc., Mequon, Wisconsin, USA) and silicic acid (i.e., silicate), nitrate (NO3-) and nitrate+nitrite (NO3- + NO2-) 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. (1994), but a brief summary of each analysis is provided. The PO43- method was modified from the molybdenum blue procedure (Bernhardt and Wilhelms 1967), in which PO43- 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 NH4+. Silicic acid was measured using the methodology of Atlas et al. (1971) in which the addition of an acidic molybdate reagent forms silicomolybdic acid that is then reduced by stannous chloride. Lastly, NO3- and NO3- + NO2- were measured based on the methods of Atlas et al. (1967) with modifications to improve precision. Sulfanilamide and N-(1-Napthyl)ethylenediamine dihydrochloride react with NO2- to form a diazo compound. For the NO3- + NO2- analysis, NO3- is first reduced to NO2- using an OTCR and imidazole buffer as described by Patton (1983). The NO2- 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 (collected in acid-washed, combusted 40-ml amber EPA vials and stored in the dark at 4ºC) 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 (Borch and Kirchman 1997) 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 (collected in acid-washed, combusted 40-ml amber EPA vials and stored in the dark at 4ºC) from each sample following the methods detailed in Nelson et al. (2015). Samples were analyzed using a Horiba Aqualog scanning fluorometer with 150 W Xe excitation lamp, Peltier-cooled CCD emission detector, 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.m) that employs parallel factor analysis (PARAFAC) to identify peaks that correspond to previously characterized fDOM components (Coble 1996, Nelson et al. 2015).

Targeted metabolomics

For targeted metabolomics analysis, the remaining filtrate from each sample (~600 ml) was acidified to pH ~3 using 12M trace-metal grade hydrochloric acid (HCL) and the volume was recorded. Note that for targeted metabolomics analysis only one T0 sample from each treatment was analyzed. Extracellular metabolites were concentrated and extracted from each acidified filtrate using solid phase extraction (SPE) with 1g/6cc BondElut PPL cartridges (Agilent, Santa Clara, CA, USA) using a vacuum manifold (Waters Corporation, Milford, MA, USA) following the protocols from Kido Soul et al. (2015) and Fiore et al. (2017). 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 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.

Targeted metabolite analysis was performed at Woods Hole Oceanographic Institute 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. (2015). The raw XCalibur files were converted to mzML files using msConvert (Chambers et al. 2012) and processed with MAVEN (Melamud et al. 2010) 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 sea water (Johnson et al. 2017).

BCO-DMO Processing Description

* Adjusted parameter names to comply with database requirements.

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Data Files

File
907866_v1_picoplankton.csv (Comma Separated Values (.csv), 5.18 KB) MD5:8b707735121f8a910a6c3ddb32153796
Primary data file for dataset 907866

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Related Publications

Armstrong, F. A. J., Stearns, C. R., & Strickland, J. D. H. (1967). The measurement of upwelling and subsequent biological process by means of the Technicon Autoanalyzer® and associated equipment. Deep Sea Research and Oceanographic Abstracts, 14(3), 381–389. doi:10.1016/0011-7471(67)90082-4
Methods

Atlas, E. L., Hager, S. W,, Gordon, L. I., & Park, P. K. (1971). A practical manual for use of the Technicon Autoanalyzer in sea water nutrient analyses. Oregon State University, Department of Oceanography. Technical report.
Methods

Bernhardt, H., and A. Wilhelms. 1967. The continuous determination of low level iron, soluble phosphate and total phosphate with the AutoAnalyzer. Technicon Symp. 1:385-89.
Methods

Borch, N. H., & Kirchman, D. L. (1997). Concentration and composition of dissolved combined neutral sugars (polysaccharides) in seawater determined by HPLC-PAD. Marine Chemistry, 57(1–2), 85–95. https://doi.org/10.1016/s0304-4203(97)00002-9 https://doi.org/10.1016/S0304-4203(97)00002-9
Methods

Coble, P. G. (1996). Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. Marine Chemistry, 51(4), 325–346. doi:10.1016/0304-4203(95)00062-3
Methods

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. Portico. https://doi.org/10.7717/peerj.2870
Methods

Gordon, L. I., J. C. Jennings, JR, A. A. Ross, and J. M. Krest. (1994). A suggested protocol for continuous flow analysis of seawater nutrients (phosphate, nitrate, nitrite, and silicic acid) in the WOCE Hydrographic Program and the Joint Global Ocean Fluxes Study. WHP Office Report 91-1. Revision 1, Nov. 1994. WOCE Hydrographic Program Office, Woods Hole, MA.
Related Research

Kido Soule, M. C., Longnecker, K., Johnson, W. M., & Kujawinski, E. B. (2015). Environmental metabolomics: Analytical strategies. Marine Chemistry, 177, 374–387. doi:10.1016/j.marchem.2015.06.029
Methods

Longnecker, K., Futrelle, J., Coburn, E., Kido Soule, M. C., & Kujawinski, E. B. (2015). Environmental metabolomics: Databases and tools for data analysis. Marine Chemistry, 177, 366–373. https://doi.org/10.1016/j.marchem.2015.06.012
Methods

Nelson, C. E., Donahue, M. J., Dulaiova, H., Goldberg, S. J., La Valle, F. F., Lubarsky, K., … Thomas, F. I. M. (2015). Fluorescent dissolved organic matter as a multivariate biogeochemical tracer of submarine groundwater discharge in coral reef ecosystems. Marine Chemistry, 177, 232–243. doi:10.1016/j.marchem.2015.06.026
Methods

Patton, C. J. (1983) Design, characterization and applications of a miniature continuous flow analysis system. Ph.D. Thesis, Mich. State Univ. U. Microfilms International, Ann Arbor, Mich. 150 pp.
Methods

This folder contains the working matlab script to analyze fDOM data from an Aqualog Fluorescent

spectrometer https://github.com/Zquinlan/fDOMmatlab
Software

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Parameters

Parameter	Description	Units
SampleID	Unique sample indentity	unitless
Sampling_Date	Date that sample was collected. Format yyyy-mm-dd	unitless
Experimental_Sampling_Time	Number of hours since experiment start time	hours
Incubation_Treatment	Two experimental treatments (Sponge Metabolome = Samples incubated in sponge exhalent water, Reef Metabolome = Samples incubated in ambient reef water)	unitless
Ultra_Violet_Humic_like	Coble Peak A - Flourescent Dissolved Organic Matter (fDOM)	Ramen Units (R.U.)
Marine_Humic_like	Coble Peak M - Flourescent Dissolved Organic Matter (fDOM)	Ramen Units (R.U.)
Visible_Humic_like	Coble Peak C - Flourescent Dissolved Organic Matter (fDOM)	Ramen Units (R.U.)
Tryptophan_like	Coble Peak T - Flourescent Dissolved Organic Matter (fDOM)	Ramen Units (R.U.)
Tyrosine_like	Coble Peak B - Flourescent Dissolved Organic Matter (fDOM)	Ramen Units (R.U.)
Phenylalanine_like	Coble Peak F - Flourescent Dissolved Organic Matter (fDOM)	Ramen Units (R.U.)
Fulvic_Acid_like	Peak D (Stedmon, 2003) - Flourescent Dissolved Organic Matter (fDOM)	Ramen Units (R.U.)
Phosphate	Concentration of dissolved phosphate in micromoles per liter of sample water	micromoles per liter (umol/l)
Total_Inorganic_Nitrogen	Concentration of dissolved inorganic nitrogen (nitrate+nitrite) in micromoles per liter of sample water	micromoles per liter (umol/l)
Silicate	Concentration of dissolved silicate in micromoles per liter of sample water	micromoles per liter (umol/l)
Nitrite	Concentration of dissolved nitrite in micromoles per liter of sample water	micromoles per liter (umol/l)
Nitrate	Concentration of dissolved nitrate in micromoles per liter of sample water	micromoles per liter (umol/l)
Ammonium	Concentration of dissolved ammonium in micromoles per liter of sample water	micromoles per liter (umol/l)
Picoplankton_Cell_Concentration	Abundance of all particles (bacteria including Synechococcus and Prochlorococcus) stained with SYBRGreen in number of cells mL-1, corrected for preservative, sample dilution, and stain volume; subgated in FSC-405 vs. 525-488 to minimize noise; Data results from flow cytometry analysis	cells per mL (cells/ml)

NPOC	Micromolar concentration of non-purgeable dissolved organic carbon	Micromolar (uM)
TN	Micromolar concentration of total nitrogen	Micromolar (uM)
Fucose	Monosaccharide; Concentration in nanogram per millliter of sample	nanogram per millliter of sample (ng/ml)
Rhamnose	Monosaccharide; Concentration in nanogram per millliter of sample	nanogram per millliter of sample (ng/ml)
Arabinose	Monosaccharide; Concentration in nanogram per millliter of sample	nanogram per millliter of sample (ng/ml)
Galactose	Monosaccharide; Concentration in nanogram per millliter of sample	nanogram per millliter of sample (ng/ml)
Glucose	Monosaccharide; Concentration in nanogram per millliter of sample	nanogram per millliter of sample (ng/ml)
Xylose_Mannose	Monosaccharides that cannot be distinguished from one another; Concentration in nanogram per millliter of sample	nanogram per millliter of sample (ng/ml)
Fructose	Monosaccharide; Concentration in nanogram per millliter of sample	nanograms per liter (ng/l)
anthranilate	Dissolved organic metabolite; Concentration in nanograms per liter of sample	nanograms per liter (ng/l)
phenylalanine	Dissolved organic metabolite; Concentration in nanograms per liter of sample	nanograms per liter (ng/l)
inosine	Dissolved organic metabolite; Concentration in nanograms per liter of sample	nanograms per liter (ng/l)
adenosine	Dissolved organic metabolite; Concentration in nanograms per liter of sample	nanograms per liter (ng/l)
4_methyl_2_oxopentanoic_acid	Dissolved organic metabolite; Concentration in nanograms per liter of sample	nanograms per liter (ng/l)
4_aminobenzoic_acid	Dissolved organic metabolite; Concentration in nanograms per liter of sample	nanograms per liter (ng/l)
tyrosine	Dissolved organic metabolite; Concentration in nanograms per liter of sample	nanograms per liter (ng/l)

S_5_adenosyl_L_homocysteine	Dissolved organic metabolite; Concentration in nanograms per liter of sample	nanograms per liter (ng/l)
desthiobiotin	Dissolved organic metabolite; Concentration in nanograms per liter of sample	nanograms per liter (ng/l)
pantothenic_acid	Dissolved organic metabolite; Concentration in nanograms per liter of sample	nanograms per liter (ng/l)
glutathione_oxidized	Dissolved organic metabolite; Concentration in nanograms per liter of sample	nanograms per liter (ng/l)
tryptophan	Dissolved organic metabolite; Concentration in nanograms per liter of sample	nanograms per liter (ng/l)
n_acetyl_muramic_acid	Dissolved organic metabolite; Concentration in nanograms per liter of sample	nanograms per liter (ng/l)
4_hydroxybenzoic_acid	Dissolved organic metabolite; Concentration in nanograms per liter of sample	nanograms per liter (ng/l)
riboflavin	Dissolved organic metabolite; Concentration in nanograms per liter of sample	nanograms per liter (ng/l)
chorismate	Dissolved organic metabolite; Concentration in nanograms per liter of sample	nanograms per liter (ng/l)
3_3_dimethyl_2oxobutanoic_acid	Dissolved organic metabolite; Concentration in nanograms per liter of sample	nanograms per liter (ng/l)
5_hydroxy_L_tryptophan	Dissolved organic metabolite; Concentration in nanograms per liter of sample	nanograms per liter (ng/l)

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Instruments

Dataset-specific Instrument Name	Alpkem RFA 300 colorimetric autoanalyzer, Alpkem, Kranj, Slovenia
Generic Instrument Name	Alpkem RFA300
Generic Instrument Description	A rapid flow analyser (RFA) that may be used to measure nutrient concentrations in seawater. It is an air-segmented, continuous flow instrument comprising a sampler, a peristaltic pump which simultaneously pumps samples, reagents and air bubbles through the system, analytical cartridge, heating bath, colorimeter, data station, and printer. The RFA-300 was a precursor to the smaller Alpkem RFA/2 (also RFA II or RFA-2).

Dataset-specific Instrument Name	Bio-Rad ZE5, Hercules, California, USA
Generic Instrument Name	Flow Cytometer
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 with 150 W Xe excitation lamp
Generic Instrument Name	Fluorometer
Dataset-specific Description	FDOM analysis: Horiba Aqualog scanning fluorometer with 150 W Xe excitation lamp, Peltier-cooled CCD emission detector, and a simultaneous absorbance spectrometer, Horiba Scientific, Piscataway, New Jersey, USA
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	DIONEX ICS3000 system, Thermo Fisher Scientific
Generic Instrument Name	Ion Chromatograph
Generic Instrument Description	Ion chromatography is a form of liquid chromatography that measures concentrations of ionic species by separating them based on their interaction with a resin. Ionic species separate differently depending on species type and size. Ion chromatographs are able to measure concentrations of major anions, such as fluoride, chloride, nitrate, nitrite, and sulfate, as well as major cations such as lithium, sodium, ammonium, potassium, calcium, and magnesium in the parts-per-billion (ppb) range. (from http://serc.carleton.edu/microbelife/research_methods/biogeochemical/ic....)

Dataset-specific Instrument Name	Shimadzu TOC-L analyzer
Generic Instrument Name	Shimadzu TOC-L Analyzer
Generic Instrument Description	A Shimadzu TOC-L Analyzer measures DOC by high temperature combustion method. Developed by Shimadzu, the 680 degree C combustion catalytic oxidation method is now used worldwide. One of its most important features is the capacity to efficiently oxidize hard-to-decompose organic compounds, including insoluble and macromolecular organic compounds. The 680 degree C combustion catalytic oxidation method has been adopted for the TOC-L series. http://www.shimadzu.com/an/toc/lab/toc-l2.html

Dataset-specific Instrument Name	Absorbance spectrometer, Horiba Scientific, Piscataway, New Jersey, USA
Generic Instrument Name	Spectrophotometer
Dataset-specific Description	FDOM analysis: Horiba Aqualog scanning fluorometer with 150 W Xe excitation lamp, Peltier-cooled CCD emission detector, and a simultaneous absorbance spectrometer, Horiba Scientific, Piscataway, New Jersey, USA
Generic Instrument Description	An instrument used to measure the relative absorption of electromagnetic radiation of different wavelengths in the near infra-red, visible and ultraviolet wavebands by samples.

Dataset-specific Instrument Name	Technicon Auto Analyzer II, SEAL Analytical Inc., Mequon, Wisconsin, USA
Generic Instrument Name	Technicon AutoAnalyzer II
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.

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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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