

# Surface water and *N.digitalis* inhalent/exhalent water sample nutrient and bacterioplankton metadata of Looe Key and Wonderland Reef conducted in 10-15 December 2023

**Website:** <https://www.bco-dmo.org/dataset/907559>

**Data Type:** Other Field Results

**Version:** 1

**Version Date:** 2024-05-02

## 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 sessile filter-feeders that can process vast amounts of water and are known to influence the chemistry of the surrounding seawater. In areas where sponges are abundant, there may be a unique nutrient profile or 'reef signal' produced by the metabolism of the reef benthic community and sponges may contribute significantly to this 'signal'. This work provides an initial test of such a hypothesis, specifically that sponges can influence the dissolved nutrient profile in the overlying seawater. We analyzed differences in dissolved nutrient concentration (total organic carbon and dissolved nitrogen, inorganic nutrients, fluorescent dissolved organic matter (fDOM), and metabolites) and bacterioplankton cell density in surface seawater between two Florida Key coral reefs with different benthic communities. Additionally, we analyzed the processing of these nutrients by one species of sponge, *Niphates digitalis*, observed at both sites. While picoplankton abundances and inorganic nutrients were not different between sites, the surface water at Wonderland reef was characterized by elevated humic-like fDOM components and concentrations of certain metabolites such as aromatic amino acids relative to Looe Key. There is also higher similarity in the metabolite profile between Looe Key reef and Wonderland reef compared to the surface water away from the reef. There was not corresponding net production of the quantified metabolites by *N. digitalis* and overall little processing of dissolved organic nutrients by this sponge species. These results provide initial support for a 'reef signal' in metabolite profiles and there may be an impact of sponge abundance on the nutrient profile at Wonderland. However, the sponge *N. digitalis* is likely not a major contributor to the dissolved organic nutrient pool. Overall, these results have implications for better understanding the influence of the benthic community on coral reef nutrient dynamics.

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## Coverage

**Spatial Extent:** N:24.5605 E:-81.4 S:24.5346 W:-81.5039

**Temporal Extent:** 2020-12-09 - 2020-12-15

## Methods & Sampling

Looe Key Reef, Florida Keys National Marine Sanctuary. Wonderland Reef, Florida Keys National Marine Sanctuary. Various locations and depths are included in the metadata set.

Inhalant/exhalant water samples were taken from the sponge *N. digitalis*, at each reef using a modified version of the vacuSIP (Morganti et al. 2016). Briefly, acid cleaned and combusted bottles were negatively pressurized and sealed. PEEK tubing lines were connected to the pressurized bottles via a needle, and this pulled seawater from the opening of the sponge ("exhalant") or nearby seawater ("inhalant") at a slow and steady rate to fill the bottles. Surface seawater samples from Looe Key (n=4) and Wonderland Reef (n=2) were collected from each reef's surface water ("reef"), 0.5 km from the reef ("mid"), and 1.0 km from the reef seaward ("away"). Nutrient data from the seawater samples were compiled and organized by reef, and by distance from the reef in an Excel file. All surface and inhalant/exhalant water samples were filtered through 0.2 µm Teflon Omnipore filters. Filtrate was then saved for nutrients and the rest was acidified and saved for organic nutrient analysis. From this filtrate, inorganic nutrient, fluorescent Dissolved Organic Matter (fDOM), and targeted and untargeted metabolomics data were collected. Flow cytometry of phytoplankton and bacteria data was collected from pre-filtered seawater and preserved in 0.5% paraformaldehyde final concentration. Seawater for total organic carbon (TOC) was also collected prior to filtration and was acidified to ~pH 2 using concentrated HCl. Metabolomics and TOC analysis were performed at Woods Hole Oceanographic Institution at the Mass Spectrometry Facility. Dissolved Organic Matter Extraction: 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.

### Targeted Metabolite Analysis by UPLC-MS:

DOM extracts were reconstituted in 200 µl MilliQ water with 50 ng/ml isotopically-labeled injection standards d 2 biotin, d 6 succinic acid, d 4 cholic acid, and d 7 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<sup>-1</sup>, 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.

### Flow Cytometry:

Samples were shipped them to the Center for Aquatic Cytometry where they were stored at -80 C until analysis. Picophytoplankton (less than 3 µm) and nanophytoplankton (3-20 µm) were analyzed using a slight modification of the method described in Lomas et al., 2010. Immediately after thawing at room temperature, 300-400 µl of sample was prescreened through 70 µm mesh and run at a flow rate of 1 µl sec<sup>-1</sup>. Particles were excited with a 488 nm blue laser and data acquisition was triggered on red fluorescence. Signals were recorded from detectors with bandpass filters for forward scatter (FSC), right angle light scatter (SSC) and fluorescence emission in red (692/80 nm) indicative of chlorophyll a, and orange (593/52 nm) for phycoerythrin. Data files were analyzed from logarithmic dot plots based on fluorescence and characteristic light scattering properties (DuRand & Olson, 1996) using FlowJo 10.6 software (Becton Dickinson & Company, San Jose, CA, USA). Total pico and nano phytoplankton populations were identified based upon cell size and red fluorescence. Phycoerythrin containing cell populations were determined by orange fluorescence. Based upon these gating criteria, the number of cells in each identified population was enumerated and converted to cell abundances using the processed sample volume and adjusted for dilution by preservative.

For total bacteria analysis, samples were thawed, diluted 1:10 with Tris EDTA (TE) Buffer pH 8.0 and stained using a 10x working stock of SYBR Green I Nucleic Acid Stain (Thermofisher Scientific, USA) at room temperature in the dark for 15 min using the protocol of Marie et al. (2005). At a flow rate of 0.5 µl sec<sup>-1</sup>, 180

µl of the diluted sample was run. Particles were excited with a 488 nm blue laser and data acquisition was triggered on green fluorescence. Signals were recorded from detectors with bandpass filters for forward scatter (FSC), right angle light scatter (SSC) and fluorescence emission in green (525/35nm). Data files were analyzed from two logarithmic scatter plots based on fluorescence and characteristic light scattering properties. Total bacteria counts were identified based on size and presence of green fluorescence and counts were converted to cell abundances using the volume of sample processed including adjustments for preservation, dilution and staining.

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-Naphthyl)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, &quot;Nitrogen Ammonia&quot;, Method 350.1 (Colorimetric, Automated Phenate) A detailed description of the continuous segmented flow procedures used can be found in Gordon et. al. (1994).

Samples for TOC analysis were stored at 4°C until analysis with a Shimadzu TOC-VCSH total organic carbon analyzer (Longnecker *et al.*, 2015).

## 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 EI-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 micromolar concentrations using the formula weight for each metabolite.

Issue with contamination of samples when processing targeted metabolites, positive reading not used in data analysis. – Not needed for targeted dataset

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## Data Files

File
<b>907559_v1_nutrients.csv</b> (Comma Separated Values (.csv), 14.95 KB) MD5:76f5766fe8408009eae3de1d11724050
Primary data file for dataset 907559

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## Related Publications

Agrawal, S., Kumar, S., Sehgal, R., George, S., Gupta, R., Poddar, S., Jha, A., & Pathak, S. (2019). EI-MAVEN: A Fast, Robust, and User-Friendly Mass Spectrometry Data Processing Engine for Metabolomics. *Methods in Molecular Biology*, 301–321. [https://doi.org/10.1007/978-1-4939-9236-2\\_19](https://doi.org/10.1007/978-1-4939-9236-2_19)

*Methods*

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](https://doi.org/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*

Chambers, M. C., Maclean, B., Burke, R., Amodei, D., Ruderman, D. L., Neumann, S., ... Mallick, P. (2012). A cross-platform toolkit for mass spectrometry and proteomics. *Nature Biotechnology*, 30(10), 918–920.

doi:[10.1038/nbt.2377](https://doi.org/10.1038/nbt.2377)

*Software*

Dittmar, T., Koch, B., Hertkorn, N., & Kattner, G. (2008). A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater. *Limnology and Oceanography: Methods*, 6(6), 230–235. doi:[10.4319/lom.2008.6.230](https://doi.org/10.4319/lom.2008.6.230)

*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.

*Methods*

Grasshoff, K., Kremling, K., and Ehrhardt, M. (1983). *Methods of Seawater Analysis*. Verlag Chemie, Florida

*Methods*

Lomas, M. W., Steinberg, D. K., Dickey, T., Carlson, C. A., Nelson, N. B., Condon, R. H., & Bates, N. R. (2010). Increased ocean carbon export in the Sargasso Sea linked to climate variability is countered by its enhanced mesopelagic attenuation. *Biogeosciences*, 7(1), 57–70. <https://doi.org/10.5194/bg-7-57-2010>

*Methods*

Longnecker, K., Kido Soule, M. C., & Kujawinski, E. B. (2015). Dissolved organic matter produced by *Thalassiosira pseudonana*. *Marine Chemistry*, 168, 114–123. doi:[10.1016/j.marchem.2014.11.003](https://doi.org/10.1016/j.marchem.2014.11.003)

*Methods*

Marie, D., Simon, N., and Vaultot, D. (2005). “Phytoplankton cell counting by flow cytometry,” in *Algal culturing techniques*. Ed. R. A. Andersen (San Diego: Academic Press), 253–267.

*Methods*

Morganti, T., Yahel, G., Ribes, M., & Coma, R. (2016). VacuSIP, an Improved InEx Method for *In Situ* Measurement of Particulate and Dissolved Compounds Processed by Active Suspension Feeders. *Journal of Visualized Experiments*, 114. <https://doi.org/10.3791/54221>

*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*

Vaultot, D., Courties, C., & Partensky, F. (1989). A simple method to preserve oceanic phytoplankton for flow cytometric analyses. *Cytometry*, 10(5), 629–635. <https://doi.org/10.1002/cyto.990100519>

*Methods*

## Parameters

Parameter	Description	Units
SampleID	unique sample indentify	unitless
Site	site of sample collection, either Looe Key (Looe) or Wonderland Reef (Wonderland)	unitless
Sample_Type	the site and distance at which each surface water sample was collected, reef= reef surface water, mid= 0.5km seaward of reef, away= 1.0km seaward of reef and/or the type of water sample taken from <i>N.digitalis</i> (inhalant or exhalant).	unitless
Coordinates	coordinates of surface water samples and of <i>N.digitalis</i> water samples.	degrees minuntes seconds
latitude	latitude of surface water samples, south is negative	decimal degrees
longitude	longitude of surface water samples, west is negative	decimal degrees
Water_Depth	depth of sea water in feet below surface water collection site	Feet of seawater (fsw)
Collection_Date	date of the sample collection	unitless
PO4	concentration of phosphate per liter of surface water sample	micromolar per liter (uM/L)
N_N	concentration of nitrite + nitrate per liter of surface water sample	micromolar per liter (uM/L)
Silica	concentration of silica per liter of surface water sample	micromolar per liter (uM/L)
NO2	concentration of nitrite per liter of surface water sample	micromolar per liter (uM/L)
NH4	concentration of ammonium per liter of surface water sample	micromolar per liter (uM/L)
NH4_N_N	concentration of total nitrogen (nitrate, nitrite, and ammonium) per liter of surface water sample	micromolar per liter (uM/L)
UVHumicLike	Coble Peak A (Ultra Violet Humic-like)	Raman units of water (RU)
MarineHumicLike	Coble Peak M (Marine Humic-like)	Raman units of water (RU)
VisibleHumicLike	Coble Peak C (Visible Humic-like)	Raman units of water (RU)
TryptophanLike	Coble Peak T (Tryptophan-like)	Raman units of water (RU)

TyrosineLike	Coble Peak B (Tyrosine-like)	Raman units of water (RU)
PhenylalanineLike	Coble Peak F (Phenylalanine-like)	Raman units of water (RU)
FulvicAcidLike	Peak D (Stedmon, 2003)	Raman units of water (RU)
TOC	Concentration of dissolved organic carbon	micromolar per liter (uM)/L
TN	Total Nitrogen	micromolar per liter (uM)/L
anthranilate	anthranilate metabolite concentration	picograms (pg)
phenylalanine	phenylalanine metabolite concentration	picograms (pg)
inosine	inosine metabolite concentration	picograms (pg)
adenosine	adenosine metabolite concentration	picograms (pg)
4_aminobenzoic_acid	4-aminobenzoic acid metabolite concentration	picograms (pg)
tyrosine	tyrosine metabolite concentration	picograms (pg)
4_methyl_2_oxopentanoic_acid	4-methyl-2-oxopentanoic acid metabolite concentration	picograms (pg)
S_5_adenosyl__L_homocysteine	S-(5'-adenosyl)-L-homocysteine metabolite concentration	picograms (pg)
desthiobiotin	desthiobiotin metabolite concentration	picograms (pg)
taurocholic_acid	taurocholic acid metabolite concentration	picograms (pg)
glutathione_oxidized	glutathione oxidized metabolite concentration	picograms (pg)
pantothenic_acid	pantothenic acid metabolite concentration	picograms (pg)
tryptophan	tryptophan metabolite concentration	picograms (pg)
n_acetyl_muramic_acid	n-acetyl muramic acid metabolite concentration	picograms (pg)
4_hydroxybenzoic_acid	4-hydroxybenzoic acid metabolite concentration	picograms (pg)
riboflavin	riboflavin metabolite concentration	picograms (pg)
chorismate	chorismate metabolite concentration	picograms (pg)
3_3_dimethyl_2oxobutanoic_acid	3,3-dimethyl-2oxobutanoic acid metabolite concentration	picograms (pg)

5_hydroxy_L_tryptophan	5-hydroxy-L-tryptophan metabolite concentration	picograms (pg)
tryptamine	tryptamine metabolite concentration	picograms (pg)
4_Methyl_5_Thiazoleethanol	4-Methyl-5-Thiazoleethanol metabolite concentration	picograms (pg)
TotPhytoCount	total phytoplankton count	count
SynCount	total Synechococcus count	count
ProCount	total Prochlorococcus count	count
TotPhyto_Conc	total phytoplankton concentration	cells per milliliter (mL-1)
Syn_Conc	Synechococcus concentration (mL-1)	cells per milliliter (mL-1)
Pro_Conc	Prochlorococcus concentration (mL-1)	cells per milliliter (mL-1)
TotBact_Count	Total Bacteria count	count
TotBact_Conc	Total Bacteria concentration (mL-1)	cells per milliliter (mL-1)

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## Instruments

<b>Dataset-specific Instrument Name</b>	Bio-Rad ZE5
<b>Generic Instrument Name</b>	Flow Cytometer
<b>Dataset-specific Description</b>	Preserved samples were analyzed using a Bio-Rad ZE5 with 405 nm, 488 nm, and 640 nm lasers activated.
<b>Generic Instrument Description</b>	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: <a href="http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm">http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm</a> )

<b>Dataset-specific Instrument Name</b>	Horiba Aqualog scanning fluorometer
<b>Generic Instrument Name</b>	Fluorescence Microscope Image Analysis System
<b>Dataset-specific Description</b>	Fluorometer was used to measure fDOM
<b>Generic Instrument Description</b>	A Fluorescence (or Epifluorescence) Microscope Image Analysis System uses semi-automated color image analysis to determine cell abundance, dimensions and biovolumes from an Epifluorescence Microscope. An Epifluorescence Microscope (conventional and inverted) includes a camera system that generates enlarged images of prepared samples. The microscope uses excitation ultraviolet light and the phenomena of fluorescence and phosphorescence instead of, or in addition to, reflection and absorption of visible light.

<b>Dataset-specific Instrument Name</b>	TSQ Vantage, Thermo Scientific
<b>Generic Instrument Name</b>	Quadrupole Mass Spectrometer
<b>Dataset-specific Description</b>	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).
<b>Generic Instrument Description</b>	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.

<b>Dataset-specific Instrument Name</b>	Technicon AutoAnalyzer II
<b>Generic Instrument Name</b>	Technicon AutoAnalyzer II
<b>Dataset-specific Description</b>	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.
<b>Generic Instrument Description</b>	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.



<b>Dataset-specific Instrument Name</b>	Accela Open Autosampler and Accela 1250 Pump, Thermo Scientific)
<b>Generic Instrument Name</b>	Ultra high-performance liquid chromatography
<b>Dataset-specific Description</b>	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).
<b>Generic Instrument Description</b>	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

Funding Source	Award
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-1924540</a>
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-1923962</a>

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