Isotopic analysis of ^{13}C and ^{15}N for sponges, coral, and zooxanthellae (family Symbiodiniaceae) used in a 'pulse-chase' experiment to examine the uptake of sponge-derived nutrients by the coral holobiont

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Data Type: Other Field Results, experimental
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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
These are raw data from isotopic analysis of ^{13}C and ^{15}N for sponges, coral, and zooxanthellae (family Symbiodiniaceae) used in a 'pulse-chase' experiment to examine the uptake of sponge-derived nutrients by the coral holobiont. Coral were collected from the Florida Keys National Marine Sanctuary and the experiments were carried out at the Climate and Acidification Ocean Simulator (CAOS) at Mote Marine Laboratory at Summerland Key, Florida, USA.

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Coverage

Spatial Extent: **N**:24.562747 **E**:-81.400455 **S**:24.558694 **W**:-81.503528
Temporal Extent: 2020-12 - 2021-05

Methods & Sampling

Sample Collection and Maintenance
Three coral species, 1 octocoral and 2 scleractinians, were utilized in this experiment. The octocoral, *Eunicea flexuosa*, was collected from Wonderland Reef (24.558694, -81.503528) within the Florida Keys National Marine

Sanctuary (FKNMS) under the state of Florida (FL) saltwater fishing permit (Permit number: I-H1R76333834 held by A.M. Reigel). Three axial branch tips were clipped from each of 10 healthy *E. flexuosa* colonies located at depths of ~5-8 meters (m). Branch tips were kept in seawater and immediately transported to the outdoor land-based nursery (Climate and Acidification Ocean Simulator (CAOS)) at Mote Marine Laboratory at Summerland Key, FL, USA where they were placed into a shaded, temperature- and pH-maintained flow-through tank and allowed to acclimate for ~24 hours. The two hard coral species, *Acropora cervicornis* and *Orbicella faveolata* were provided by Mote Marine Laboratory's field (*A. cervicornis;* Coordinates: 24.562747, -81.400455) and land-based (*O. faveolata*) nurseries as permitted under the FKNMS-2015-163-A3. All hard coral fragments were placed in the same flow-through CAOS tanks as the *E. Flexuosa* samples and allowed to acclimate for ~24 hours.

To develop a representative sponge community for the Florida Keys reefs, we collected 5 individuals of each of 6 sponge species (*Niphates digitalis*, Verongula rigida, *Aplysina fulva*, *Aplysina cauliformis*, *Xestospongia muta*, *Callyspongia aculeata*) from Wonderland Reef under FL saltwater fishing permit (Permit #: I-H1R76333834 held by A.M. Reigel). Sponges were kept in seawater and immediately transported to the lab where they were placed in a shaded CAOS flow-through tank to acclimate for ~24 hours. Corals and sponges were not in the same CAOS tanks during the acclimation period.

Stable Isotope Pulse-Chase Incubations
Following 24 hours of acclimation time, sponges (n=2 per species) and coral fragments (n=4-5 per species) were removed from their respective acclimation tanks and immediately placed into labeled, sterile WhirlPak© bags and stored in the -20º Celsius (C) freezer (i.e., initial, or T_0, samples). The remaining sponges were moved from the acclimation tank to a 'pulse' tank for a 3-hour incubation. The 'pulse' tank was pre-filled with 84 liters of 0.22 micrometer (µm) filtered seawater spiked with isotopically labeled inorganic compounds in the following final concentrations: 0.1 grams per liter (g L^{-1}) sodium bicarbonate ($NaH^{13}CO_3$), 0.01g L^{-1} sodium nitrate ($Na^{15}NO_3$), and 0.01g L^{-1} ammonium chloride ($^{15}NH_4Cl$). At the end of the 3-hour 'pulse', a subset of sponges ('pulse' samples) was destructively sampled following the same procedure detailed above. The remaining enriched sponges (n=5 per species) were transferred to five separate flow-through tanks, such that each tank contained one individual of each sponge species, for a 1-hour 'rinse' period to flush away any labeled compounds that were not incorporated into the sponge tissue. At the end of the rinse, two fragments from each coral species were placed into each of eight experimental tanks, five enriched sponge-containing tanks, and three no-sponge control tanks, for the 6-hr 'chase'. During the duration of the 'chase', the flow-through system was turned off and a submersible aquarium pump was added to each tank to maintain circulation and gas exchange. A single fragment from each coral species was destructively sampled from each experimental tank twice during the 'chase': at the half point (T_3) and at the end (T_6). All enriched sponges were also destructively sampled at the end of the 'chase'.

Coral and Zooxanthellae Separations
To prepare for downstream analyses the coral fractions, host and zooxanthellae, were manually separated. Scleractinian fragments were thawed and airbrushed with an aerosolized jet of 0.22 micrometer (µm) filtered seawater to physically separate the coral tissue and skeleton and suspend the coral tissue material into a homogenate. To separate host tissue from zooxanthellae cells, the homogenate was centrifuged at 2000g for 3-5 minutes. Centrifugation formed a pellet comprised of zooxanthellae cells and a homogenate of host material. The host homogenate was pipetted into a separate sterile 50 milliliter (ml) Falcon tube. The homogenate, zooxanthellae pellet, and skeletal fragments were frozen and transported to Appalachian State University where they were stored at -20 degrees Fahrenheit (F) until further processing. At Appalachian State, host homogenates and zooxanthellae pellets were thawed and checked for purity. Impure fractions were combined, homogenized with a tissue homogenizer (maximum speed for ~15 seconds) to physically separate zooxanthellae cells from host tissue, and centrifuged (3000 x g, 6 minutes) to pellet the zooxanthellae cells. Separated fractions were combined with original fractions each time and checked for purity under the microscope. The process was repeated until at least 80% purity was reached.

E. flexuosa fractions were separated using a different process. First, the frozen coral branches were lyophilized (Labconco™ FreeZone™ Bulk Tray Dryer) for 22-24 hours, until they were completely dry. Following lyophilization, the axial skeleton was removed and the tissue was ground up using a mortar and pestle (note: separate mortar and pestle sets were used for control and enriched samples). The ground tissue was weighed and then rehydrated in 10ml of MilliQ water in a sterile 15ml Falcon tube. Very quickly following rehydration, the sclerites (skeletal fragments) sank to the bottom of the tube and the remaining host homogenate was pipetted into a new tube taking care not to transfer the sclerites. The homogenate was homogenized using a tissue homogenizer for ~15 seconds at maximum speed and centrifuged at 4000g for 5 minutes to separate the fractions. The centrifugation step was repeated as necessary until the host homogenate and zooxanthellae pellets were pure. Following both octocoral and scleractinian fraction separations, 50 microliters (µl) of pure zooxanthellae from each sample was transferred to a cryovial with 50µl of 10% paraformaldehyde (PFA) to fix

the cells and stored in the refrigerator for future zooxanthellae counts. The pure host homogenates and remaining zooxanthellae pellets were stored in the -20F freezer.

Stable Isotope Analysis
All sponge and zooxanthellae tissues and the octocoral host homogenate were lyophilized, weighed, and the dried material was homogenized with a mortar and pestle. Homogenized samples were decalcified via exposure to 12M HCL in desiccator chambers. Subsamples of ~1-2.5 milligrams (mg) of decalcified tissue from each sample were packed into silver cups. Scleractinian coral homogenates, made as previously detailed, contained filtered seawater, and if lyophilized, the salts could contaminate the host tissue. To remove the salts, host homogenates were passed through a sterile pre-weighed 1.6 µm GF/F filter that captured host material while seawater passed through and was discarded. The filters containing host material were frozen, dried overnight in a drying oven, weighed, decalcified as detailed above, and sent to the Marine Biological Laboratory (MBL) Stable Isotope Laboratory where they were also packed into silver cups.

Packed samples were analyzed at the MBL Stable Isotope Laboratory for $\delta^{15}N$ and $\delta^{13}C$ using a Europa 20-20 continuous-flow isotope ratio mass spectrometer interfaced with a Europa ANCA-SL elemental analyzer. The analytical precision based on replicate analyses of isotopically homogeneous international standards is +/- 0.1 ‰ for both $\delta^{15}N$ and $\delta^{13}C$ measurements.

Sampling and Analysis Dates
Following the 08-Dec-2020 sampling, all field-collected samples were acclimated in the aquarium system for 24 hours and the stable isotope 'pulse-chase' experiment was conducted over 11 hours on 09-Dec-2020. The host and zooxanthellae fractions were frozen and then the separations of the fractions from *A. cervicornis* and *O. faveolata* happened on 17-Dec-2020 and both fractions were frozen again. The other experimental steps occurred over several months in Spring 2021.

Known Issues/Problems
Four control (i.e., non-enriched) coral samples (Of_T6_7, AC_T3_3, AC_T6_1, and EF_T6_1) and their corresponding zooxanthellae have $\delta^{15}N$ and $\delta^{13}C$ values that suggest they may have been contaminated by the labeled isotopes at some point during the sample preparation process. These samples are found at the bottom of the data file and are identified as 'Control-Contaminated' in the Treatment column. These samples should be excluded or used with caution.

Data Processing Description

BCO-DMO Processing:
- converted dates to YYYY-MM-DD format;
- removed spaces and special characters from parameter names;
- created separate columns for latitude and longitude values.

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Data Files

File	
isotopes_raw_data.csv	(Comma Separated Values (.csv), 17.88 KB) MD5:993fa55e193a968155b922eac55002c8
Primary data file for dataset ID 889857	

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Related Datasets

IsRelatedTo

Reigel, A. M., Easson, C. G., Apprill, A., Freeman, C. J., Bartley, M. M., Fiore, C. L. (2022) **Coral fragment surface area calculations utilizing two methods (tin foil and Image J) and corresponding**

zooxanthellae count data. Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2022-09-21 doi:10.26008/1912/bco-dmo.880711.1 [view at BCO-DMO]
Relationship Description: Coral Surface Area and Zooxanthellae Count Data for the coral fragments included in dataset 889857 can be found in dataset 880711.

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Parameters

Parameter	Description	Units
Sample_ID	Sample ID number	unitless
Tissue_Type	Type of organism the tissue sample was obtained from: Sponge = tissue from a sponge; Zoox = tissue of coral-associated dinoflagellates obtained by centrifuge separation from coral host tissue; coral = coral tissue (includes material from coral host and + associated microbial community); blank = experimental control samples.	unitless
Species	Species name	unitless
Treatment	Control or enriched with stable isotopes. Those labeled with Control_Contaminated = control samples that appear to have been contained with enriched isotopes during the sample preparation process and should be removed or cautiously utilized by other researchers.	unitless
TimePoint	Time point. T0 = initial; pulse = at the end of the 3-hr pulse; T3= 3 hrs into the chase; T6 = 6 hrs into the chase.	unitless
Field_Sampling_Date	Date when the samples were collected from in-situ nursery (Acropora cervicornis samples) or natural reef habitat (Eunicea flexuosa and all sponge samples).	unitless
Latitude	Latitude coordinate of in-situ collection location	decimal degrees North
Longitude	Longitude coordinate of in-situ collection location	decimal degrees North
Tissue_Weight_mg	Weight of tissue used in analysis of isotope content	milligrams (mg)
d13C	Measure of the ratio of 13C to 12C in the sample as compared to the same ratio in the standard Vienna Pee Dee Belemnite (PDB) reference material PDB	parts per thousand (per mil, o/oo)
Carbon_umol	Total carbon content in the sample	micromoles (umol)

d15N	Measure of the ratio of 15N to 14N in the sample as compared to the same ratio in the standard Atmosphere (AIR) reference material	parts per thousand (per mil, o/oo)
Nitrogen_umol	Total nitrogen content in the sample	micromoles (umol)
Molar_C_N_ratio	Molar ratio of C:N in the sample	unitless

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Instruments

Dataset-specific Instrument Name	Europa ANCA-SL elemental analyzer
Generic Instrument Name	Elemental Analyzer
Generic Instrument Description	Instruments that quantify carbon, nitrogen and sometimes other elements by combusting the sample at very high temperature and assaying the resulting gaseous oxides. Usually used for samples including organic material.

Dataset-specific Instrument Name	Europa 20-20 continuous-flow isotope ratio mass spectrometer
Generic Instrument Name	Isotope-ratio Mass Spectrometer
Generic Instrument Description	The Isotope-ratio Mass Spectrometer is a particular type of mass spectrometer used to measure the relative abundance of isotopes in a given sample (e.g. VG Prism II Isotope Ratio Mass-Spectrometer).

Dataset-specific Instrument Name	Labconco FreeZone Bulk Tray Dryer
Generic Instrument Name	Lyophilizer
Generic Instrument Description	A lyophilizer, also known as freeze dryer or liofilizador, is a device that is used to freeze-dry material.

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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
NSF Division of Ocean Sciences (NSF OCE)	OCE-1924540
NSF Division of Ocean Sciences (NSF OCE)	OCE-1923962

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