Coral fragment surface area calculations utilizing two methods (tin foil and Image J) and corresponding zooxanthellae count data

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Version: 1
Version Date: 2022-09-21

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
This dataset presents coral fragment surface area calculations utilizing two methods (tin foil and Image J) and corresponding zooxanthellae count data. Three coral species were utilized in this experiment: the octocoral, Eunicea flexuosa, and two hard coral species, Acropora cervicornis and Orbicella faveolata.

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Coverage

Spatial Extent: **N**:24.562747 **E**:-81.400455 **S**:24.558694 **W**:-81.503528
Temporal Extent: 2020-12-09

Methods & Sampling

Sample Collection and Maintenance
Three coral species (one octocoral and two 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 FL saltwater fishing permit (Permit #: 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. Branch tips were kept in seawater and immediately transported to the outdoor land-based

nursery (CAOS) at Mote Marine Laboratory at Summerland Key, FL 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.

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 um 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 ml Falcon tube. The homogenate, zooxanthellae pellet, and skeletal fragments were frozen and transported to Appalachian State University where they were stored at -20F 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 sec) to physically separate zooxanthellae cells from host tissue, and centrifuged (3000 x g, 6 min.) 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 ul of pure zooxanthellae from each sample was transferred to a cryovial with 50 ul 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.

Coral Surface Area Measurements
Coral fragment surface area was calculated following two well-documented methods: Image J measurements and the aluminum foil method (Marsh 1970). ImageJ coral surface area measurements were completed utilizing planar photography and ImageJ software (Schneider et al. 2012). For *A. cervicornis* and *O. faveolata*, fragment surface area was measured using the frozen, airbrushed skeleton for each sample, while for *E. flexuosa*, the entire branch, prior to lyophilization as detailed above, was used. To obtain images of *A. cervicornis* and *E. flexuosa*, the fragments were held at an upright position, similar to their natural growth direction, and photographed from four sides (rotated 90°). *O. faveolata* are dome-shaped mounding corals, so photographs were only taken from above. A ruler was held in alignment with the fragments for scaling purposes. Photographs were individually uploaded to ImageJ and pixel dimensions were set using the straight-line tool and ‘set scale’ option. Using the polygon tool to drag an outline around the perimeter of the fragment, the enclosed area was calculated with the ‘measure’ function (in cm^2). The area of all four sides was summed to estimate the surface area of the skeletal fragment.

The aluminum foil method was completed as documented in Marsh (1970), but briefly, small pieces of aluminum foil were cut and carefully measured (cm^2) and weighed (g) to obtain a standard weight per unit of area (g/cm^2) for the foil. The foil used in this study had a standard weight per unit of area of 0.00618 g/cm^2. Coral skeletal fragments or octocoral branches were carefully covered with aluminum foil and all excess foil was trimmed until there was no overlap. Each foil wrapping was carefully removed from the fragment and weighed. The fragment surface area was calculated using the standard weight per unit area of the aluminum foil (Surface area of coral fragment = mass of coral fragment foil*0.006180525794 g/cm^2). The surface area

calculations from both methods were compared for all fragments to ensure a relative consensus between the methods. Results were largely similar between methods and we chose to utilize the surface area estimates from the ImageJ method for downstream analyses as it resulted in slightly higher surface area values, which we deemed more conservative.

Zooxanthellae Counts
To obtain counts of zooxanthellae cells per coral fragment, 20 ul of the fixed zooxanthellae cells from each sample were stained with either 2 ul (*E. flexuosa*) or 5 ul (*A. cervicornis* and *O. faveolata*) of Trypan Blue to increase their visibility under the microscope. If the zooxanthellae cells were too numerous to be counted accurately, they were further diluted with 175 ul of MilliQ water. 10 ul of the dilution was then placed into the cell counting chamber of a hemocytometer (Marienfeld Superior Neubauer Improved Chamber) and cells were counted in 4 quadrants following the standard Neubauer protocol as suggested by Electron Microscopy Sciences (https://www.emsdiasum.com/microscopy/technical/datasheet/68052-14.aspx). We used the following calculations to obtain whole fragment zooxanthellae counts and densities for each coral sample:

Zooxanthellae cells/ml of dilution = (Zooxanthellae cell count*Dilution Factor*10,000 cells/ml)/4 quadrants

Total zooxanthellae cells/fragment = Zooxanthellae cells/ml of dilution*Total Volume of host homogenate

Zooxanthellae cells/cm^2 of coral fragment = Total zooxanthellae cells/fragment*fragment surface area (cm^2)

Known Issues/Problems
Three coral samples (Ef-iso-T3-7, Ef-iso-T6-2, Ac-iso-I-2) were not utilized for zooxanthellae counts because either no zooxanthellae cells were seen in the sample or, during the host and symbiont separations, the ethanol cleaning step was not fully rinsed, which causes the zooxanthellae to burst and therefore they are unable to be counted. These details are noted in the "Notes" column of the dataset.

Data Processing Description

BCO-DMO Processing:
- replaced "NA" with "nd" as missing data value ("no data");
- converted dates to format YYYY-MM-DD:
- renamed fields to comply with BCO-DMO naming conventions;
- created separate columns for Latitude and Longitude;
- replaced commas with semi-colons in the Notes column.

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Data Files

File
coral_data.csv (Comma Separated Values (.csv), 17.18 KB) MD5:0abf8a7a324b1d619537d577ec8ed7da Primary data file for dataset ID 880711

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Related Datasets

IsRelatedTo

Reigel, A. M., Easson, C. G., Apprill, A., Freeman, C. J., Bartley, M. M., Fiore, C. L. (2023) **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.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2023-02-16 doi:10.26008/1912/bco-dmo.889857.1 [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 identifier	unitless
Coral_Species	Species name	unitless
TimePoint	Time point of the experiment. T0 = initial, T3= 3 hrs into the chase, T6 = 6 hrs into the chase	unitless
Treatment	Control or enriched with stable isotopes	unitless
Field_Sampling_Date	Field sampling date in format YYYY-MM-DD	unitless
Latitude	Latitude of field sampling site	degrees North
Longitude	Longitude of field sampling site	degrees East
Skeletal_fragment_diameter	Skeletal fragment diameter	centimeters (cm)
Skeletal_fragment_length	Skeletal fragment length	centimeters (cm)
ImageJ_side_1	Surface area of side 1 of coral fragment	square centimeters (cm^2)
ImageJ_side_2	Surface area of side 2 of coral fragment	square centimeters (cm^2)
ImageJ_side_3	Surface area of side 3 of coral fragment	square centimeters (cm^2)
ImageJ_side_4	Surface area of side 4 of coral fragment	square centimeters (cm^2)
ImageJ_total_surface_area	Combined surface area of sides 1-4 from ImageJ	square centimeters (cm^2)
Mass_of_tin_foil	Mass of tinfoil used to wrap skeletal fragment	grams (g)
Foil_Fragment_Surface_Area	Surface area of coral fragment as determined by the tin foil method. Weight of tinfoil/ standard weight per cm2 of tin foil (0.006180525794 g/cm2)	square centimeters (cm^2)
Zooxanthellae_Cell_Count	Zooxanthellae cells count, counted using a hemocytometer	number of cells
Quadrants_counted	Number of grids counted on the hemocytometer	number of grids

Total_Volume_of_host_homogenate	Total volume of host homogenate	milliliters (mL)
Volume_of_fixed_zooxanthellae_homogenate_in_Dilution	Volume of fixed zooxanthellae homogenate in dilution	milliliters (mL)
Volume_of_Trypan_Added_to_Dilution	Volume of Trypan added to dilution	milliliters (mL)
Volume_Of_MilliQ_Water_added_to_Dilution	Volume of MilliQ water added to dilution	milliliters (mL)
Total_volume_added_to_dilution	Total volume (Trypan + MilliQ) added to dilution	milliliters (mL)
Dilution_Factor	Fixed zoox volume/Total vol added to dilution	milliliters (mL)
Zoox_cells_per_mL_dilution	(Zooxanthellae cell count*dilution factor*10000 cells/ml standard for hemocytometer)/Grids Counted	cells per milliliter (cells/mL)
Zoox_cells_per_fragment	Total zoo cells in host homogenate Zoox cells/ml*Total volume host homogenate	cells per milliliter (cells/mL)
Surface_area	Total surface area from ImageJ	square centimeters (cm^2)
Cells_per_cm2	Total zoox cells in host homogenate*surface area (ImageJ)	cells per square centimeter (cells/cm2)
Notes	Additional notes from zooxanthellae counting	unitless

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Instruments

Dataset-specific Instrument Name	
Generic Instrument Name	Centrifuge
Generic Instrument Description	A machine with a rapidly rotating container that applies centrifugal force to its contents, typically to separate fluids of different densities (e.g., cream from milk) or liquids from solids.

Dataset-specific Instrument Name	Labconco™ FreeZone™ Bulk Tray Dryer
Generic Instrument Name	Drying Oven
Generic Instrument Description	a heated chamber for drying

Dataset-specific Instrument Name	Marienfeld Superior Neubauer Improved Chamber
Generic Instrument Name	Hemocytometer
Generic Instrument Description	A hemocytometer is a small glass chamber, resembling a thick microscope slide, used for determining the number of cells per unit volume of a suspension. Originally used for performing blood cell counts, a hemocytometer can be used to count a variety of cell types in the laboratory. Also spelled as "haemocytometer". Description from: http://hlsweb.dmu.ac.uk/ahs/elearning/RITA/Haem1/Haem1.html.

Dataset-specific Instrument Name	mortar and pestle
Generic Instrument Name	Homogenizer
Generic Instrument Description	A homogenizer is a piece of laboratory equipment used for the homogenization of various types of material, such as tissue, plant, food, soil, and many others.

Dataset-specific Instrument Name	
Generic Instrument Name	Microscope - Optical
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of reflection and absorption of visible light. Includes conventional and inverted instruments. Also called a "light microscope".

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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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