Proximate biochemistry of sponge species collected in 2017 and 2018 across the Caribbean Basin in Curacao, Belize, Grand Cayman, St. Croix

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Project
» Collaborative Research: Dimensions: Evolutionary Ecology of Sponges and Their Microbiome Drives Sponge Diversity on Coral Reefs (DimensionsSponge)

Program
» Dimensions of Biodiversity (Dimensions of Biodiversity)

Contributors	Affiliation	Role
Slattery, Marc	University of Mississippi	Principal Investigator
Gochfeld, Deborah J.	University of Mississippi	Co-Principal Investigator
York, Amber D.	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
Proximate biochemistry of sponge species collected in 2017 and 2018 across the Caribbean Basin in Curacao, Belize, Grand Cayman, St. Croix. These data were published in Clayshulte Abraham et al. (2021).

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Coverage

Spatial Extent: **N**:19.5656 **E**:-64.7722 **S**:12.1294 **W**:-88.2522
Temporal Extent: 2017-03 - 2018-08

Methods & Sampling

Sample collection

To assess the effect of the environment on sponge biochemical and energetic content, individuals (n = 2-21) of *Agelas conifera, Agelas tubulata, Amphimedon compressa, Aplysina cauliformis, Niphates amorpha, Niphates erecta,* and *Xestospongia muta* were collected at 15 m depth from 2-4 sites at each of 4 locations across the broader Caribbean Basin: Belize (May 2017), Curaçao (March 2017) , Grand Cayman (January 2018), St. Croix (August 2018). Although not every species was found in every region, target species were generally common and were selected to represent a range of antipredator chemical defenses (defended, undefended, variably defended), relative microbial abundance (HMA=high microbial abundance, LMA=low

microbial abundance) and relative abundance of photoautotrophic symbionts (high, intermediate, low).

Tissue samples were excised from individual sponges *in situ* using scissors, placed into individual resealable plastic bags, kept submerged in seawater in a shaded cooler, and returned to shore where they were frozen at -20°C for transport. In the lab, sample wet mass and volume were recorded, and samples were freeze dried to determine dry mass, and ground to a powder.

Proximate Biochemical Analysis

The proximate biochemical composition (PBC) of sponge tissue was quantified as described in Clayshulte Abraham et al. (in press). Briefly, carbohydrates were extracted from 10 mg of ground freeze-dried tissue in 5% trichloroacetic acid (TCA) and concentration was determined using the phenol-sulfuric acid method in microplate format, as described in Masuko et al. (2005). Absorbance was measured using a BioTek Synergy HT Multi-Detection Microplate Reader. The concentration of carbohydrates in samples was calculated relative to a glucose standard curve.

Protein was extracted from ground freeze-dried tissue in 1 M sodium hydroxide (NaOH) and the soluble protein concentration was analyzed using the Bradford Method (Bradford, 1976). Absorbance was measured using an Eppendorf Biophotometer. Soluble protein concentration in sponge samples was calculated relative to a standard curve using Bovine Serum Albumin (BSA).

Lipids were extracted using a modified version of the protocol described by Freeman et al. (1957). Briefly, ground freeze-dried tissue was sonicated, chloroform:methanol solution, and filtered into a conical tube containing distilled water. The organic layer was then pipetted into a pre-weighed vial, and this process was repeated three times. The organic solvent was then evaporated via vacuum centrifugation, and the final mass of dry lipid was recorded.

Ash was measured using methods in McClintock et al. (1991). Briefly, ground freeze-dried sponge tissue was placed into a pre-weighed foil pan, ashed at 500°C in a muffle furnace for 5 h, and the final mass of ash was recorded.

Refractory material was calculated by subtracting the combined masses of all of the measured components (carbohydrates, soluble protein, lipids, and ash) from the total sponge tissue mass to obtain ash-free dry weight (AFDW).

The proportion of each biochemical constituent was calculated by dividing each constituent's mass by the sample's AFDW. The total sponge tissue energetic content was calculated by multiplying the proportional dry mass of each biochemical constituent by the kilojoule (kJ) coefficients detailed in Gnaiger & Bitterlich (1984). For purposes of energetic calculations, refractory material was assumed to consist predominantly of insoluble protein.

To quantify the concentration of total sponge extract obtained from each sample, ground freeze-dried sponge tissue was extracted in methanol:methylene chloride and sonicated. This was repeated twice, and the three extracts were combined in a pre-weighed vial. The solvent was removed via vacuum centrifugation to yield extract dry mass. Natural extract concentrations were calculated by based on initial volume to dry mass relationships for each sample.

Data Processing Description

See Clayshulte Abraham et al. (2021) for data analysis and results that used this dataset.

BCO-DMO data manager processing notes:
* Data submitted in Excel file DOB_biochemistry_to_upload.xlsx sheet 1 extracted as csv.
* Latitude and longitude rounded from 8 to 3 decimal places
* Year and month added to dataset from metadata for the regions.
* Species names checked using the World Register of Marine Species Taxa Match tool. A species list with taxonomic identifiers (AphiaID,TSN,LSID) was attached as a supplemental file. Matched all names in dataset exactly on 2022-01-14.

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Data Files

File
sponge_biochem.csv (Comma Separated Values (.csv), 52.71 KB) MD5:d45dea9377955176b756b2cd2bb58d0b Primary data file for dataset ID 868047

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Supplemental Files

File
Species List filename: species_list.csv (Comma Separated Values (.csv), 538 bytes) MD5:23c75b63dea0b12ad459281fe23751b2 This data table is a species list for the dataset "Biochemistry of sponges" with taxonomic identifiers (AphiaID, TSN, LSID). The genus and species columns provided in the dataset "biochemistry of sponges" were checked using the World Register of Marine Species taxa match tool (on 2022-01-14) and matched exactly to the names in this species list. Columns in this table are: ScientificName, AphiaID, TSN, LSID

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Related Publications

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72(1-2), 248–254. doi:10.1016/0003-2697(76)90527-3 https://doi.org/10.1006/abio.1976.9999
Methods

Clayshulte Abraham, A., Gochfeld, D. J., Macartney, K., Mellor, A., Lesser, M. P., & Slattery, M. (2021). Biochemical variability in sponges across the Caribbean basin. Invertebrate Biology, 140(3). doi:10.1111/ivb.12341
Results

Freeman, N. K., Lindgren, F. T., Ng, Y. C., & Nichols, A. V. (1953). Infra-red spectra of some lipoproteins and related lipides. Journal of Biological Chemistry, 203(1), 293-304. PMID: 13069513. https://pubmed.ncbi.nlm.nih.gov/13069513/
Methods

Gnaiger, E., & Bitterlich, G. (1984). Proximate biochemical composition and caloric content calculated from elemental CHN analysis: a stoichiometric concept. Oecologia, 62(3), 289–298. doi:10.1007/bf00384259 https://doi.org/10.1007/BF00384259
Methods

Masuko, T., Minami, A., Iwasaki, N., Majima, T., Nishimura, S.-I., & Lee, Y. C. (2005). Carbohydrate analysis by a phenol–sulfuric acid method in microplate format. Analytical Biochemistry, 339(1), 69–72. doi:10.1016/j.ab.2004.12.001
Methods

McClintock, J. B., Heine, J., Slattery, M., & Weston, J. (1991). Biochemical and energetic composition, population biology, and chemical defense of the antarctic ascidian Cnemidocarpa verrucosa lesson. Journal of Experimental Marine Biology and Ecology, 147(2), 163–175. doi:10.1016/0022-0981(91)90180-5
Methods

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Parameters

Parameter	Description	Units
Genus	Sponge genus	unitless
Species	Sponge species	unitless
Chemical_defense	Defended, undefended, or variable levels of chemical defense	unitless
Microbial_abundance	High microbial abundance (HMA) or low microbial abundance (LMA)	unitless
Photosymbiont_abundance	Low, high, or intermediate relative photosymbiont abundance	unitless
Region	Region where collected	unitless
Site	Site location of collection	unitless
Latitude	Latitude (north is positive)	decimal degrees
Longitude	Longitude (east is positive)	decimal degrees
carbohydrates_AFDW	Carbohydrates (AFDW = ash-free dry weight). Milligrams of carbohydrates per gram of ash free dry weight of sponge.	milligrams per gram (mg/g)
soluble_protein_AFDW	Soluble protein (AFDW = ash-free dry weight). Milligrams of protein per gram of ash free dry weight of sponge	milligrams per gram (mg/g)
lipids_AFDW	Lipids (AFDW = ash-free dry weight). Milligrams of lipids per gram of ash free dry weight of sponge	milligrams per gram (mg/g)
ash	Ash. Milligrams of ash per gram of sponge	milligrams per gram (mg/g)
refractory_material_AFDW	Refractory material (AFDW = ash-free dry weight). Milligrams of refractory material per gram of ash free dry weight of sponge.	milligrams per gram (mg/g)
total_energetic_content	Total energetic content. kilojoules per gram of sponge.	kilojoules per gram (kJ/g)
total_energetic_content_AFDW	Total energetic content (AFDW = ash-free dry weight). kilojoules per gram of ash free dry weight of sponge.	kilojoules per gram (kJ/g)

crude_extract	Crude extract. Milligrams of crude extract per ml of sponge.	milligrams per milliliter (mg/ml)
year_month	Collection year and month in format yyyy-mm	unitless

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Instruments

Dataset-specific Instrument Name	BioTek Synergy HT Multi-Detection Microplate Reader
Generic Instrument Name	plate reader
Generic Instrument Description	Plate readers (also known as microplate readers) are laboratory instruments designed to detect biological, chemical or physical events of samples in microtiter plates. They are widely used in research, drug discovery, bioassay validation, quality control and manufacturing processes in the pharmaceutical and biotechnological industry and academic organizations. Sample reactions can be assayed in 6-1536 well format microtiter plates. The most common microplate format used in academic research laboratories or clinical diagnostic laboratories is 96-well (8 by 12 matrix) with a typical reaction volume between 100 and 200 uL per well. Higher density microplates (384- or 1536-well microplates) are typically used for screening applications, when throughput (number of samples per day processed) and assay cost per sample become critical parameters, with a typical assay volume between 5 and 50 µL per well. Common detection modes for microplate assays are absorbance, fluorescence intensity, luminescence, time-resolved fluorescence, and fluorescence polarization. From: http://en.wikipedia.org/wiki/Plate_reader, 2014-09-0-23.

Dataset-specific Instrument Name	Eppendorf Biophotometer
Generic Instrument Name	Spectrophotometer
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.

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Project Information

Collaborative Research: Dimensions: Evolutionary Ecology of Sponges and Their Microbiome Drives Sponge Diversity on Coral Reefs (DimensionsSponge)

Coverage: Curacao, Belize, Florida, Cayman Islands

NSF Award Abstract:
Coral reefs, the tropical rain forests of the marine environment, are under significant threat from a variety of stressors such as pollution, overfishing, coastal development and climate change. There is increasing interest

by the coral reef research community in the ecology and evolution of other groups of organisms besides corals on coral reefs with sponges being of particular interest. Sponges are a very old group of organisms essential to reef health because of their roles in nutrient cycling, providing food and homes for many other reef organisms, and their ability to synthesize diverse chemical compounds of ecological importance on the reef, and of interest to the biomedical community. Many of these important functions would not be possible without the symbiotic microbes (e.g., bacteria) that live within sponges. In this project, the investigators will examine relationships between the sponge host and its microbiome in the ecological roles described above. Like the human microbiome, understanding the sponge micobiome may be the key to understanding their ecology and biodiversity. The investigators will use a combination of classical ecological approaches combined with sophisticated biochemical and molecular analyses to unravel the role of the symbionts in the ecology and evolution of sponges. Both the University of New Hampshire and the University of Mississippi will provide training opportunities for undergraduate and graduate students as well as veterans and post-doctoral researchers, especially from underrepresented groups. Additionally, the investigators will develop unique outreach programs for public education on the importance of coral reef ecosystems.

The goal of this study is to examine the relationships between marine sponges and their microbiomes, and reveal the phylogenetic, genetic, and functional biodiversity of coral reef sponges across the Caribbean basin. This research will provide a better understanding of sponges as a major functional component of the biodiversity of coral reef communities. This transformative project will examine important paradigms relative to sponge communities worldwide that will provide unique insights into the integrative biodiversity of sponges on coral reefs and enhance our understanding of the ecology and evolution of this extensive, yet understudied, group of marine organisms. This is essential because sponges continue to emerge as the dominant taxon on many coral reefs, particularly following regional declines in coral cover over the last three decades, and their ecological importance to shallow coral reef communities is unequivocal. In addition, many marine sponges host a diverse assemblage of symbiotic microorganisms that play critical functional roles in nutrient cycling within sponges themselves and in the coral reef communities where they reside, and many sponges can potentially transfer photoautotrophically derived energy to higher trophic levels. As shallow coral reefs continue to decline, the phylogenetic, genetic, and functional diversity of coral reefs will increasingly be found in taxa other than scleractinian corals, such as soft corals and sponges. The investigators predict that co-evolution of the sponge host and microbiome leads to emergent functional properties that result in niche diversification and speciation of sponges. To assess this, they will quantify trophic modes (e.g., DOM and POC uptake, photo-autotrophy) of sponges in the Caribbean, as well as the production of chemical defenses. These character states will be analyzed in the context of the phylogenetic composition of the sponge hosts and their microbiomes, and the functional activities of the host and symbionts at the genetic level (i.e., transcriptomics and metatranscriptomics). These data will provide unique insights into the co-evolution of sponges and their microbiomes, and how these symbioses influence the functional attributes of sponges within coral reef communities.

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Program Information

Dimensions of Biodiversity (Dimensions of Biodiversity)

Website: http://www.nsf.gov/funding/pgm_summ.jsp?pims_id=503446

Coverage: global

(adapted from the NSF Synopsis of Program)
Dimensions of Biodiversity is a program solicitation from the NSF Directorate for Biological Sciences. FY 2010 was year one of the program. [MORE from NSF]

The NSF Dimensions of Biodiversity program seeks to characterize biodiversity on Earth by using integrative, innovative approaches to fill rapidly the most substantial gaps in our understanding. The program will take a broad view of biodiversity, and in its initial phase will focus on the integration of genetic, taxonomic, and functional dimensions of biodiversity. Project investigators are encouraged to integrate these three dimensions to understand the interactions and feedbacks among them. While this focus complements several core NSF programs, it differs by requiring that multiple dimensions of biodiversity be addressed simultaneously, to

understand the roles of biodiversity in critical ecological and evolutionary processes.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1638289

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