Microbial Symbionts, Carbon, and Nutrient Cycling in Caribbean Coral Reef Sponges from Conch Reef, Key Largo (Florida, USA) and Carrie Bow Cay (Belize) in June and July 2016

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Project
» Testing the sponge-loop hypothesis for Caribbean coral reefs (Sponge_Loop)

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Abstract
This dataset examines microbial symbionts, carbon, and nutrient cycling in Caribbean coral reef sponges collected from Conch Reef, Key Largo, Florida, USA and from Carrie Bow Cay, Belize in June and July 2016.

Table of Contents

Coverage

Spatial Extent: **N**:24.948 **E**:-80.453 **S**:16.8025 **W**:-88.07979
Temporal Extent: 2016-06-01 - 2016-07-19

Methods & Sampling

Sample Collection:
Ambient seawater and sponge tissue were collected from two geographically distant locations (~1203

kilometers apart): Conch Reef, Florida (24° 56.9' N, 80° 27.2′ W), and Carrie Bow Cay, Belize (16° 48.14' N, 88° 4.79′ W). Ten of the most common Caribbean coral reef sponges were sampled in Key Largo, Florida (Conch Reef), and eight of these species were sampled in Belize (Carrie Bow Cay) from depths of 13 to 23 meters in June and July 2016, respectively (see Supplemental File named "Microbial communities in sponge species" (Table_1_Finelli_Dataset.pdf)). Only apparently healthy sponge individuals (i.e., no evidence of disease, tissue damage, algal colonization, or epibionts) with a single osculum were sampled (except in the case of *Agelas tubulata* which had multiple oscula) (McMurray et al. 2018). All sponge tissue samples were collected in separate bags, preserved in 100% ethanol, and stored at -20°C until processing. Seawater samples (1 liter) were collected at each sampling site and day of sponge tissue sample collection, concentrated onto 0.2 micrometer (µm) filters, preserved in 100% ethanol, and stored at -20°C until processing.

Sponge barcoding
All ten sponge species represent common Caribbean coral reef species and were identified morphologically following Zea et al. (2014). To confirm identifications made in the field, PCR amplification of the partial mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified using the forward primer LCO1490 and reverse primer HCO2198 for species barcoding (Folmer et al. 1994). PCR amplification reactions contained 0.5 µl of each primer 10 µM, 12.5 µl (0.5 units) of MyTaq™ Red Mix DNA polymerase (Bioline), 1 µl of DNA template, and PCR water for a total reaction volume of 25 µl. The thermocycler conditions included an initial denaturation step (95 °C, 1 minute) followed by 35 cycles of denaturation (95 °C, 15 s), annealing (45 °C, 15 s), and extension (72 °C, 10 s), with a final extension step (72 °C, 1 min) and 6 °C hold. The COI amplicons were used in a sequencing PCR with BigDye version 3.1 (Applied Biosystems) and a thermocycler program consisting of an initial denaturation step (96 °C, 1min), 25 cycles of annealing (50 °C, 5 s), extension (60 °C, 4 min), and denaturation (96 °C, 10 s), followed by a final annealing (50 °C, 5 s), extension (60 °C, 4 min), and 10 °C hold. Amplicons were cleaned using BigDye® XTerminator™ Purification Kit (Thermo Fischer), following the manufacturer's protocol, and sequenced on an AB 3500 Gene Analyzer (Applied Biosystems) at the UNCW Center for Marine Science. Forward and reverse sequences were aligned in Geneious version 8.1.9 (Kearse et al. 2012) to create consensus sequences and compared to the GenBank database using the nucleotide Basic Local Alignment Search Tool (BLASTn). Sequence data were deposited in GenBank under the accession numbers MH297440 to MH297461.

Sample metadata
For each sponge tissue sample that was collected, metadata on sponge pumping rates, sponge volumes, and carbon fluxes were collected on in situ colonies (prior to tissue sampling) and processed as reported previously (McMurray et al. 2018). Briefly, sponge pumping rates were measured using an acoustic Doppler velocimeter (SonTek) (McMurray et al. 2014) and sponge tissue volume was estimated using measurements of the dimensions of each sponge (McMurray et al. 2018). Paired incurrent (ambient) and excurrent seawater samples (1.5 L) were collected via syringe and subsequently filtered (Whatman GF/F). POC on filters was quantified using a CE Elantech NC2100 elemental analyzer, and DOC in the filtrate of each sample was quantified using a Shimadzu TOC 5050 analyzer (McMurray et al. 2016). In Belize, an additional 40 mL of the filtrate from each incurrent and excurrent seawater sample was collected and stored frozen until quantification of NOx, NH4, and PO4 using a Bran + Luebbe AutoAnalyzer III following standard protocols (EPA 1997). The specific filtration rate (SFR, µmol C, or nutrient/s/L sponge) for each carbon and nutrient species (i.e., POC, DOC, NOx, NH4, and PO4) was calculated as: SFR ((Cin−Cex) x Q)/Vsponge where Cin and Cex are the incurrent and excurrent concentrations of each carbon pool or nutrient type (C/mL), Vsponge is the sponge tissue volume (L), and Q is the pumping rate for each sponge (mL/s); thus, positive values indicate net consumption and negative values indicate net production of a particular carbon pool or nutrient type.

DNA extraction and sequence processing
Ethanol-preserved tissue samples were dissected into 2 mm^3 cubes that included interior and exterior sponge tissue and were extracted using the DNeasy® Blood & Tissue Kit (Qiagen) following the manufacturer protocols. Partial (V4) 16S rRNA gene sequences were amplified using the 515f forward primer and 806r reverse primer (Caporaso et al. 2011) and sequenced on an Illumina MiSeq platform at Molecular Research LP (Shallowater, TX). Illumina sequence reads were processed in mothur v1.38.0 (Schloss et al. 2009) using a modified version of the bioinformatics pipeline described in Weigel and Erwin (2016). Briefly, raw sequences (n = 13.9 million) were demultiplexed, quality-filtered, aligned, classified, and clustered into operational taxonomic units (OTUs) at 97% sequence identity (nOTU = 25,712). Sequence libraries for each sample were subsampled to the lowest read count (n = 15,825), and all data analyses were based on the subsampled dataset. Sequence data were deposited in the Sequence Read Archive (SRA) for microbial data (16S ribosomal RNA gene sequences, Illumina MiSeq platform) of the National Center for Biotechnology under Accession number SRP142647 (https://www.ncbi.nlm.nih.gov/sra/SRP142647).

GenBank Database for sponge data (Mitochondrial cytochrome oxidase subunit I genes, Sanger platform). Accession numbers MH297440 to MH297461. https://www.ncbi.nlm.nih.gov/nuccore/?

term=Microbial+Symbionts%2C+Carbon+and+Nutrient+Cycling+in+Caribbean+Coral+Reef+Sponges

Data Processing Description

Data analysis
Microbial community diversity
Diversity statistics (Shannon-Weaver, OTU richness, Simpson) were calculated in mothur using OTU relative abundance data. Two-way nested analyses of variance (ANOVA) were used to test for significant differences in diversity indices for two factors: "source" (sponge species or seawater) and “location” (Key Largo or Belize) nested within source in JMP (version 12.0), followed by Tukey’s honest significant difference (HSD) tests to assess multiple post hoc comparisons of means.

Microbial community structure
Bray-Curtis similarity matrices were constructed using square root transformed OTU relative abundance data to give more even representation of rare and abundant taxa in community comparisons using Primer-e (version 6.1.11) and visualized in a cluster dendrogram and a two-dimensional non-metric multidimensional scaling (nMDS) plot. A permutational multivariate analysis of variance (PERMANOVA, version 1.0.1) was conducted to test for significant differences in microbial community structure across two factors: “source” and “location” nested within source, with significance determined by Monte Carlo asymptotic P values.

OTU-level analyses
Similarity percentage (SIMPER) analysis was used to identify individual OTUs driving the overall dissimilarity between microbial communities within each sponge species, using OTU relative abundance matrices and a cumulative dissimilarity cutoff percentage of 0.70. Significant differences in OTU relative abundances were assessed using Metastats (White et al. 2009) in mothur with 1000 permutations. OTUs of interest were then taxonomically identified and compared to OTUs identified in nutrient correlations (see below).

Correlations between microbial community diversity and carbon/nutrient flux
Spearman rank-order correlations were conducted in SigmaPlot (version 11) to assess relationships between microbial diversity indices (Shannon-Weaver, OTU richness, Simpson) and carbon/nutrient flux data (DOC, POC, NOx, NH4, and PO4). Correlations between microbial community structure and carbon/nutrient flux Distance-based linear models (DistLM) were conducted in Primer-e to assess correlations between microbial community (Bray-Curtis) similarity and carbon/nutrient flux data (DOC, POC, NOx, NH4, and PO4) and visualized with distance-based redundancy analysis (dbRDA) plots. Specifically, carbon and nutrient flux measurements estimated from specific filtration rates (SFR, µmol C, or nutrient/s/L sponge) were tested as marginal predictor variables for microbial community similarity within and among sponge species. Analyses were repeated using Bray-Curtis similarity matrices constructed from untransformed (raw) data, showing identical significance patterns and thus minimal impact of data transformation on statistical results. Analyses were also repeated using carbon and nutrient uptake (Cin–Cex) instead of specific filtration rates, showing similar statistical patterns when considering a metric not influenced by pumping rates. Inter-specific comparisons were conducted at three levels: all sponge species, within each sponge category (HMA, LMA), and between each pairwise species comparison. Pearson correlations were run in JMP to compare nutrient flux data (POC and NH4) in the form of SFR and OTU abundance counts for the first 1000 OTUs within each sponge species.

Functional gene PCR screening
To test for the presence of signature functional genes involved in nitrogen cycling processes (nitrification: ammonia monooxygenase, amoA/amoB; nitrogen fixation: nitrogenase, nifH; and denitrification: nitrite reductase, nirS), the following primer pairs and cited procedures were used: Arch-amoAF and Arch-amoAR (Francis et al. 2005), AmoBMF and AmoBMR (Calvo et al. 2004), nif1/2 and nif3/4 (Mohamed et al. 2008), nirS1F and nirS6R (Yang et al. 2012). For all sponge species, at least two replicates were tested for the presence of each functional gene, except for Aplysina archeri (only one sample available). If positive, all remaining replicates of that species were tested at both sites. If negative, the PCR was repeated twice for verification.

BCO-DMO Processing:
- Converted dates to format YYYY-MM-DD;
- Adjusted field/parameter names to comply with BCO-DMO naming conventions;
- Added a conventional header with dataset name, PI names, version date;
- Split lat/lon into separate columns and converted longitude from West to East to comply with BCO-DMO standards.

[table of contents | back to top]

Data Files

File
Sponge_Symbiont.csv (Comma Separated Values (.csv), 22.18 KB) MD5:55596f8cb415ee46282b705fbc96adaa Primary data file for dataset ID 876787

[table of contents | back to top]

Supplemental Files

File
Microbial communities in sponge species filename: Table_1_Finelli_Dataset.pdf (Portable Document Format (.pdf), 45.70 KB) MD5:03f04c257ca6fb0fecde82a8e78e5801 Measurements of microbial communities in 10 sponge species and ambient seawater showing sponge abundance category (HMA or LMA) and replicates per location (Conch Reef, Florida vs. Carrie Bow Cay, Belize).

[table of contents | back to top]

Related Publications

Calvó, L., & Garcia-Gil, L. J. (2004). Use of amoB as a new molecular marker for ammonia-oxidizing bacteria. Journal of Microbiological Methods, 57(1), 69–78. https://doi.org/10.1016/j.mimet.2003.11.019
Methods

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., Fierer, N., & Knight, R. (2010). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences, 108(supplement_1), 4516–4522. https://doi.org/10.1073/pnas.1000080107
Methods

EPA US. Methods for the determination of chemical substances in marine and estuarine environmental matrices. 2nd ed. Washington, DC: Edited by Agency USEP; 1997.
Methods

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular marine biology and biotechnology, 3(5), 294–299. PMID: 7881515. https://pubmed.ncbi.nlm.nih.gov/7881515/
Methods

Francis, C. A., Roberts, K. J., Beman, J. M., Santoro, A. E., & Oakley, B. B. (2005). Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. Proceedings of the National Academy of Sciences, 102(41), 14683–14688. doi:10.1073/pnas.0506625102
Methods

Gantt, S. E., McMurray, S. E., Stubler, A. D., Finelli, C. M., Pawlik, J. R., & Erwin, P. M. (2019). Testing the relationship between microbiome composition and flux of carbon and nutrients in Caribbean coral reef sponges. Microbiome, 7(1). https://doi.org/10.1186/s40168-019-0739-x
Results

JMP Statistical software (2021): JMP (pronounced "jump") is a suite of computer programs for statistical analysis developed by JMP, a subsidiary of SAS Institute. https://www.jmp.com/en_us/home.html https://hdl.handle.net/JMP
Software

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., … Drummond, A. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics, 28(12), 1647–1649. doi:10.1093/bioinformatics/bts199

Methods

McMurray, S. E., Johnson, Z. I., Hunt, D. E., Pawlik, J. R., & Finelli, C. M. (2016). Selective feeding by the giant barrel sponge enhances foraging efficiency. Limnology and Oceanography, 61(4), 1271–1286. doi:10.1002/lno.10287
Methods

McMurray, S., Pawlik, J., & Finelli, C. (2014). Trait-mediated ecosystem impacts: how morphology and size affect pumping rates of the Caribbean giant barrel sponge. Aquatic Biology, 23(1), 1–13. doi:10.3354/ab00612
Methods

McMurray, S., Stubler, A., Erwin, P., Finelli, C., & Pawlik, J. (2018). A test of the sponge-loop hypothesis for emergent Caribbean reef sponges. Marine Ecology Progress Series, 588, 1–14. https://doi.org/10.3354/meps12466
Results

Mohamed, N. M., Colman, A. S., Tal, Y., & Hill, R. T. (2008). Diversity and expression of nitrogen fixation genes in bacterial symbionts of marine sponges. Environmental Microbiology, 10(11), 2910–2921. https://doi.org/10.1111/j.1462-2920.2008.01704.x
Methods

Primer-e software: version 6 (2019, Primer-E, Ivybridge, UK) A core package for sharp, sophisticated nonparametric multivariate analysis. https://www.primer-e.com/
Software

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., … Weber, C. F. (2009). Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. Applied and Environmental Microbiology, 75(23), 7537–7541. doi:10.1128/aem.01541-09 https://doi.org/10.1128/AEM.01541-09
Software

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Software

SigmaPlot (Systat Software Inc.) Version 11: a proprietary software package for scientific graphing and data analysis that runs on Microsoft Windows.
Software

Weigel, B. L., & Erwin, P. M. (2016). Intraspecific Variation in Microbial Symbiont Communities of the Sun Sponge, Hymeniacidonheliophila, from Intertidal and Subtidal Habitats. Applied and Environmental Microbiology, 82(2), 650–658. https://doi.org/10.1128/aem.02980-15 https://doi.org/10.1128/AEM.02980-15
Methods

White, J. R., Nagarajan, N., & Pop, M. (2009). Statistical Methods for Detecting Differentially Abundant Features in Clinical Metagenomic Samples. PLoS Computational Biology, 5(4), e1000352. https://doi.org/10.1371/journal.pcbi.1000352
Software

Yang, Z., & Li, Z. (2012). Spatial distribution of prokaryotic symbionts and ammoxidation, denitrifier bacteria in marine sponge Astrosclera willeyana. Scientific Reports, 2(1). https://doi.org/10.1038/srep00528
Software

Zea, S., Henkel, T. P., & Pawlik, J. R. (2014). The Sponge Guide: a picture guide to Caribbean sponges. Available at https://spongeguide.uncw.edu/ [Verified January 2018].
Methods

[table of contents | back to top]

Related Datasets

IsRelatedTo

University of North Carolina Wilmington. Microbial Symbionts, Carbon and Nutrient Cycling in Caribbean Coral Reef Sponges. 2018/04. In: BioProject [Internet]. Bethesda, MD: National Library of Medicine (US), National Center for Biotechnology Information; 2011-. Available from: http://www.ncbi.nlm.nih.gov/bioproject/PRJNA453627. NCBI:BioProject: PRJNA453627.

[table of contents | back to top]

Parameters

Parameter	Description	Units
SRAStudy	NCBI SRA Study number	unitless
Submission	submission number	unitless
ProjectID	project identification number	unitless
Sample	unique identifier for each sample	unitless
BioSample	NCBI BioSample number	unitless
BioProject	NCBI BioProject number	unitless
Experiment	experiment number	unitless
sample_name	unique sample identifier	unitless
organism	organism	unitless
host	host sponge species or seawater	unitless
isolation_source	source of isolation	unitless
collection_date	date of sample collection in format YYYY-MM-DD	unitless
Latitude	latitude North of sample location	decimal degrees
Longitude	longitude East of sample location	decimal degrees
geo_loc_name	location of samples collected	unitless
Platform	platform used	unitless
Model	model of platform used	unitless
LibraryStrategy	library strategy used	unitless
LibrarySelection	library selection used	unitless
LibrarySource	source of library used	unitless
LibraryLayout	layout of library used	unitless

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	SonTek acoustic doppler velocimeter
Generic Instrument Name	Acoustic Doppler Velocimeter
Generic Instrument Description	ADV is the acronym for acoustic doppler velocimeter. The ADV is a remote-sensing, three-dimensional velocity sensor. Its operation is based on the Doppler shift effect. The sensor can be deployed either as a moored instrument or attached to a still structure near the seabed. Reference: G. Voulgaris and J. H. Trowbridge, 1998. Evaluation of the Acoustic Doppler Velocimeter (ADV) for Turbulence Measurements. J. Atmos. Oceanic Technol., 15, 272–289. doi: http://dx.doi.org/10.1175/1520-0426(1998)0152.0.CO;2

Dataset-specific Instrument Name	Illumina MiSeq platform
Generic Instrument Name	Automated DNA Sequencer
Generic Instrument Description	General term for a laboratory instrument used for deciphering the order of bases in a strand of DNA. Sanger sequencers detect fluorescence from different dyes that are used to identify the A, C, G, and T extension reactions. Contemporary or Pyrosequencer methods are based on detecting the activity of DNA polymerase (a DNA synthesizing enzyme) with another chemoluminescent enzyme. Essentially, the method allows sequencing of a single strand of DNA by synthesizing the complementary strand along it, one base pair at a time, and detecting which base was actually added at each step.

Dataset-specific Instrument Name	
Generic Instrument Name	Bran Luebbe AA3 AutoAnalyzer
Generic Instrument Description	Bran Luebbe AA3 AutoAnalyzer See the description from the manufacturer.

Dataset-specific Instrument Name	CE Elantech NC2100 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	AB 3500 Applied Biosystems Gene Analyzer
Generic Instrument Name	Gene Analyzer
Generic Instrument Description	An automated analyzer designed for a wide range of sequencing and fragment analysis applications with the ability to perform comparative sequencing, linkage analysis, STR analysis, SNP detection, discovery and validation, mutation detection, and many other applications.

Dataset-specific Instrument Name	Shimadzu TOC 5050 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

[table of contents | back to top]

Project Information

Testing the sponge-loop hypothesis for Caribbean coral reefs (Sponge_Loop)

Coverage: Conch Reef, Key Largo, Florida, USA; Carrie Bow Cay, Belize

NSF Abstract:
Sponges are bottom-dwelling animals that dominate Caribbean reefs now that reef-building corals have been declining for decades. Sponges feed by filtering huge volumes of seawater, providing a mechanism for recycling organic material back to the reef. A new theory has been proposed called the "sponge-loop hypothesis" that is potentially the most important new concept in marine ecology in many years, because it seeks to explain Darwin's Paradox: how do highly productive and diverse coral reefs grow in desert-like tropical seas? The sponge loop hypothesis proposes that sponges on coral reefs absorb the large quantities of dissolved organic carbon (molecules such as carbohydrates) that are released by seaweeds and corals and return it to the reef as particles in the form of living and dead cells, or other cellular debris. This project will use a rigorous set of techniques to test the sponge-loop hypothesis in the field on ten of the largest and most common sponges on Caribbean reefs. For each species, the contributions of particles and dissolved organic carbon to sponge nutrition will be measured, as well as the production of cellular particles in the seawater flowing out of the sponge. For selected sponge species, the concentration of dissolved organic carbon entering the sponge will be experimentally enhanced to determine the capacity of the sponge to absorb this potential food source, and to gauge its effect on the production of cellular particles. This project will provide STEM education and training for postdoctoral, graduate and undergraduate students and public outreach in the form of easily accessible educational videos. Further, this project is important for understanding the carbon cycle on coral reefs where the effects of climate change and ocean acidification may be tipping the competitive balance toward non-reef-building organisms, such as sponges.

The cycling of carbon from the water-column to the benthos is central to marine ecosystem function; for coral reefs, this process begins with photosynthesis by seaweeds and coral symbionts, which then exude a substantial portion of fixed carbon as dissolved organic carbon (DOC) that may be lost to currents and tides. But if sponges, with their enormous water filtering capacity, can return DOC from the water column to the reef, it would represent a major unrecognized source of carbon cycling. The "sponge-loop hypothesis" has the potential to transform our understanding of carbon cycling on coral reefs. Building on preliminary data from studies of the giant barrel sponge, this project will investigate each of the three components of the sponge-loop hypothesis for ten common barrel, vase and tube-forming species that span a range of associations with microbial symbionts, from high microbial abundance (HMA) to low microbial abundance (LMA) in the sponge tissue. Specifically, the experimental approach will include InEx techniques (comparative sampling of seawater immediately before and after passage through the sponge), velocimetry, and flow cytometry to determine whether each species consumes DOC and produces particulate organic carbon (POC) in the form of cellular detritus. Then, for species that consume DOC, the same techniques will be used in manipulative experiments that augment the amount of DOC from three categories (labile, semi-labile and refractory) to determine the types of DOC consumed by sponges. In addition to testing the sponge-loop hypothesis, this project will use molecular techniques to investigate the differences among HMA and LMA sponge species, targeting the microbial symbionts that may be responsible for DOC uptake.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1558580

[table of contents | back to top]