Site descriptions and physical environmental conditions in coral microbiomes in the Florida Keys during 2013 (Coral Microbial Relationships project)

Website: https://www.bco-dmo.org/dataset/661942
Data Type: experimental
Version: Final
Version Date: 2016-10-19

Project
» Fundamental Coral-Microbial Associations (Coral Microbial Relationships)

Contributors	Affiliation	Role
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Abstract
Site descriptions and physical environmental conditions in coral microbiomes in the Florida Keys during 2013 (Coral Microbial Relationships project)

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Coverage

Spatial Extent: **N**:24.605683 **E**:-81.381333 **S**:24.552583 **W**:-81.501267
Temporal Extent: 2013-04-30 - 2013-05-02

Dataset Description

This dataset describes seawater microbial biogeochemistry parameters.

Methods & Sampling

Sample collections:

Triplicate colonies of the corals Orbicella (formerly Montastrea) faveolata (Budd et al., 2012), Montastrea cavernosa, Diploria strigosa, Porites astreoides and Porites porites were sampled via SCUBA diving using hammer and chisel at four sites within the Florida Keys, offshore of Summerland Key at depths ranging from 2.4 – 7.6 m (Reef flat, 24 deg 33.155', 81 deg 22.88'; Open water patch reef, 24 deg 33.164, 81 deg 26.225; Mid-channel patch reef 24 deg 33.620, 81 deg 30.076; Nearshore reef, 24 deg 36.341, 81 deg 25.756 and an offshore Nursery site, 24 deg 33.745, 81 deg 24.013, where corals had been transplanted from

nearby reefs). Fragments from each colony were placed in sterile Whirl-Pak bags (Nasco, Fort Atkinson WI, USA) underwater and transferred to a cooler containing ice after dive completion. Within 1-4 hours, each fragment was rinsed with 0.1 um filtered seawater. Coral mucus was collected by siphoning the mucus from the coral surface with a pipette, and mucus fractions were frozen at -80 deg C. The coral fragment was then divided using a sterilized chisel and hammer into two smaller fragments; one frozen at -80 deg C and the second placed in a 4% paraformaldehyde-0.2 um filter sterilized phosphate buffered saline solution, fixed at 4 deg C for 1 hour, and stored at -20 deg C.

At each site, seawater temperature, dissolved oxygen, and pH were measured from a depth just above the corals (~5 m) and from the surface (0-1 m) using an EXO water quality sonde (YSI Inc., Yellow Springs, OH, USA). Seawater samples (4 l) from the same depths were also collected for microbial biomass, inorganic nutrient analyses and direct cell enumeration, and stored on ice for 1-4 hours. Upon arrival at the laboratory, water for nutrient analyses was frozen at -20 deg C. For direct cell counts, duplicate 1 ml aliquots of seawater were preserved at a final concentration of 4% paraformaldehyde for 20 minutes at 4 deg C and then frozen at -80 deg C. Finally, ~2 l of water was pressure filtered onto duplicate 25 mm, 0.22 um Durapore membrane filters (Millipore, Boston, MA, USA) using a peristaltic pump for collection of seawater microbial biomass and stored at -80 deg C.

Nutrient and pigment analyses and direct cell counts:

Concentrations of dissolved inorganic nutrients (NH_4^+, NO_3^- + NO_2^-, NO_2^-, PO_4^{3-}, and silicate) were measured using a continuous segmented flow system with methods previously described (Apprill and Rappé, 2011), with NO_3^- was derived from subtracting the contribution of NO_2^-. NH_4^+ was also measured in the field on unfrozen samples using the ortho-phthaldialdehyde fluorescence method (Holmes et al., 1999;Taylor et al., 2007). Pigment analysis of the phytoplankton community was performed using high-performance liquid chromatography with known standards on extracts from the frozen filters, and quantified as described previously (Van Heukelem and Thomas, 2001). Seawater microbial cell counts were measured using flow cytometry (Apprill and Rappé, 2011).

Preparation of nucleic acids:

In order to examine mucus- and tissue-associated microbes, coral samples were processed using three approaches (Fig. 1A). The first approach harvested the mucus that was collected as previously described (hereafter referred to as 'mucus' samples). For the second approach, the frozen coral tissue (which still contained some mucus) was removed from the skeleton using an airbrush with 80 psi air pressure and 0.22 um filtered phosphate buffered saline solution. The tissue homogenate was then vortexed for 2 min and centrifuged at 20,000 x g for 20 min at 4 deg C. After removal of the supernatant, the cells were stored at -80 deg C for 1 week or less (referred to as 'holobiont' samples). Lastly, the third biomass substrate analyzed was decalcified coral tissue, referred to as 'tissue' samples, which were devoid of mucus and skeleton. To obtain these samples, the coral subsample that was initially preserved in paraformaldehyde solution was placed in a 20% EDTA solution (Acros Organics Thermo Fisher Scientific, NJ, USA) for 2-3 weeks at 4C on a slow rocker. The EDTA solution was exchanged daily until complete skeleton dissolution, (similar to Ainsworth et al., 2015).

Nucleic acids were extracted from the seawater membrane filters (1/4 of the 25 mm filter), the holobiont cells, and the decalcified tissue samples using the PowerPlant Pro DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA, USA), with modifications that included adaptations that included 350 mg of 0.1mm silica beads (MP Biomedicals, Irvine, CA, USA) to the bead solution and 25 ul of proteinase K (Qiagen Inc., Valencia, CA, USA) followed by incubation at 65 deg C for 60 min. Because tissue samples were initially preserved in paraformaldehyde, they were subject to an extended 30-minute proteinase K digestion at 55 deg C followed by an additional high-heat incubation step at 90 deg C for 60 minutes before beadbeating. The PowerPlant Pro extraction method did not result in high-quality DNA from the mucus. Therefore, the PowerBiofilm DNA Isolation kit (Mo Bio Laboratories), containing inhibitor removal steps but otherwise a similar bead mixture and proteinase K digestion at 65 deg C for 60 min, was used for mucus samples. All nucleic acids were quantified using the Qubit dsDNA BR fluorescent assay (Invitrogen Corp., Carlsbad, CA, USA) on a Qubit 2.0 fluorometer.

Sequencing of V4 region of archaeal and bacterial SSU rRNA genes:

Barcoded primers targeting the V4 hypervariable region of the SSU rRNA gene, 515F and 806R, were utilized for sequencing analysis as detailed in Kozich and colleagues (2013). This sequencing and data analysis occurred prior to the modifications of these primers to capture additional SAR11 clade bacteria (Apprill et al., 2015) and Thaumarchaeota (Parada et al., 2015), and therefore SAR11 are likely underrepresented by 15-25% in the seawater samples; Thaumarchaeota are probably not heavily underestimated in this study because the archaeal amoA gene abundances suggest their low abundance in reef seawater. Triplicate 25 ul PCR reactions contained 1.25 U of GoTaq Flexi DNA Polymerase (Promega Cooperation, Madison, WI, U.S.A.), 5X Colorless

GoTaq Flexi Buffer, 2.5 mM MgCl2, 200 uM of each dNTP, 200 nM of each barcoded primer, and 0.15 ng - 2.0 ng of genomic template for most samples, with some samples diluted or concentrated to encompass a range of 0.0057 to 4.40 ng. The reaction conditions included an initial denaturation step at 95 ºC for 2 min, followed by 32-39 cycles of 95 ºC for 20 s, 55 ºC for 15 s, and 72 ºC for 5 min, concluding with an extension at 72 ºC for 10 min. The reactions were carried out on a thermocycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Reaction products (5 ul) were screened on a 1% agarose-TBE gel. Samples were optimized for PCR at the lowest number of cycles that resulted in an amplified PCR product detected on a gel with the HyperLadder II standard (generally 5 ng ul-1) (Bioline, Taunton, MA, USA), thus minimizing biases from over-amplification. The three replicate reactions were excised from the gel, combined, and purified using the Qiagen QIAquick Gel Extraction Kit (Qiagen Inc., Valencia, California, USA), and quantified using the Qubit dsDNA HS fluorescent assay. Barcoded amplicons were pooled in equimolar ratios (5 ng each) and shipped to the University of Illinois W.M. Keck Center for Comparative and Functional Genomics for construction of libraries and sequenced using 2x250 bp paired-end MiSeq (Illumina, San Diego, CA, USA)**.**

Data Processing Description

BCO-DMO Data Processing Notes:

-added accession links column for each accession number listed
-removed spaces and replaced with underscores
-filled in all blank cells with nd
-reformatted column names to comply with BCO-DMO standards

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Data Files

File
seawater_data.csv (Comma Separated Values (.csv), 3.44 KB) MD5:31480a99604f84e2f141b6e5e7aa42e3
Primary data file for dataset ID 661942

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Related Publications

Apprill, A., & Rappé, M. (2011). Response of the microbial community to coral spawning in lagoon and reef flat environments of Hawaii, USA. Aquatic Microbial Ecology, 62(3), 251–266. doi:10.3354/ame01471
Methods

Apprill, A., McNally, S., Parsons, R., & Weber, L. (2015). Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquatic Microbial Ecology, 75(2), 129–137. doi:10.3354/ame01753
Methods

BUDD, A. F., FUKAMI, H., SMITH, N. D., & KNOWLTON, N. (2012). Taxonomic classification of the reef coral family Mussidae (Cnidaria: Anthozoa: Scleractinia). Zoological Journal of the Linnean Society, 166(3), 465–529. doi:10.1111/j.1096-3642.2012.00855.x
Methods

D Ainsworth, T., Krause, L., Bridge, T., Torda, G., Raina, J.-B., Zakrzewski, M., … Leggat, W. (2015). The coral core microbiome identifies rare bacterial taxa as ubiquitous endosymbionts. The ISME Journal, 9(10), 2261–2274. doi:10.1038/ismej.2015.39
Methods

Holmes, R. M., Aminot, A., Kérouel, R., Hooker, B. A., & Peterson, B. J. (1999). A simple and precise method for measuring ammonium in marine and freshwater ecosystems. Canadian Journal of Fisheries and Aquatic Sciences, 56(10), 1801–1808. doi:10.1139/f99-128
Methods

Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., & Schloss, P. D. (2013). Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform. Applied and Environmental Microbiology, 79(17), 5112–5120. doi:10.1128/aem.01043-13
Methods

Parada, A. E., Needham, D. M., & Fuhrman, J. A. (2015). Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environmental Microbiology, 18(5), 1403–1414. doi:10.1111/1462-2920.13023
Methods

Taylor,B. D., Keep,C. F., Hall,R. O., Koch, B. J., Tronstad, L. M., Flecker,A. S. & Ulseth, A. J. (2007). Improving the fluorometric ammonium method: matrix effects, background fluorescence, and standard additions. Journal of the North American Benthological Society 26(2), 167-177. doi:10.1899/0887-3593(2007)26[167:ITFAMM]2.0.CO;2
Methods

Van Heukelem, L., & Thomas, C. S. (2001). Computer-assisted high-performance liquid chromatography method development with applications to the isolation and analysis of phytoplankton pigments. Journal of Chromatography A, 910(1), 31–49. doi:10.1016/s0378-4347(00)00603-4
Methods

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Parameters

Parameter	Description	Units
site	Site description where sampling took place	unitless
date	Date of sampling; mm.dd.yy	unitless
time_local	Local time of sampling; HH:MM	unitless
reef_description	Reef habitat description where sampling took place	unitless
depth	Depth where sampling occurred	meters
lat	Latitude; North is positive	decimal degrees
lon	Longitude; West is positive	decimal degrees
temperature	Temperature at sampling site	celsius
salinity	Salinity at sampling site	practical salinity units (PSU)
DO	Dissolved oxygen percent saturation at sampling site	percent
pH	pH at sampling site	pH
PO4	Phosphate concentration at sampling site	uM/L
NO3_NO2	Nitrate + Nitrite concentration at sampling site	uM/L
SiO4	Silicate concentration at sampling site	uM/L
NO2	Nitrite concentration at sampling site	uM/L
NO3	Nitrate concentration at sampling site	uM/L
NH4_indophenolBlue	Ammonium concentration measured on site; indophenol blue method	uM/L
NH4_fluorometric	Ammonium concentration measured on site; ortho-phthaldialdehyde fluorescence method	um/L
prochlorococcus_A	Prochlorococcus cell count measured from first duplicate of 1 ml aliquots of seawater.	cells per mL
prochlorococcus_B	Prochlorococcus cell count measured from second duplicate of 1 ml aliquots of seawater.	cells per mL
synechococcus_A	Synechococcus cell count measured from first duplicate of 1 ml aliquots of seawater.	cells per mL
synechococcus_B	Synechococcus cell count measured from second duplicate of 1 ml aliquots of seawater.	cells per mL
picoeukaryote_A	Picoeukaryote cell count measured from first duplicate of 1 ml aliquots of seawater.	cells per mL
picoeukaryote_B	Picoeukaryote cell count measured from second duplicate of 1 ml aliquots of seawater.	cells per mL
nonpigmentedPicoplankton_A	Non-pigmented picoplankton cell count measured from first duplicate of 1 ml aliquots of seawater.	cells per mL
nonpigmentedPicoplankton_B	Non-pigmented picoplankton cell count measured from second duplicate of 1 ml aliquots of seawater.	cells per mL
nonpurgeableOrganicCarbon	Non-purgeable organic carbon concentration	uM/L
total_nitrogen	Total nitrogen concentration	uM/L
accession_numbers	NCBI GenBank accession numbers (16S rRNA genes)	unitless
accession_links	Links to NCBI GenBank accession numbers	unitless

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Instruments

Dataset-specific Instrument Name	Airbrush with 80 psi air pressure
Generic Instrument Name	Airbrush
Dataset-specific Description	Frozen coral tissue was removed from skeleton using airbrush.
Generic Instrument Description	Device for spraying liquid by means of compressed air.

Dataset-specific Instrument Name	MiSeq
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	MiSeq (Illumina, San Diego, CA, USA)
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	Centrifuge
Generic Instrument Name	Centrifuge
Dataset-specific Description	Tissue homogenate was then vortexed for 2 min and centrifuged at 20,000 x g for 20 min at 4 deg C
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	Continous Segmented Flow System
Generic Instrument Name	Continuous Flow Analyzer
Dataset-specific Description	Dissolved inorganic nutrients measured.
Generic Instrument Description	A sample is injected into a flowing carrier solution passing rapidly through small-bore tubing.

Dataset-specific Instrument Name	Bio-Rad thermocycler
Generic Instrument Name	Thermal Cycler
Dataset-specific Description	Reactions carried out on a Bio-Rad thermocycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA)
Generic Instrument Description	A thermal cycler or "thermocycler" is a general term for a type of laboratory apparatus, commonly used for performing polymerase chain reaction (PCR), that is capable of repeatedly altering and maintaining specific temperatures for defined periods of time. The device has a thermal block with holes where tubes with the PCR reaction mixtures can be inserted. The cycler then raises and lowers the temperature of the block in discrete, pre-programmed steps. They can also be used to facilitate other temperature-sensitive reactions, including restriction enzyme digestion or rapid diagnostics. (adapted from http://serc.carleton.edu/microbelife/research_methods/genomics/pcr.html)

Dataset-specific Instrument Name	EXO Water Quality Sonde
Generic Instrument Name	Water Quality Multiprobe
Dataset-specific Description	Temperature, DO, and pH measured just above the coral colony and at surface.
Generic Instrument Description	An instrument which measures multiple water quality parameters based on the sensor configuration.

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Deployments

Apprill_2013

Website	https://www.bco-dmo.org/deployment/565728
Platform	BIOS
Start Date	2012-09-15
End Date	2016-08-31
Description	High-throughput sequencing of small subunit ribosomal RNA (SSU rRNA) genes from marine environments. Coral-bacterioplankton mesocosm experiments.

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Project Information

Fundamental Coral-Microbial Associations (Coral Microbial Relationships)

Coverage: Florida Keys, Federated States of Micronesia, Red Sea, & Bermuda

Description from NSF award abstract:
Reef-building corals are in decline worldwide due in part to climate change and other human activities, and it is becoming increasingly important to understand what aspects of coral biology are degraded by environmental stress which then leads to coral mortality. It is now widely known that corals harbor communities of bacteria

and archaea that are believed to play important roles in maintaining the health of their hosts, but we lack any appreciable understanding about the identity of the microbial associates regularly residing within healthy, reef-building corals. This project asks the central question: do reef-building corals harbor fundamental or persistent microbial associates that are symbiotic within their tissues? In order to address this hypothesis, the investigator will assess the identity of the bacterial and archaeal microbes using a variety of molecular and microscopy approaches that includes the identification and localization of a widespread group of coral bacterial associates belonging to the genus Endozoicomonas. The results of this study will then be used to develop additional questions about the role of these microbial associates in nutrient cycling and how they contribute to the health and survival of corals.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1233612

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