Diel, daily, and spatial variation of coral reef seawater microbial communities from US Virgin Islands, 2017

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Project
» Signature exometabolomes of Caribbean corals and influences on reef picoplankton (Coral Exometabolomes)

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Abstract
Bacterial and archaeal diversity and composition, microbial cell abundances, inorganic nutrient concentrations, and physicochemical conditions were determined and measured in coral reef seawater over a three-day, diel time series on one reef in St. John, U.S. Virgin Islands.

Table of Contents

Coverage

Spatial Extent: **N**:41.5265 **E**:-64.70403 **S**:18.30204 **W**:-70.6731

Temporal Extent: 2017-08 - 2017-11

Dataset Description

Bacterial and archaeal diversity and composition, microbial cell abundances, inorganic nutrient concentrations, and physicochemical conditions were determined and measured in coral reef seawater over a three-day, diel time series on one reef in St. John, U.S. Virgin Islands.

Acquisition Description

Sample collection:
Five Porites astreoides colonies and a sand patch were selected and marked with flagging tape by divers on Ram Head reef (18º18’07.3” N, 64º42’14.5” W; 8 m depth in sand) in St. John, U. S. Virgin Islands. Colonies of various sizes (3 – 16 inches in diameter) from a range of heights above the seafloor (1 – 27 cm) were selected and these colonies were labeled A through E. Additionally, colonies were evenly distributed across the reef in order to minimize location effects (range of 3.6 to 14 meters between each colony). All colonies were located directly next to sand patches based on colony size constraints and the space needed for deployment of the custom made Coral Ecosphere Sampling Devices (CESD). Six CESD made out of aluminum strut material were deployed adjacent to each sampling location with sand screws. The last CESD was placed in a wide sand patch with no corals or benthic organisms located in its vicinity and this sampling location was used as a ‘no-coral’ control. Divers positioned the CESD so that a 60 ml syringe with an attached filter holder could be placed 5 cm away from the middle of the colony. Light and temperature loggers (8K HOBO/PAR loggers; Onset, Wareham, MA) were zip-tied to the end of each CESD and programmed to collect temperature and relative light intensity measurements every 5 minutes over the course of the three-day study. An hour after CESD deployment, scuba divers collected the first set of samples (Day 1, 3:00 pm). Filter holders were pre-loaded with 0.22 µm pore size Supor® filters (Pall Corporation, Ann Arbor, MI, USA) and were contained within sterile Whirl-pack® bags prior to sampling. Divers also descended with acid-washed polyethylene nutrient bottles (30 ml volume) to collect seawater samples for unfiltered inorganic nutrient analysis and flow cytometry. At depth, seawater samples (60 ml) collected for amplicon-based microbial community analysis were conducted at 2 different stationary locations relative to the CESD device (with the exception of collections completed at the sand-patch location). Reef-depth samples were collected first at the top of the CESD (2 m from the colony) in order to minimize stirring close to the coral ecosphere sampling area. To collect the sample, a diver attached a piece of acid-cleaned Masterflex silicone tubing to connect the end of the filter holder to the mouth of the syringe and then used reverse filtration to pull seawater through the filter. The filter-holder was then placed in an individual Whirl-pack® bag and sealed. After collection of

microbial biomass with the syringe, a nutrient sample was collected. After collection of the reef-depth sample, a diver attached the filter holder to the syringe, slowly descended closer to the coral colony, but behind the CESD to maintain sufficient distance from the sampling area and then placed the syringe into the syringe holder located on the horizontal arm of the CESD. As before, the diver first collected the coral ecosphere sample (5 cm from the colony) onto the filter followed by a nutrient sample in the same location. Replicate samples collected for DNA analysis were collected from both seawater environments surrounding each colony on the first dive, but were not collected on the following dives due to time constraints. Surface seawater samples (< 1 m) were collected using 60 mL syringes at each time point from the dive boat.

This sampling scheme was repeated at approximately 3 am and 3 pm for the next three days, totaling up to 6 sampling time points. Divers sampled each colony and collected samples in the same order (reef-depth followed by coral ecosphere) during all time points. After collection, samples were placed in a cooler equipped with blue-ice packs for the transit from the reef to the lab and then samples were processed immediately. Over the course of sampling, 85 seawater samples were collected.

After the last time point, coral tissue was collected from each colony (close to the area where the coral ecosphere seawater was sampled) using a hammer and chisel and the CESD were removed. Sand was also collected in the location where the sand control CESD device was deployed.

Sample processing:
In the laboratory, sterile syringes were used to remove residual seawater trapped within filter holders and then filters were placed into cryovials, flash-frozen in a dry shipper charged with liquid nitrogen, and then transferred into a -20 C freezer.

Seawater collected for flow cytometric analysis was subsampled from unfiltered nutrient samples and preserved with paraformaldehyde (Electron Microscopy Sciences, Allentown, PA) to a final concentration of 1% (by volume). Nutrient, DNA, and flow cytometry samples were shipped frozen back to Woods Hole Oceanographic Institution and ultimately stored at -80 C prior to analysis. The coral tissue and sand samples were stored in a second dry shipper and ultimately at -80 C until they were processed.

Macronutrient analysis and flow cytometry:
Frozen and unfiltered nutrient samples were analyzed with a continuous segmented flow-system using previously described methods (as in Apprill and Rappe 2011). The concentrations of NO2- + NO3-, NO2-, PO43-, NH4+, and silicate were measured in all of the samples. Nitrate concentrations were obtained by subtracting the nitrite concentration from the nitrite + nitrate measurements for each sample.

Samples collected for flow cytometry were analyzed using colinear analysis (laser excitation wavelength of 488 nm, UV) on an Altra flow cytometer (Beckman Coulter, Pasadena, CA.).

Unstained subsamples were used to enumerate the abundances of picocyanobacteria (Prochlorococcus, Synechococcus) and picoeukaryotes. Stained (Hoechst stain, 1 µg ml-1 final concentration) subsamples were analyzed to estimate the abundance of unpigmented cells (an estimate of heterotrophic bacterial abundance) (Marie et al. 1997). FlowJo (v. 6.4.7) software was used to estimate the abundance of each cell type. The abundance of total cells was calculated by adding the cell counts obtained for each of the respective picoplankton classes together for each sample.

DNA extraction, amplification, pooling, and sequencing:
DNA was extracted from filters using a sucrose-lysis extraction method and Qiagen spin-columns (Santoro et al. 2010) Control extractions were also completed with unused filters (control filters without biomass) in order to account for contamination from the filters or extraction reagents. Lastly, diluted DNA from a synthetic staggered mock community (BEI Resources, Manassas, VA, USA) was used to account for amplification and sequencing errors in downstream microbial community analysis. Coral tissue was removed from the skeleton using air-brushing with autoclaved 1% phosphate-buffered-saline (PBS) solution (Apprill et al. 2016; Weber et al. 2017). The coral tissue slurry was pelleted using a centrifuge and the PBS supernatant was discarded. DNA was extracted from each pellet (300 mg of tissue) using a modified version of the DNeasy DNA extraction kit protocol (Qiagen, Germantown, MD). The lysis buffer in the kit was added to each tube followed by approximately 300 mg of garnet beads (from a MOBIO DNA extraction kit) and 300 mg of Lysing B matrix beads (MP Biomedicals, Solon, OH). The tubes were subjected to a bead-beating step for 15 minutes so that the beads could break up the coral tissue (Weber et al. 2017). After bead-beating, 20 µl of proteinase-k was added to each tube and the samples were incubated with gentle agitation for 10 minutes at 56 °C. After these modifications, the DNeasy protocol (Qiagen) was followed to complete extractions.

Extracts were amplified with barcoded primers targeting the V4 hypervariable region of the bacterial and archaeal small subunit ribosomal RNA gene (Kozich et al. 2013). The forward primer: 5’ TATGGTAATTGTGTGYCAGCMGCCGCGGTAA 3’ (Parada et al. 2016) and reverse primer: 3’ AGTCAGTCAGCCGGACTACNVGGGTWTCTAAT 5’ (Apprill et al. 2015) were used, along with the barcodes, to amplify and tag each sample prior to pooling. We used forward and reverse primers with degeneracies in order to eliminate amplification biases against Crenarchaeota/ Thaumarchaeota (Parada et al. 2016) and SAR 11 (Apprill et al. 2015). Triplicate Polymerase Chain Reactions (25 l volume) were run with 2 l of DNA template from each sample using the same barcodes in order to minimize the formation of chimeras during amplification. The reaction conditions included: a 2-minute hot start at 95 °C followed by 36 cycles of 95 °C for 20 seconds, 55 °C for 15 seconds, and 72 °C for 5 minutes. The final extension step was 72 °C for 10 minutes. Triplicate barcoded amplicons were pooled and screened using gel electrophoresis to assess the quality and the relative concentration of amplicons. Amplicons were purified using the MinElute Gel Extraction Kit (Qiagen) and pooled

to form the sequencing library. The library was sequenced (paired-end 2x250 bp) at the Georgia Genomics and Bioinformatics Core with a Miseq (Illumina, San Diego, CA) sequencer and raw sequence reads are available at the NCBI Sequence Read Archive under BioProject # PRJNA550343.

Microbial community analyses:

Raw sequences were quality-filtered and grouped into amplicon sequence variants (ASVs) using DADA2 (Callahan et al. 2016). Reads were filtered, trimmed, dereplicated and error rates were calculated using the program's parametric error model. The DADA2 algorithm was used to infer the number of different ASVs (8357 distinct ASVs), paired reads were merged, an ASV table was constructed, and chimeras were removed (1% of all ASVs). Taxonomy was assigned to each ASV using the Silva v.132 reference database (Quast et al. 2013). Mock communities were used to assess the performance of the program as well as sequencing error rates. DADA2 inferred 15, 17, and 17 strains within the mock community (compared to the 20 expected stains present at different concentrations within the staggered community) and 13, 14, and 14 of the strains were exact matches to the expected sequences from the mock community reference file. Sequence recovery is slightly lower than expected, but is comparable to normal performance of DADA2 on this staggered mock community (Callahan et al. 2016).

The R packages Phyloseq (McMurdie and Holmes 2013), Vegan (Oksanen et al. 2017), DESeq2 (Love et al. 2014), and ggplot2 (Wickham 2016) were used for downstream analysis of the microbial community. Sequences were not subsampled, but samples with less than 1000 reads (2 samples) were removed. In addition, ASVs identifying as chloroplasts were removed. Sequences representing ASVs that identified as "NA" at the Phylum level were checked using the SINA aligner and classifier (v.1.2.11) (Pruesse et al. 2012) and then removed if not identified as bacteria or archaea at 70% similarity. The average number of reads across all seawater samples used in microbial community analyses was 58,398 (± 32,184 standard deviation) with a range of 11,502 – 206,689 reads. The average number of reads in coral tissue samples was 38,096 (±23,854) with a range of 11,538 – 59,437 reads. DNA extraction control communities were initially inspected and then removed because they fell out as outliers compared to the highly similar seawater microbial communities. Taxonomic bar plots, metrics of alpha diversity (observed richness of ASVs), and boxplots of alpha diversity were made and calculated using Phyloseq. Alpha diversity was also calculated for samples after Prochlorococcus and Synechococcus ASVs were removed in order to understand how much their dynamics influenced observed richness. Constrained analysis of principal coordinates (CAP) based on Bray – Curtis dissimilarity was completed (using 'capscale' in Vegan) and variance partitioning was used to identify which of the measured environmental parameters significantly ($p<0.01$) contributed to shifts in the microbial community composition over time. Permutational Multivariate Analysis of Variance using distance matrices (PERMANOVA/Adonis) tests identified categorical factors that significantly ($p<0.05$) contributed to a similarity between the microbial communities. DESeq2 was used to identify

differentially abundant ASVs between day and night as well as reef-associated (reef-depth and coral ecosphere) compared to surface microbial communities (using the “local” fitType parameter to estimate gene dispersion). Lastly, the Rhythmicity Analysis Incorporating Non-parametric methods (RAIN) R package was used to identify ASVs that experienced rhythmic change in relative abundance over a period of 24 hours (Thaben and Westermark 2014). This analysis was completed separately for reef-depth and coral ecosphere seawater and the input ASV matrix was center log-ratio transformed and detrended following previous methods (Hu et al. 2018). Only ASVs with significant p-values ($p<0.05$) after adaptive Benjamini-Hochberg correction were reported to control for false recovery rates (Benjamini and Hochberg 2000).

Statistical analyses:
A Principal Coordinates Analysis (PCA) was completed to summarize changes in picoplankton abundances, inorganic nutrient concentrations, and relative light and temperature information collected from the HOBO loggers and reduce the dimensionality of this data. Separate PCAs were also generated using samples collected during either day or night to observe trends specific to these times. Kruskal-Wallis rank sums tests were used to test for significant differences ($p<0.05$) in alpha diversity between the different sample groupings. Pairwise post-hoc Dunn’s tests with Bonferonni corrections were used to identify which groups were significantly different from each other. These tests were also used to test for significant differences in picoplankton cell abundance overtime, between day and night samples, and between coral ecosphere and reef-depth samples.

Processing Description

BCO-DMO Processing Notes:
- added conventional header with dataset name, PI name, version date
- modified parameter names to conform with BCO-DMO naming conventions
- split lat and lon into separate columns
- replaced blank cells with NaN

[table of contents | back to top]

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

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806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquatic Microbial Ecology, 75(2), 129–137. doi:10.3354/ame01753

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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

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Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M., H., H. Stevens, and H. Wagner. 2015. Vegan: Community Ecology Package. R package version 2.3-0. http://CRAN.R-project.org/package=vegan.

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Weber, L., DeForce, E., & Apprill, A. (2017). Optimization of DNA extraction for advancing coral microbiota investigations. Microbiome, 5(1). doi:10.1186/s40168-017-0229-y

Wickham, H. (2016). ggplot2: elegant graphics for data analysis. New York: Springer-Verlag Use R! doi:10.1007/978-3-319-24277-4

[table of contents | back to top]

Parameters

Parameter	Description	Units
Sample_ID	sample identifier	unitless
NCBI_BioProject_accession_number	NCBI BioProject accession number	unitless
NCBI_BioSample_accession_number	NCBI BioSample accession number	unitless
Sample_type	Sample type	unitless
Coral_Colony_or_sand	Coral Colony or sand identifier	unitless
Collection_time	Collection time (day or night) and day relative to the start of the study	unitless
Collection_Date	Collection Date; fomatted as Mon-yyyy	unitless
Collection_location	Collection location	unitless
lat	latitude; north is positive	decimal degrees
lon	longitude; east is postive	decimal degrees
Prochlorococcus_cells_mL	concentration of Prochlorococcus	cell/milliliter
Synechococcus_cells_mL	concentration of Synechococcus	cell/milliliter
Picoeukaryotes_cells_mL	concentration of Picoeukaryotes	cell/milliliter
Unpigmented_cells_cells_mL	concentration of unpigmented cells	cell/milliliter
Phosphate_uM	concentration of Phosphate_uM	micromoles
Silicate_uM	concentration of Silicate_uM	micromoles
Nitrate_uM	concentration of Nitrate_uM	micromoles
Nitrite_uM	concentration of Nitrite_uM	micromoles
Ammonium_uM	concentration of Ammonium_uM	micromoles
Temperature_F	Temperature	degrees Fahrenheit
Depth_Feet	Depth	feet
Relative_light_levels	Relative_light_levels	lumens/foot^2

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	A continuous segmented flow-system
Generic Instrument Name	Nutrient Autoanalyzer
Dataset-specific Description	Used to analyze nutrient samples.
Generic Instrument Description	Nutrient Autoanalyzer is a generic term used when specific type, make and model were not specified. In general, a Nutrient Autoanalyzer is an automated flow-thru system for doing nutrient analysis (nitrate, ammonium, orthophosphate, and silicate) on seawater samples.

Dataset-specific Instrument Name	Miseq (Illumina, San Diego, CA) sequencer
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	Used to obtain genetic data.
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	Flow Cytometer
Dataset-specific Description	Used for measuring cell concentrations. Samples collected for flow cytometry were analyzed using colinear analysis (laser excitation wavelength of 488 nm, UV) on an Altra flow cytometer (Beckman Coulter, Pasadena, CA.).
Generic Instrument Description	Flow cytometers (FC or FCM) are automated instruments that quantitate properties of single cells, one cell at a time. They can measure cell size, cell granularity, the amounts of cell components such as total DNA, newly synthesized DNA, gene expression as the amount messenger RNA for a particular gene, amounts of specific surface receptors, amounts of intracellular proteins, or transient signalling events in living cells. (from: http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm)

Dataset-specific Instrument Name	Light temperature loggers (8K HOBO/PAR loggers; Onset, Wareham, MA)
Generic Instrument Name	Temperature Logger
Dataset-specific Description	Measured temperature and relative light level.
Generic Instrument Description	Records temperature data over a period of time.

[table of contents | back to top]

Project Information

Signature exometabolomes of Caribbean corals and influences on reef picoplankton (Coral Exometabolomes)

Coverage: U.S. Virgin Islands

NSF abstract: Coral reefs are some of the most diverse and productive ecosystems in the ocean. Globally, reefs have declined in stony (reef-building) coral abundance due to

environmental variations, and in the Caribbean this decline has coincided with an increase in octocoral (soft coral) abundance. This phase shift occurring on Caribbean reefs may be impacting the interactions between the sea floor and water column and particularly between corals and picoplankton. Picoplankton are the microorganisms in the water column that utilize organic matter released from corals to support their growth. These coral-picoplankton interactions are relatively unstudied, but could have major implications for reef ecology and coral health. This project will take place in the U.S. territory of the Virgin Islands (USVI) and will produce the first detailed knowledge about the chemical diversity and composition of organic matter released from diverse stony coral and octocoral species. This project will advance our understanding of coral reef microbial ecology by allowing us to understand how different coral metabolites impact picoplankton growth and dynamics over time. The results from this project will be made publically accessible in a freely available online magazine, and USVI minority middle and high school students will be exposed to a lesson about chemical-biological interactions on coral reefs through established summer camps. This project will also contribute to the training of USVI minority undergraduates as well as a graduate student. Coral exometabolomes, which are the sum of metabolic products of the coral together with its microbiome, are thought to structure picoplankton communities in a species-specific manner. However, a detailed understanding of coral exometabolomes, and their influences on reef picoplankton, has not yet been obtained. This project will utilize controlled aquaria-based experiments with stony corals and octocorals, foundational species of Caribbean reef ecosystems, to examine how the exometabolomes of diverse coral species differentially influence the reef picoplankton community. Specifically, this project will capitalize on recent developments in mass spectrometry-based metabolomics to define the signature exometabolomes of ecologically important and diverse stony corals and octocorals. Secondly, this project will determine how the exometabolomes of these corals vary with factors linked to coral taxonomy as well as the coral-associated microbiome (Symbiodinium algae, bacteria and archaea). With this new understanding of coral exometabolomes, the project will then apply a stable isotope probe labeling approach to the coral exometabolome and will examine if and how (through changes in growth and activity) the seawater picoplankton community incorporates coral exometabolomes from different coral species over time. This project will advance our ability to evaluate the role that coral exometabolomes play in contributing to benthic-picoplankton interactions on changing Caribbean reefs.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1736288

[table of contents | back to top]