Flow cytometry and nutrient analyses data from a tidal study over 48 hours of mangrove, seagrass, and seawater from the US Virgin Islands in July of 2017

Website: https://www.bco-dmo.org/dataset/783679
Data Type: Other Field Results
Version: 1
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Project
» Signature exometabolomes of Caribbean corals and influences on reef picoplankton (Coral Exometabolomes)

Contributors	Affiliation	Role
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Abstract
Data from a tidal study over 48 hours of mangrove, seagrass, and seawater from the US Virgin Islands in 2017. These data include tidal height, depth, temperature, salinity, Prochlorococcus counts, Synechococcus counts, Picoeukaryote abundances, nutrient concentrations at accession numbers for sequences at The National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA).

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Coverage

Spatial Extent: **N**:18.32065 **E**:-64.72223 **S**:18.30964 **W**:-64.76453
Temporal Extent: 2017-07-22 - 2017-07-24

Dataset Description

Data from a tidal study over 48 hours of mangrove, seagrass, and seawater from the US Virgin Islands in 2017. These data include tidal height, depth, temperature, salinity, Prochlorococcus counts, Synechococcus counts, Picoeukaryote abundances, nutrient concentrations at accession numbers for sequences at The National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA).

Acquisition Description

Materials and Methods

Sampling. Sampling occurred from July 22-24, 2017 and coincided with the spring tides. A new moon occurred on July 23 at 5:45 (EST). While St. John tidal cycles are typically semidiurnal, during spring tide the tide cycle is diurnal. Sampling time points coincided with the low, flood, high, and ebb tides over a 48 hour (hr) window, resulting in 8 total sampling time points. Samples were collected ±1 hr from the designated time point, placed on ice, and processed within two hours of collection. Samples from nine locations across Lameshur Bay and Fish Bay on the south shore of St. John, U.S. Virgin Islands represented coral reef, seagrass bed and mangrove biomes. The Lameshur Bay mangrove location included two distinct sampling sites: an inland area that was only submerged and sampled during high tide and an outlet area that was always submerged and sampled at each time point. The majority of sampling sites were within the boundaries of the Virgin Islands National Park, which is largely undeveloped except for a small research station. The Fish Bay mangrove, Fish Bay seagrass, and Ditliff reef sites are outside the boundary of the park, and the land surrounding Fish Bay is inhabited.

At all sites, a CTD (Castaway, SonTek, San Diego, CA, USA) was deployed from the surface to the bottom depth in reef and seagrass seawater, and single point measurements were collected from mangrove seawater, to capture the temperature and salinity at each timepoint over the course of the 48 hr sampling window. Only temperature and salinity at the surface of the cast was used for analysis. Samples for inorganic nutrients were collected by filling 30ml of surface seawater into acid-washed and seawater-rinsed vials (HDPE, Nalgene), followed by freezing to 20C. Seawater (875µl) was transferred to a 2ml cryovial (Corning) for analysis of microbial abundances and fixed to a final concentration of 1% paraformaldehyde (Electron Microscopy Sciences), allowed to fix for 20 min at 4C, then flash-frozen in an LN2 dry shipper. To capture seawater microbial communities, acid-washed 4L bottles (LDPE, Nalgene, ThermoFisher Scientific, Waltham, MA, USA) were rinsed three times with seawater prior to collection of 3L of surface seawater. The specific 4L bottle used for a site at the first timepoint remained consistent across all sampling timepoints, and the bottles were rinsed with freshwater between uses. Following collection, 1L of seawater was pumped using a Masterflex peristaltic pump (Cole-Palmer, Vernon Hills, IL, USA) through Masterflex silicone tubing (L/S, platinum-cured, #96410-24 size, Cole-Parmer) to rinse the tubing. The remaining 2L of seawater was filtered through a 0.22µm Supor filter (25mm; Pall, Ann Arbor, MI, USA). For the mangrove and seagrass sites, 2L could not always be filtered completely and therefore 0.3 - 2L and 1.2 - 2L of water was filtered through the membrane, respectively. For the coral reef sites, 1.5 - 2L passed through the filter membrane. All filters were placed into 2ml cryovials (Corning, Corning, NY, USA) and flash-frozen in a liquid nitrogen dry shipper until returned to Woods Hole, MA and placed at 80C.

Flow cytometry and Nutrient analyses. Samples for microbial abundance were analyzed at the University of Hawaii with an EPICS Altra flow cytometry (Beckman Coulter Life Sciences, Inc, Indianapolis, IN) as described in Furby et al (Furby et al. 2014), with some modifications. Briefly, to obtain concentrations of cyanobacteria (Prochlorococcus and Synechococcus) and eukaryotic phytoplankton (picoeukaryotes), an unstained aliquot was run on the instrument and excited by visible wavelengths. To enumerate unpigmented cells, which is a proxy for heterotrophic bacteria and archaea (Marie et al. 1997), an aliquot was stained with a Hoechst DNA stain and run on the flow cytometer with excitation at 488nm. The number of cells per ml was estimated following analysis of fluorescence spectra using FlowJo software (v 6.4.7, Tree Star, Inc., Ashland, OR, USA).

Samples for nutrient analysis were analyzed at Oregon State University using methods described in Furby et al. (Furby et al. 2014) to measure dissolved concentrations (µM) of phosphate, ammonium, nitrite, nitrite + nitrate, and silicate.

DNA Extraction, PCR amplification, and Sequencing. DNA was extracted from the filters using a sucrose-EDTA lysis method similar to Santoro et al. (Santoro et al. 2010) that combines lysis with filter column purification. Briefly, the 25mm filter was subjected to physical and chemical lysis using 0.1mm glass beads (Lysing Matrix B, MP Biomedicals, Irvine, CA, USA), sucrose-EDTA lysis buffer (0.75M Sucrose, 20mM EDTA, 400mM NaCl, 50 mM Tris) and 10% sodium dodecyl sulfate (Teknova, Hollister, CA, USA), followed by a proteinase-K digestion (20 mg/ml Promega, Madison, WI, USA). Lysate was then purified using the DNeasy Blood and Tissue Kit (Qiagen, Germantown, MD, USA) spin column filters. Purified DNA was fluorometrically quantified using a high sensitivity dsDNA assay on a Qubit 2.0 fluorometer (ThermoFisher

Scientific).

Sample DNA was diluted 1:100 in UV-sterilized PCR-grade H2O and 1µl was used in a PCR reaction. Barcoded primers recommended by the Earth Microbiome Project, 515FY and 806RB, were used to amplify the V4 region of the SSU rRNA gene in bacteria and archaea (Apprill et al. 2015, Parada et al. 2016). Triplicate 25µl reactions contained 1.25 units of GoTaq DNA Polymerase (Promega, Madison, WI, USA), 0.2µM forward and reverse primers, 0.2mM deoxynucleoside triphosphate (dNTP) mix (Promega), 2.5mM MgCl2, 5µl GoTaq 5X colorless flexi buffer (Promega), and nuclease-free water. The reactions were run on a Bio-Rad Thermocycler (Hercules, CA, USA) using the following criteria: denaturation at 95C for 2 min; 28C cycles of 95C for 20 s, 55C for 15 s, and 72C for 5 min; and extension at 72C for 10 min. Successful amplification was verified by running 5µl of product on a 1% agarose-TBE gel stained with SYBR Safe gel stain (Invitrogen, Carlsbad, CA, USA). Triplicate PCR products were pooled and purified using the MinElute PCR purification kit (Qiagen). Concentration of purified products was quantified using the high sensitivity dsDNA assay on the Qubit 2.0 fluorometer (ThermoFisher Scientific). Barcoded PCR products were diluted to equal concentrations and pooled for sequencing. Samples were shipped to the Georgia Genomics and Bioinformatics Core at the University of Georgia for sequencing on an Illumina MiSeq using paired-end 250bp sequencing.

Data analysis. All sequence processing and data analysis was performed in R Studio (v 1.1.463) running R (v 3.4.0, 2017-04-21). Sequence reads were inspected for quality, filtered, trimmed, and dereplicated in the DADA2 R package (v.1.10.0) (Callahan et al. 2016). Specific filtering parameters used included the following: truncLen = c(240, 200), maxN = 0, maxEE = c(2,2), rm.phix = TRUE, and compress = TRUE. DADA2 was then used to generate amplicon sequence variants (ASVs) and remove chimeras. Taxonomy was assigned in DADA2 using the SILVA SSU rRNA database down to the species level where applicable (v.132, (Quast et al. 2012)). ASV counts in each sample were transformed to relative abundance for further data analysis.

To understand the variability in microbial communities over time at all sites, Bray-Curtis dissimilarity was calculated between each sample in the R package vegan (v2.5.4) (Oksanen et al. 2019) and illustrated using non-metric multidimensional scaling (NMDS) in the R package, ggplot2 (v3.2.1) (Wickham 2016). Environmental vectors that significantly associated (cutoff $p<0.01$) with the ordination were produced using the function envfit() in the vegan R package. Pairwise dissimilarity was plotted to represent the range of dissimilarity in microbial communities over 48 hrs at each site. A higher average dissimilarity would suggest that the site experiences more variable microbial communities than a site with a lower average dissimilarity. A Kruskal-Wallis test was used to examine if there is a significant difference between sites (significance level $p<0.05$). To determine which pairs of sites had significantly different dissimilarities, a pairwise Wilcoxon Rank Sum test was used with a Benjamini-

Hochberg correction for multiple testing and a cutoff of 0.05.

Differential abundance (DA) and of ASVs in relation to the tide was evaluated at mangrove sites using the corncob R package (v0.1.0) (Martin et al. 2019). The following analyses were conducted on a subset of the data representing mangrove communities. All ASV relative abundances were modeled in corncob using a logit-link for mean and dispersion. DA was modeled as a linear function of sea level (a continuous covariate that is representative of the tide cycle) while controlling for differential variance and the effect of site and day or night on DA. Controlling for the effect of day or night was imperative because over the 48 hr period low and flood tide only occurred during the day and high and low tide only occurred during dusk and night, respectively. The parametric Wald test was used to test the hypotheses that the relative abundance or variance of a given ASV changed significantly with respect to sea level and the Benjamini-Hochberg false discovery rate (FDR) correction was applied to account for multiple comparisons, with the cutoff at 0.05.

Processing Description

BCO-DMO Data Manager Processing Notes:

* added a conventional header with dataset name, PI name, version date

* modified parameter names to conform with BCO-DMO naming conventions (spaces, +, and - changed to underscores). Units in parentheses removed and added to Parameter Description metadata section.

* Date and time in UTC converted to an ISO DateTime timestamp.

* Latitude and Longitude rounded to 5 decimal places

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Related Publications

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

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods, 13(7), 581–583. doi:10.1038/nmeth.3869

FlowJo software version 6.4.7 (2005, November 16). Tree Star, Inc., Ashland, OR, USA. Retrieved from http://v9docs.flowjo.com/html/version.html#6.4.7

Furby, K. A., Apprill, A., Cervino, J. M., Ossolinski, J. E., & Hughen, K. A. (2014). Incidence of lesions on Fungiidae corals in the eastern Red Sea is related to water temperature and coastal pollution. Marine Environmental Research, 98, 29–38. doi:10.1016/j.marenvres.2014.04.002

Marie, D., Partensky, F., Jacquet, S., and Vaulot, D. (1997) Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. Applied and Environmental Microbiology 63: 186-193.

Martin BD, Witten D, Willis AD (2019) Modeling microbial abundances and dysbiosis with beta-binomial regression. arXiv:190202776 [stat].

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens HH, Szoecs E, Wagner H (2019) Vegan: Community Ecology Package. R package version 25-4. https://cran.r-project.org/package=vegan

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

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F. O. (2012). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research, 41(D1), D590–D596. doi:10.1093/nar/gks1219

Santoro, A. E., Casciotti, K. L., & Francis, C. A. (2010). Activity, abundance and diversity of nitrifying archaea and bacteria in the central California Current. Environmental Microbiology, 12(7), 1989–2006. doi:10.1111/j.1462-2920.2010.02205.x

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

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Parameters

Parameter	Description	Units
Sample_ID	Sample identifier	unitless
NCBI_BioProject_accession_number	BioProject accession number for the NCBI Sequence read archive	unitless

NCBI_BioSample_accession_number	BioSample accession number for the NCBI Sequence read archive	unitless
Sample_type	Sample type as reported to NCBI for sequence upload	unitless
Sequencing_Strategy	Describes what type of sequencing library preparation we did	unitless
Sequencing_Instrument_model	This is the model type of sequencer used for the dataset uploaded to NCBI	unitless
Sequencing_strategy	This is a more detailed description of how we sequenced. This is included in the methods in more depth.	unitless
Site_Name	This is the local name of the site where samples were collected	unitless
Biome	This is a qualitative assessment of the type of ecosystem	unitless
Latitude	Latitude	decimal degrees
Longitude	Longitude	decimal degrees
Collection_Date	Local date when samples were collected (UTC-4)	unitless
Collection_Time	Local time when samples were collected (UTC-4)	unitless

ISO_DateTime_UTC	ISO Datetime (UTC) when samples were collected in ISO 8601:2004(E) format yyyy-mm-ddTHH:MM:SSZ	unitless
Tide_Height	Tide height corresponding to verified mean low low water (MLLW), as taken from the NOAA station 9751381 in Lameshur Bay, USVI.	meters (m)
Time_elapsed_between_tide_timepoint_and_collection	Time that elapsed between the designated collection timepoint and when the sample was actually collected during the time series.	minutes
Collection_Depth	Collection depth of all samples	meters (m)
Site_Depth	Approximate depth of the collection site. Generally taken from the CTD cast.	meters (m)
Temperature	Temperature of seawater as taken from the CastAway CTD (SonTek)	degrees Celsius (ºC)
Salinity	Salinity of seawater as taken from the CastAway CTD (SonTek)	Practical Salinity Units (PSU)
Prochlorococcus	Prochlorococcus cell counts as determined by flow cytometry	cells per milliliter (cells/ml)

Synechococcus	Synechococcus cell counts as determined by flow cytometry	cells per milliliter (cells/ml)
Picoeukaryotes	Picoeukaryote abundances as determined by flow cytometry	cells per milliliter (cells/ml)
Unpigmented_cells	unpigmented cells as determined by flow cytometry of Hoescht stained cells.	cells per milliliter (cells/ml)
Phosphate	Phosphate concentrations in seawater	micromolar (µM)
Silicate	Silicate concentrations in seawater	micromolar (µM)
Nitrate_Nitrite	Nitrate and Nitrite concentrations in seawater	micromolar (µM)
Nitrite	Nitrite concentrations in seawater	micromolar (µM)
Ammonium	Ammonium concentrations in seawater	micromolar (µM)
Tide	Qualitative description of the tide level during the sample timepoint	unitless

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Instruments

Dataset-specific Instrument Name	CTD (Castaway, SonTek, San Diego, CA, USA)
Generic Instrument Name	CTD profiler
Generic Instrument Description	The Conductivity, Temperature, Depth (CTD) unit is an integrated instrument package designed to measure the conductivity, temperature, and pressure (depth) of the water column. The instrument is lowered via cable through the water column and permits scientists observe the physical properties in real time via a conducting cable connecting the CTD to a deck unit and computer on the ship. The CTD is often configured with additional optional sensors including fluorometers, transmissometers and/or radiometers. It is often combined with a Rosette of water sampling bottles (e.g. Niskin, GO-FLO) for collecting discrete water samples during the cast. This instrument designation is used when specific make and model are not known.

Dataset-specific Instrument Name	Qubit 2.0 fluorometer (ThermoFisher Scientific)
Generic Instrument Name	Fluorometer
Generic Instrument Description	A fluorometer or fluorimeter is a device used to measure parameters of fluorescence: its intensity and wavelength distribution of emission spectrum after excitation by a certain spectrum of light. The instrument is designed to measure the amount of stimulated electromagnetic radiation produced by pulses of electromagnetic radiation emitted into a water sample or in situ.

Dataset-specific Instrument Name	Illumina MiSeq
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	Samples were shipped to the Georgia Genomics and Bioinformatics Core at the University of Georgia for sequencing on an Illumina MiSeq using paired-end 250bp sequencing
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	EPICS Altra flow cytometry (Beckman Coulter Life Sciences, Inc, Indianapolis, IN)
Generic Instrument Name	Flow Cytometer
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)

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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.

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Funding

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

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