Incubation experiments were conducted in St. John, US Virgin Islands to investigate the response of reef seawater microbial communities to the mixed exudates released from the coral species Porites astreoides and Gorgonia ventalina.

Website: https://www.bco-dmo.org/dataset/865739
Data Type: experimental
Version: 1
Version Date: 2022-11-10

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

Contributors	Affiliation	Role
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Abstract
Incubation experiments were conducted in St. John, US Virgin Islands to investigate the composition of exudates released from different species of benthic organisms, and the response of reef seawater microbial communities to mixed exudates released from different species and to specific metabolites. Exudates were collected from the stony coral Porites astreoides, and the octocoral Gorgonia ventalina after an 8 hour incubation. Reef seawater microbial communities were incubated separately in the presence of exudates from P. astreoides and G. ventalina for 48 hours and samples were collected to monitor changes in microbial abundance via flow cytometry and microbial community composition via 16S rRNA gene sequencing. Complementary Targeted and Untargeted metabolomic data from these incubation experiments is available on the MetaboLights database under accession number MTBLS2855.

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Coverage

Spatial Extent: **Lat**:18.315773476842 **Lon**:-64.727413483487

Dataset Description

The flow cytometry data in this dataset is the raw data and has not been normalized (as is presented in the manuscript Weber et al., 2022).

Other relevant files and publications:

The targeted and untargeted metabolomics data and metadata associated with this study are located on the

MetaboLights database under accession numbers MTBLS2855 and MTBLS3286.

The 16S rRNA gene sequencing data and metadata associated with this study are located on the NCBI Sequence Read Archive (SRA) under BioProject PRJNA739882. BioSample accession numbers are not linked with the data submitted to BCO-DMO because samples for flow cytometry and macronutrients were not always collected at the same time as samples collected for microbial community analyses, meaning that only some of the samples collected for microbial community analyses have affiliated microbial abundances and nutrient concentrations.

Methods & Sampling

Experiments were conducted to monitor responses of reef seawater microbial communities to concentrated exudates from *P. astreoides* and *G. ventalina*. After the organism incubations were conducted, excess filtrate (2 l) from 3 of the 6 colony/fragment incubations (selected randomly) were pooled into an acid-washed, 10 l carboy and mixed. Excess filtrate (~2 l) from the three control incubations were also pooled into a second, acid-washed, 10 l carboy and mixed.

For the *P. astreoides* experiment, surface seawater was collected from the offshore site and coarsely filtered through a GF/A filter (1.6 µm nominal pore size) using peristalsis to remove larger cells and minimize heterotrophic grazing, but retain bacteria and archaea. Approximately 2.4 l of this inoculum was added separately to each of the 10 l carboys (pooled coral and control filtrate) to create a 5:2 ratio of filtrate: inoculum. After this addition, each carboy was mixed and a suite of samples were collected for different analyses including cell counts, inorganic and organic macronutrient quantification, and 16S rRNA gene sequencing. This collection marked the first timepoint (0 hours [hrs]) for the exudate uptake experiment. For the *G. ventalina* experiment, reef seawater inoculum was collected from Tektite reef (Table S1).

For each experiment, coral metabolite or control filtrate seawater were transferred into separate 1 l acid-washed polycarbonate bottles (6 bottles per treatment). Within each treatment (coral and control), 3 of the 6 bottles were blackened to block light. The bottles were placed into a flow-through seawater table. PAR readings in the seawater table for the *P. astreoides* and *G. ventalina* experiments ranged from 250 – 1000 and 164 - 530 µmol quanta m -2s -1, respectively, with variation caused by shading and cloud cover. Over the course of 48 hours, samples were collected for cell counts (1 ml) at all timepoints (0, 12, 24, 36, and 48 hrs), macronutrient analyses (30 – 40 ml) at 0 and 48 hrs, and microbial community analyses (60 – 300 ml) at 0, 24, and 48 hrs.

Samples collected for cell counts were fixed to 1% (v/v) paraformaldehyde, refrigerated for 20 minutes in the dark, and frozen in a charged dry shipper.

Seawater samples (60 ml for *P. astreoides*) collected for microbial community analyses were obtained using 60 ml sterile, Luer-lock syringes (Becton Dickinson, Franklin Lakes, NJ, USA) and filtered using positive pressure onto 25 mm, 0.2 m pore-size filters. The amount of volume filtered was increased for the *G. ventalina* exudate experiment because of biomass concerns and ranged from 180 (0 and 24 hrs) to 300 ml (48 hrs). Previous work has demonstrated that sample volume does not significantly influence microbial community composition when beta diversity comparisons are made [6]. Alpha diversity analysis was avoided due to the differences in seawater volumes. Filters were then transferred into cryovials and placed in the charged dry shipper, followed by -80 °C storage until DNA extraction.

Data Processing Description

Microbial abundances of *Prochlorococcus*, *Synechococcus*, picoeukaryotes, and unpigmented cells were enumerated via flow cytometry. Briefly, samples were thawed and stained with Hoechst 34442 (1 µg/mL, final concentration) [1–3] and analyzed using a Beckman-Coulter Altra Flow Cytometer endowed with two argon ion lasers, tuned to UV (200 mW) and 488 nm (1 W) excitation wavelengths. Side and forward scatter as well as fluorescence signals were collected using the appropriate filters designated for Hoechst-bound DNA, phycoerythrin, and chlorophyll. FlowJo software (Tree Star, Inc.) was used to bin populations and estimate the abundances of *Prochlorococcus*, *Synechococcus*, picoeukaryotes, and unpigmented cells (primarily heterotrophic bacteria and archaea). Cell abundances collected at 12, 24, 36, and 48 hrs were normalized using initial cell abundances collected at the beginning of the experiment (0 hrs).

DNA was extracted from the filters using the Qiagen PowerBiofilm extraction kit (Qiagen, Germantown, MD, USA) following the default instructions. Five DNA extraction controls were created alongside the samples by

performing the extractions without filter biomass. Purified DNA from a mock community (BEI Resources, NIAID, NIH as part of the Human Microbiome Project: Genomic DNA from Microbial Mock Community B (Even, Low Concentration), v5.1L, for 16S rRNA Gene Sequencing, HM-782D) was included in the sequencing library to assess PCR performance. Samples were amplified using the primers 515F-Y [7] and 806R-B [8] with conditions outlined in the Supplementary Methods and sequenced using a benchtop iSeq 100 sequencer (Illumina, San Diego, CA, USA). Data were analyzed using the R packages DADA2 [9], Decontam [10], Corncob [11], and Vegan [12] (see Supplementary Methods for details). The raw fastq files containing sequences for these samples can be accessed in the NCBI Sequence Read Archive (SRA) under BioProject PRJNA739882.

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Data Files

File
exudate_microbial.csv (Comma Separated Values (.csv), 8.99 KB) MD5:0ff4166277bd3468b97e682f7b1a17d8 Primary data file for dataset ID 865739

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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

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
Methods

Campbell, L., & Vaulot, D. (1993). Photosynthetic picoplankton community structure in the subtropical North Pacific Ocean near Hawaii (station ALOHA). Deep Sea Research Part I: Oceanographic Research Papers, 40(10), 2043–2060. doi:10.1016/0967-0637(93)90044-4
Methods

Campbell, L., Nolla, H. A., & Vaulot, D. (1994). The importance of Prochlorococcus to community structure in the central North Pacific Ocean. Limnology and Oceanography, 39(4), 954–961. doi:10.4319/lo.1994.39.4.0954
Methods

Davis, N. M., Proctor, D. M., Holmes, S. P., Relman, D. A., & Callahan, B. J. (2018). Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. Microbiome, 6(1). doi:10.1186/s40168-018-0605-2
Methods

Hansell, D. A., & Carlson, C. A. (2001). Biogeochemistry of total organic carbon and nitrogen in the Sargasso Sea: control by convective overturn. Deep Sea Research Part II: Topical Studies in Oceanography, 48(8-9), 1649–1667. doi:10.1016/s0967-0645(00)00153-3 https://doi.org/10.1016/S0967-0645(00)00153-3
Methods

Martin BD, Witten D, Willis AD (2019) Modeling microbial abundances and dysbiosis with beta-binomial regression. arXiv:1902.02776 [stat].
Methods

Monger, B. C., & Landry, M. R. (1993). Flow Cytometric Analysis of Marine Bacteria with Hoechst 33342 †. Applied and Environmental Microbiology, 59(3), 905–911. doi:10.1128/aem.59.3.905-911.1993
Methods

Oksanen J. Vegan: ecological diversity. R Packag Version 24-4 . 2017. https://cran.r-project.org/src/contrib/Archive/vegan/vegan_2.4-4.tar.gz
Software

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 https://doi.org/http://doi.org/10.1111/1462-2920.13023
Methods

Weber, L., Gonzalez-Díaz, P., Armenteros, M., & Apprill, A. (2019). The coral ecosphere: A unique coral reef habitat that fosters coral–microbial interactions. Limnology and Oceanography, 64(6), 2373–2388. doi:10.1002/lno.11190
Methods

Weber, L., Soule, M. K., Longnecker, K., Becker, C. C., Huntley, N., Kujawinski, E. B., & Apprill, A. (2022). Benthic exometabolites and their ecological significance on threatened Caribbean coral reefs. ISME Communications, 2(1). https://doi.org/10.1038/s43705-022-00184-7
Results

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Parameters

Parameter	Description	Units
Sample_ID	Sample ID	unitless
sample_type	Sampling type	unitless
Incubation	Pastreoides or Gventalina	unitless
Light_conditions	Samples were either incubated in the presence or absence of light.	unitless
Time	This metadata category reflects the timepoints when each sample was collected. The timepoints include na (before the beginning of the experiment)	unitless
Prochlorococcus	Prochlorococcus abundance	cells per milliliter (cells/ml)
Synechococcus	Synechococcus abundance	cells per milliliter (cells/ml)
Picoeukaryotes	Picoeukaryote abundance	cells per milliliter (cells/ml)
Unpigmented_cells	Unpigmented cells abundance	cells per milliliter (cells/ml)

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Instruments

Dataset-specific Instrument Name	Illumina sequencers
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	Illumina iSeq 100 sequencer Illumina MiSeq sequencer (2x250 bp)
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	Shimadzu TOC-VCSH total organic carbon analyzer with a TNM-1 module
Generic Instrument Name	Shimadzu TOC-V Analyzer
Dataset-specific Description	Shimadzu TOC-VCSH total organic carbon analyzer with a TNM-1 module
Generic Instrument Description	A Shimadzu TOC-V Analyzer measures DOC by high temperature combustion method.

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Project Information

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

Coverage: U.S. Virgin Islands

NSF Award 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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