Biogeochemistry, metabolomics, and metagenomics of Florida's Coral Reef from sampling conducted over 15 days in June 2019

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

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Abstract
This comparative 'omics dataset was collected over 15 days in June 2019 along Florida's Coral Reef. We assessed 85 reefs for the prevalence of stony coral tissue loss disease (SCTLD), nutrients (total organic carbon (TOC), total organic nitrogen (TON), inorganic nutrients), and abundances of microbial functional groups (Prochlorococcus, Synechococcus, picoeukaryotes, and heterotrophic microbes (unpigmented bacteria and archaea)), from reef depth waters. At 45 of the reefs, high-resolution photomosaics were used to examine the composition of benthic organisms. At 13 geographically dispersed reefs, we collected seawater (1.7 liters in biological triplicates) for both targeted and untargeted metabolomics analyses. Seawater (2 liters in duplicate) was collected at 26 sites, including the 13 examined for metabolomics, for taxonomic (bacteria and archaea 16S ribosomal RNA gene) and functional (shotgun metagenome) microbiome analyses, and chlorophyll. Given the stony coral tissue loss disease outbreak, we also targeted healthy and diseased coral tissue and near-coral seawater for taxonomic microbiome (16S rRNA gene) analysis (11 sites). Significance: Microorganisms and the dissolved metabolites they process are central to the functioning of ocean ecosystems. These 'invisible' ocean components are poorly understood in biodiverse and productive coral reef ecosystems, where they contribute to nutrient cycling and signaling cues between reef organisms. Microbes and dissolved metabolites offer a new means to examine reef features and have applications for conservation, monitoring, and restoration efforts in these changing ecosystems.

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Coverage

Spatial Extent: **N**:41.5334 **E**:-70.6479 **S**:24.2519 **W**:-82.9496
Temporal Extent: 2019-06-05 - 2019-06-19

Methods & Sampling

Study Area: We sampled coral reef environments during a research cruise aboard the M/V Alucia between June 5 and June 19, 2019. During this time, we conducted surveys and sampled biogeochemical seawater parameters at 85 reefs across 8 zones in Florida's Coral Reef, from the North Key Largo/Biscayne Bay area, designated as Zone 1, to the Dry Tortugas National Park (Zone 8). We selected reefs based on input from the Florida Fish and Wildlife Conservation Commission and existing reefs in long-term monitoring programs (e.g., Coral Reef Evaluation and Monitoring Project, CREMP).

Sample Collection and Ship-board Processing: We conducted diver-based surveys to evaluate the prevalence of stony coral tissue loss disease (SCTLD) at each of the 85 reefs. At each site, one diver performed a 30-minute roving diver survey to determine the richness of scleractinian species, the presence or absence of stony coral tissue loss disease, and the size of all observed coral colonies. The diver assigned coral colonies to four size classes, based on diameter/length: <10 centimeters (cm), 10-25cm, 25-50cm, and >50cm. The area in square meters (m2) surveyed by each diver was estimated to calculate the density of corals at each site.

We generated 100 m2 plots at each of the 85 reefs for benthic surveys via high-resolution 2D orthophotomosaics. We analyzed 45 of these reefs for benthic composition data after determining this would sufficiently cover the observed variability in the different reefs.

We collected discrete seawater samples at all 85 reefs to measure inorganic nutrient (phosphate, ammonium, silicate, nitrite plus nitrate) concentrations, total organic carbon (TOC) and total nitrogen (TN) concentrations, and cell abundances (heterotrophic microbes (unpigmented bacteria and archaea), *Prochlorococcus*, *Synechococcus*, and picoeukaryotes). We collected samples via SCUBA with acid-washed and combusted 40-milliliter (mL) borosilicate glass vials for TOC and TN collections and 30 mL acid-washed square bottles (HDPE, Nalgene, ThermoFisher Scientific, Waltham, MA, USA) for nutrient collections, and filled both vials while at reef depth. Samples were kept on ice in a cooler for less than 4 hours prior to processing. Once on board the M/V Alucia, we processed all samples. We added 75 microliters (µL) of phosphoric acid to the 40 mL glass vials to fix the samples for TOC and TN, and kept these samples at room temperature or 4°C until laboratory analysis. We removed 1.4 mL of seawater from the nutrient bottles, mixed it with 8% paraformaldehyde (1% final concentration, Electron Microscopy Sciences), fixed it in the dark for 20 minutes at 4°C, then froze it at -80°C. We capped the 30 mL inorganic nutrient bottles and placed them at -80°C until analysis.

Metabolomic Analyses: We collected seawater for targeted and untargeted metabolomic analyses at 13 reefs across the 8 zones of Florida’s Coral Reef. At each reef, we collected seawater in 1.7L Niskin bottles via SCUBA at three distinct locations on the reef for biological replication. These Niskin bottles were kept in a cooler for less than 4 hours prior to processing on the M/V Alucia. Once back on board the M/V Alucia, we transferred seawater from the Niskin bottles into acid-washed 2L polycarbonate bottles using acid-washed PharMedBPT tubing (Masterflex, Cole-Parmer, Vernon Hills, IL, USA). These water samples were processed as described previously by Weber et al. (2020). Briefly, we prefiltered the seawater through a 47-millimeter (mm) 0.1-micrometer (µm) pore size polytetrafluoroethylene filter (Omnipore, EMD Millipore Corporation, Billerica, MA, USA) to remove all microbial biomass via peristalsis and placed the filtered seawater directly into a second acid-washed 2L polycarbonate bottle. We acidified this filtrate with 2 mL OPTIMA-grade 12 M (molar) hydrochloric acid prior to solid phase extraction (SPE). We used SPE to concentrate metabolites (primarily low molecular weight dissolved organic matter) from the filtered seawater. We used a Waters vacuum manifold to slowly pass the seawater through 1 gram per 6 cubic centimeters (g/cc) SPE cartridges (Bond Elut PPL; Agilent, Santa Clara, CA, United States) pre-conditioned with HPLC-grade methanol and weighed bottles with seawater prior to and following SPE to calculate the volume of seawater filtered. SPE cartridges were wrapped in combusted aluminum foil and frozen to -80°C prior to analysis at the Woods Hole Oceanographic Institution.

Microbial Biomass and Chlorophyll Analyses: We collected seawater for microbial biomass and chlorophyll

analysis at 27 reefs across the 8 zones of Florida's Coral Reef. At each reef, we employed a groundwater pump (Mini-Monsoon 12V, Proactive Environmental Products, Bradenton, Florida, USA) to pump seawater from just above the reef benthos into acid-washed or 10% bleach-rinsed 4L LDPE bottles (Nalgene). Samples were kept in a cooler on ice until processing less than 4 hours following collection. Once back on board the M/V Alucia, we used peristalsis to filter 2L of seawater to obtain duplicates from each reef through a 0.2 µm Supor filter (Pall, Port Washington, New York, USA) for microbial biomass housed in a 25 mm filter holder (Swinnex-25, Millipore Corporation), as described previously (Becker et al. 2020). Chlorophyll samples were obtained by filtering 2L of seawater in duplicate with the same peristalsis setup, but using a GF/F filter. We placed filters (GF/F or 0.2 µm) into 2 mL cryovials and froze them at -80°C prior to further processing at the Woods Hole Oceanographic Institution.

Coral Tissue Analyses: On reefs with active stony coral tissue loss disease, we collected coral tissue and near-coral seawater samples from apparently healthy and actively diseased coral colonies. We aimed to only sample reefs where at least three healthy coral colonies were present in addition to at least three diseased colonies. Sample collection proceeded on near-coral seawater followed by coral tissue as described previously (Becker et al. 2021). Prior to tissue collection, we collected near-coral seawater via a 60 mL syringe within 1-5 cm of the lesion margin on diseased corals or healthy tissue from apparently healthy colonies. Following near-coral seawater collections, we sampled tissue of diseased corals at the lesion margin between apparently healthy tissue and bleached and sloughing tissue or apparently healthy colonies (without any indication of disease or other affliction) randomly on the coral head. We collected tissue samples with Luer slip 10 mL syringes. Syringes were quickly placed into small Whirl-Pak bags to contain any mucus and tissue leaking from the syringe. Following the collections, all tissue and near-coral seawater syringe samples were placed on ice prior to processing. We transferred samples of coral tissue and mucus into 15 ml conical tubes and froze them at -80°C until analysis. We attached filter holders containing 25 mm 0.2 µm Supor filters to the 60 mL luer-lock syringes and depressed them by hand to capture microbial biomass on the filters. We placed filters into labeled 2 mL cryovials and placed them in a -80°C freezer until analysis.

Data Processing Description

Disease prevalence and benthic composition: Stony coral tissue loss disease (SCTLD) prevalence was calculated by dividing the number of coral colonies showing active disease by the total number of colonies on the reef. Benthic composition was analyzed by using 2,500 stratified random points that were placed over the high-resolution photomosaic and classified to generate reef-wide cover estimates of calcified macroalgae, crustose coralline algae, cyanobacteria, fleshy macroalgae, hard coral, invertebrates, non-biological substrate, other, other invertebrates, seagrass, soft coral, sponge, and turf algae. Full methods for benthic coverage estimates are in Fox et al. (2019).

Flow cytometry, organic nutrient, inorganic nutrient, and chlorophyll analyses for water quality: Flow cytometry samples were processed and analyzed by the University of Hawaii SOEST Flow Cytometry Facility as described previously (Becker et al 2020). Briefly, each sample was stained with Hoechst 33342 DNA stain and excited with both 488 nanometers (nm) (1W) and UV (~350 nm, 200 mW) lasers co-linearly on a Beckman-Coulter Altra flow cytometer (Beckman Coulter Life Sciences). Signals of forward and side scatter and fluorescence were analyzed to distinguish populations and abundances (cells ml−1) of four cell types: *Prochlorococcus*, *Synechococcus*, eukaryotic picophytoplankton (picoeukaryotes), and non-pigmented bacteria. Non-pigmented prokaryotes were used as a proxy for heterotrophic bacterial and archaeal cells (Monger and Landry 1993, Marie et al. 1997).

Non-purgeable total organic carbon (TOC) samples and total nitrogen (TN) samples were run with a Shimadzu TOC-VCSH TOC analyzer (Hansell and Carlson, 2001) using a TNM-1 module. We shipped inorganic nutrient samples to Oregon State University for analysis of phosphate, ammonium, silicate, nitrite, and nitrate, as in Apprill and Rappé (2011). Briefly, samples were run on a Technicon AutoAnalyzer II (SEAL Analytical) and an Alpkem RFA 300 Rapid Flow Analyzer to generate nutrient concentrations (µM). We generated total organic nitrogen (TON) concentrations by subtracting concentrations of inorganic nitrogen (ammonium and nitrite plus nitrate) from total nitrogen.

Chlorophyll was extracted with acetone using standard methods (JGOFS, 1996). Filters were thawed individually and immediately placed in a glass test tube with 5 mL or 10 ml of 90% acetone, with 10 ml used in the case the filter appeared particularly dark, and capped. The filters were left to extract for 24 hours in the dark at 4°C. After the extraction, the tubes were vortexed and centrifuged to concentrate any particulate matter at the bottom of the tube. Prior to analysis, blanks including air, 90% acetone, and a black standard were run on an AquaFluor fluorometer (Turner Designs handheld 800446) fitted with a red-sensitive photomultiplier.

Approximately 3 mL of solvent was analyzed on the fluorometer at wavelength of 664 nm, followed by acidifying the sample with two drops of 10% hydrochloric acid, then measuring again to assess phaeopigment concentration. Readings were corrected for the volume filtered and concentration of chlorophyll was measured by referencing a standard curve.

Targeted and untargeted metabolomic laboratory processing and mass spectrometry: Untargeted samples were run 2/5/2020. We eluted dissolved organic matter (DOM) from the SPE cartridges and prepared samples for analyses as outlined by Weber and colleagues (2020). To summarize, 4 bed-volumes of 0.01 M HCl were added to the cartridges to remove salt. The cartridges were then dried for five minutes, and eluted into combusted glass vials using 6 mL of 100% methanol. Extracts were frozen at -20ºC until they were dried down using a vacuum centrifuge. Extracts were then resuspended with a 95:5 (v/v) MilliQ water: acetonitrile (ACN) solution with deuterated biotin (final concentration 0.05 milligrams per milliliter (mg per mL) (200 µL total) and vortexed. Pooled samples were made for all mass spectrometry runs by combining equal volumes of all extracts into one vial. The pooled samples were used for monitoring instrument drift and run quality. After preparation, all extracts were stored at -20◦C until analysis. For targeted metabolomics, 100 µL aliquots of each extract was placed in a separate vial with a combusted glass insert. For the untargeted metabolomics analysis, 600 µL of the deuterated biotin standard and water: ACN solution was used to dilute a 25 µL aliquot of each extract. Untargeted metabolite analysis was performed using an ultrahigh-performance liquid chromatography system (Vanquish UHPLC, Thermo ScientificTM) coupled with an Orbitrap Fusion Lumos Tribid mass spectrometer (Thermo ScientificTM). A Vanguard pre-column and Waters Acquity HSS T3 column (2.1 mm × 100 mm, 1.8 µm), was used for chromatographic separation at 40ºC. The column was eluted at 0.5 mL per minute with the following solvents: A) 0.1% formic acid in water and B) 0.1% formic acid in ACN. The chromatographic gradient was: 1% B for 1 min, 15% B for 1-3 minutes, 50% B for 3-6 minutes, 95% B for 6-9 minutes, and 95% B for 10 minutes. Between injections, the column was washed and re-equilibrated with 1% B for 2 minutes. Individual autosampler injections (5 µL each) were made for negative and positive ion mode analyses. In negative ion mode, the electrospray voltage was set to 2600 volts (V). Settings for source gases were 55 (sheath), 20 (auxiliary), and 1 (sweep) in arbitrary units. The temperatures of the heated capillary and vaporizer were 350ºC and 400ºC, respectively. MS data were collected in the Orbitrap analyzer with a mass resolution of 120,000 FWHM at m/z 200. The automatic gain control (AGC) target was 4e5, with a 50 sec maximum injection time, and a scan range of 100 to 1000 m/z. Data-dependent MS/MS spectra were collected at 7,500 resolution in the Orbitrap analyzer. Parent ions were isolated with a 1 m/z width in the quadrupole, and fragmented with a HCD (higher energy collisional dissociation) energy of 35%. All data were collected in profile mode. Samples were run in random order and after every seven samples, a pooled sample was run. Raw data files from the instrument were converted into mzML files using msConvert and then processed using XCMS (Smith et al., 2006). Peak-picking was performed with the CentWave algorithm and a Gaussian fit with the following parameters: noise = 10000, peak-width = 3-15, ppm = 15, prefilter = c(2,168.600), integrate = 2, mzdiff = -0.005, snthresh = 10. Retention times were then adjusted using Orbiwarp and correspondence between the peaks was conducted. The coefficient of variation across the eight untargeted pooled sample features was 0.044, demonstrating good agreement between the pooled samples, and the pooled samples were removed from further analyses. Untargeted peak intensities were normalized by dividing the peak intensities by the total seawater volume. In the untargeted analysis, only MS1 features were analyzed, and were defined as unique combinations of mass-to-charge ratios (m/z) and retention times (RT). This analysis yielded a table of MS1 features (m/z x RT) and their peak intensities across each sample.

Extracts prepared for targeted metabolomics were run on a triple-stage quadrupole mass spectrometer (TSQ Vantage, Thermo Fisher ScientificTM) using UPLC (Accela Open Autosampler and Accela 1250 Pump, Thermo ScientificTM) coupled to a heated electrospray ionization source (H-ESI) and operated in selective reaction monitoring (SRM) mode. The same chromatography column, conditions, gradient, and flow rates were used for targeted analyses as those described for untargeted analyses. Separate autosampler injections of 5 µL each were made for positive and negative modes. Additionally, as with the untargeted analysis, samples were run in random order and pooled samples were run every seven samples. SRM parameters were optimized for each compound using a standard as described in Kido Soule et al. (2015) and two SRM transitions (precursor-product ion pairs) were monitored for quantification and confirmation. Target metabolites included compounds found in central carbon metabolism and metabolites that are environmentally relevant in marine habitats or are produced by marine microorganisms (Fiore et al. 2015, Fiore et al. 2017, Kido Soule et al. 2015). The resulting XCalibur raw files (MS/MS data) were converted into mzML files using msConvert (Chambers et al., 2012) and processed with the open-source program El-MAVEN (v.774)(Agrawal et al., 2019) . Using El-MAVEN, 8-point calibration curves based on integrated peak area were generated for each compound. Environmental concentrations of metabolites were determined by dividing each concentration by the original sample collection volume. Next, metabolites that passed the limits of detection and quantification for the UPLC-MS/MS analysis (Kido Soule, Longnecker, Swarr, unpublished) were corrected for extraction efficiency based on published data for each metabolite in seawater (Johnson et al., 2017).

DNA extraction and sequencing for 16S rRNA and shotgun metagenomes: We extracted DNA from 25 mm filters used for 2L seawater collections using Qiagen PowerBiofilm kits (Qiagen, Germantown, MD, USA). To begin, we added the filter directly to the bead tube, then proceeded with the extraction following manufacturer protocols. We also included four DNA extraction controls by extracting DNA from unused filters. Resulting DNA from these extractions was used as the template for both 16S rRNA gene sequencing and shotgun metagenomic sequencing.

For 16S rRNA gene sequencing of bacteria and archaea, we included 2 µL of template DNA into a 50 µL (total volume) PCR reaction. We added a PCR negative control by including one PCR reaction with 2 µL of PCR grade H2O instead of DNA. Earth Microbiome Project primers, 515F (Parada et al., 2016) and 806R (Apprill et al., 2015), were used to amplify the V4 region of the small subunit (SSU) rRNA gene in bacteria and archaea and included sample-specific barcodes with an 8 bp barcode, 10 bp pad, and 2 bp link, similar to Kozich et al. (2013). The 50 µL reactions were diluted in UV-sterilized nuclease-free water and contained 2.5 units of GoTaq DNA Polymerase (Promega, Madison, WI, USA), barcoded primers at 0.2 µM, 0.2 mM dNTP mix (Promega), 2.5 mM MgCl2, and 1X colorless GoTaq flexi buffer (Promega). The reactions were run on a Bio-Rad Thermocycler using the following criteria: denaturation at 95°C for 2 minutes; 28 cycles at 95°C for 20 seconds, 55°C for 15 seconds, and 72°C for 5 minutes; and extension at 72°C for 10 minutes. We used gel electrophoresis to verify successful amplification using 5 µL of product on a 1% agarose-Tris-borate-EDTA (TBE) gel stained with SYBR Safe gel stain (Invitrogen, ThermoFisher Scientific). We used the QIAquick 96 PCR Purification Kit (Qiagen) with the QIAvac 96 (Qiagen) and vacuum pressure to purify the remaining 45 µL of PCR products following manufacturer's protocols. We applied the HS dsDNA assay on the Qubit 2.0 fluorometer (ThermoFisher Scientific) to quantify the DNA concentrations then converted to nM assuming an average library size of 450 bp, and average molar mass of DNA nucleotides of 660 g/mol. We diluted individual barcoded PCR products to 10 nM, pooled all samples, and shipped the pooled, ready-to-run library to the Georgia Genomics and Bioinformatics Core at the University of Georgia for sequencing on an Illumina MiSeq using paired-end 250 bp sequencing.

We prepared a library for shotgun metagenomic sequencing following the Illumina DNA Prep Reference Guide (Illumina, San Diego, CA, USA, Document # 1000000025416 v09 June 2020). DNA input for all samples was between 100-500 ng. Concentration of the four DNA extraction control samples was below detection, so we included 30 µL of each in the procedure and processed in the same way as all seawater samples. We used IDT for Illumina DNA/RNA UD Indexes Set A, Tagmentation (Illumina, 96 samples, Cat # 20027213), to apply sample-specific indices to each sample. Following the procedure, we eluted samples in 30 µL resuspension buffer. To verify successful processing, we used a fluorometric assay (HS dsDNA) on a Qubit 2.0 fluorometer to measure DNA concentrations of a subset of samples. All final concentrations were greater than 4 ng/µL, and therefore deemed sufficient for pooling. All samples were pooled, and the final concentration was 5.30 ng/µL. The final library was run at the Georgia Genomics and Bioinformatics Core at the University of Georgia on an Illumina NextSeq 2000 with the P3 flow cell and paired-end 150 bp sequencing.

On-ship near-coral seawater sample processing and sequencing: To expedite the turnaround time between sample collection and sample processing, we performed on-ship DNA extraction, PCR, and sequencing with the Illumina iSeq 100 System on near-coral seawater samples following methods for in-the-field microbiome preparation described previously (Becker et al., 2021). Seawater samples targeted for on-ship sequencing included those sampled from 5 reefs over two days (June 9-10, 2019).

DNA extraction and sequencing of near-coral seawater and coral microbiomes: All processing of near-coral seawater and coral tissue slurries proceeded as described previously to identify bacteria and archaea within each environment (Becker et al., 2021). Briefly, we extracted DNA from all seawater and tissue samples using the DNeasy PowerBiofilm kit (Qiagen). PCR occurred in a two-stage procedure. In stage one, we used Earth Microbiome Project primers, 515F (Parada et al., 2016) and 806R (Apprill et al., 2015), to amplify the V4 region of the small subunit (SSU) ribosomal RNA gene of bacteria and archaea. In stage two, we attached unique index primers to each sample using the Nextera XT v2 set A kit (Illumina). For PCR that occurred at the Woods Hole Oceanographic Institution, we used larger benchtop centrifuges (Eppendorf 5418) and thermocyclers (Bio-Rad), rather than the small and portable versions used on the M/V Alucia. Following purification of stage two PCR products, we diluted and pooled samples such that we included approximately 40 samples. Seawater and tissue samples were randomized across all library pools. Pooled libraries were diluted to approximately 90 picomolar (pM), and a 10% PhiX Control v3 (Illumina) spike-in was added to increase base diversity. All libraries were run on the Illumina iSeq 100 System (Illumina) with the i1 cartridge pack, over a total of 6 sequencing runs.

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Data Files

File
alucia_reef_biogeochem.csv (Comma Separated Values (.csv), 209.59 KB) MD5:4352f08ed2eda592e4bc17f16d318b8a Primary data file for dataset ID 890979

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Related Publications

Agrawal, S., Kumar, S., Sehgal, R., George, S., Gupta, R., Poddar, S., Jha, A., & Pathak, S. (2019). El-MAVEN: A Fast, Robust, and User-Friendly Mass Spectrometry Data Processing Engine for Metabolomics. Methods in Molecular Biology, 301–321. https://doi.org/10.1007/978-1-4939-9236-2_19
Methods

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Methods

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Methods

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Methods

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Methods

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Methods

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Methods

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Methods

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Methods

JGOFS (1996) Protocols for the joint global ocean flux study (JGOFS) core measurements (Report 19), Bergen, Norway: IOC SCOR.
Methods

Johnson, W. M., Kido Soule, M. C., & Kujawinski, E. B. (2017). Extraction efficiency and quantification of dissolved metabolites in targeted marine metabolomics. Limnology and Oceanography: Methods, 15(4), 417–

428. doi:10.1002/lom3.10181
Methods

Kido Soule, M. C., Longnecker, K., Johnson, W. M., & Kujawinski, E. B. (2015). Environmental metabolomics: Analytical strategies. Marine Chemistry, 177, 374–387. doi:10.1016/j.marchem.2015.06.029
Methods

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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
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Methods

Smith, C. A., Want, E. J., O'Maille, G., Abagyan, R., & Siuzdak, G. (2006). XCMS: Processing Mass Spectrometry Data for Metabolite Profiling Using Nonlinear Peak Alignment, Matching, and Identification. Analytical Chemistry, 78(3), 779–787. doi:10.1021/ac051437y
Methods

Weber, L., Armenteros, M., Kido Soule, M., Longnecker, K., Kujawinski, E. B., & Apprill, A. (2020). Extracellular Reef Metabolites Across the Protected Jardines de la Reina, Cuba Reef System. Frontiers in Marine Science, 7. doi:10.3389/fmars.2020.582161
Methods

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Parameters

Parameter	Description	Units
sample_name	Generic name of a sample. Could be an individual water or tissue sample for sequencing or the collection of metadata from a site that was not used for sequencing.	unitless
analysis_type	Quick description of the type of sample included. One of: metabolome, environmental metadata, 16S rRNA microbiome, or shotgun metagenome	unitless
Project_Accession	Project accession number for either the MetaboLights database or NCBI Sequence Read Archive. For the environmental metadata, this is not relevant. "Not applicable" = data is not relevant	unitless
biosample_accession	Accession number for a specific sample. Only relevant for the sequencing data uploaded to the NCBI Sequence Read Archive. "Not applicable" = data is not relevant	unitless
sample_type	Sample type is one of, seawater metabolome, environmental metadata, seawater metagenome, or coral metagenome	unitless

stationID	Generic name of the reef site used for the study. Not applicable is used for any laboratory-added control samples not collected at a reef. "Not applicable" = data is not relevant	unitless
collection_date	Date of collection, typically in format YYYY-MM-DD; years of 2020 or 2021 indicate controls that were introduced into the analysis during laboratory processing at Woods Hole Oceanographic Institution (WHOI).	unitless
depth	Depth of reef. "Not applicable" = data is not relevant	meters
geo_loc_name	Broad location of the collection (Country: State); samples with "Massachusetts" in this column are controls that were introduced into the analysis during laboratory processing at Woods Hole Oceanographic Institution (WHOI).	unitless
latitude	Station latitude, south is negative. "Not applicable" = data is not relevant	decimal degrees
longitude	Station longitude, west is negative. "Not applicable" = data is not relevant	decimal degrees
replicate	Replicate if there is duplicate metadata for a sample	unitless
depth_ft	Depth of reef. "Not applicable" = data is not relevant	feet
host	Species of coral host of microbiome sample, only relevant for coral metagenome samples and near-coral seawater samples. "Not applicable" = data is not relevant	unitless
ZoneNum	Zone of Florida's Coral Reef sampled. One of eight zones. "Not applicable" = data is not relevant	unitless
FL_Region	Name of the Florida's Coral Reef where sample was collected. "Not applicable" = data is not relevant	unitless
Collaborator_siteID	Name of reef if it falls under an existing monitoring program. "Not applicable" = data is not relevant	unitless
Habitat	Reef type. "Not applicable" = data is not relevant	unitless
prochlorococcus_cells_per_ml	Number of prochlorococcus cyanobacteria cells per milliliter in reef seawater. "Not applicable" = data is not relevant; "Not collected" = data not collected	cells per milliliter
synechococcus_cells_per_ml	Number of Synechococcus cyanobacteria cells per milliliter in reef seawater. "Not applicable" = data is not relevant; "Not collected" = data not collected	cells per milliliter
picoeukaryotes_cells_per_ml	Number of photosynthetic picoeukaryote cells per milliliter in reef seawater. "Not applicable" = data is not relevant; "Not collected" = data not collected	cells per milliliter

unpigmented_cells_per_ml	Number of unpigmented bacteria and archaea, also referred to as heterotrophic microbes, cells per milliliter in reef seawater. "Not applicable" = data is not relevant; "Not collected" = data not collected	cells per milliliter
npoc_uM	Non-purgeable organic carbon, referred to as total organic carbon, in reef seawater. "Not applicable" = data is not relevant; "Not collected" = data not collected	micromolar (uM)
tn_uM	Total nitrogen, which includes organic and inorganic nitrogen. "Not applicable" = data is not relevant; "Not collected" = data not collected	micromolar (uM)
phosphate_uM	Phosphate in reef seawater. "Not applicable" = data is not relevant; "Not collected" = data not collected	micromolar (uM)
nitriteandnitrate_uM	Nitrite plus nitrate concentrations in reef seawater. "Not applicable" = data is not relevant; "Not collected" = data not collected	micromolar (uM)
silicate_uM	Silicate concentrations in reef seawater. "Not applicable" = data is not relevant; "Not collected" = data not collected; "below detection" = below detection limit	micromolar (uM)
nitrite_uM	Nitrite concentrations in reef seawater. "Not applicable" = data is not relevant; "Not collected" = data not collected	micromolar (uM)
ammonium_uM	Ammonium concentrations in reef seawater. "Not applicable" = data is not relevant; "Not collected" = data not collected	micromolar (uM)
nitrate_uM	Nitrate concentrations in reef seawater. "Not applicable" = data is not relevant; "Not collected" = data not collected; "below detection" = below detection limit	micromolar (uM)
Temperature_C	Temperature of seawater taken from Exo2 sonde or CastAway CTD (SonTek). "Not applicable" = data is not relevant; "Not collected" = data not collected	degrees Celsius
Dissolved_Oxygen_at_depth_percent_saturation	Dissolved oxygen of seawater taken from Exo2 sonde (SonTek). "Not applicable" = data is not relevant; "Not collected" = data not collected	Percent saturation
Dissolved_Oxygen_at_depth_mg_per_liter	Dissolved oxygen of seawater taken from Exo2 sonde (SonTek). "Not applicable" = data is not relevant; "Not collected" = data not collected	milligrams per liter (mg/L)
SCTLDprevalence	Percent of live coral on the reef with stony coral tissue loss disease. "Not applicable" = data is not relevant	percent
Calcified_Macroalgae	Amount of calcified macroalgae on the reef. "Not applicable" = data is not relevant; "Not calculated" = image data was collected but has not been used to calculate the parameter	percent
Crustose_Coralline_Algae	Amount of crustose coralline algae on the reef. "Not applicable" = data is not relevant; "Not calculated" = image data was collected but has not been used to calculate the parameter	percent

Cyanobacteria	Amount of cyanobacterial mats on the reef. "Not applicable" = data is not relevant; "Not calculated" = image data was collected but has not been used to calculate the parameter	percent
Fleshy_Macroalgae	Amount of fleshy macroalgae on the reef. "Not applicable" = data is not relevant; "Not calculated" = image data was collected but has not been used to calculate the parameter	percent
Hard_coral	Amount of hard coral on the reef	percent
Invertebrates	Amount of invertebrates on the reef. "Not applicable" = data is not relevant; "Not calculated" = image data was collected but has not been used to calculate the parameter	percent
Non_biological	Amount of non-biological substrate on the reef. "Not applicable" = data is not relevant; "Not calculated" = image data was collected but has not been used to calculate the parameter	percent
Other	Amount of other organisms and substrates on the reef. "Not applicable" = data is not relevant; "Not calculated" = image data was collected but has not been used to calculate the parameter	percent
Other_invertebrates	Amount of other invertebrates on the reef. "Not applicable" = data is not relevant; "Not calculated" = image data was collected but has not been used to calculate the parameter	percent
Seagrass	Amount of seagrass on the reef. "Not applicable" = data is not relevant; "Not calculated" = image data was collected but has not been used to calculate the parameter	percent
Soft_coral	Amount of soft coral on the reef. "Not applicable" = data is not relevant; "Not calculated" = image data was collected but has not been used to calculate the parameter	percent
Sponge	Amount of sponge on the reef. "Not applicable" = data is not relevant; "Not calculated" = image data was collected but has not been used to calculate the parameter	percent
Turf_Algae	Amount of turf algae on the reef. "Not applicable" = data is not relevant; "Not calculated" = image data was collected but has not been used to calculate the parameter	percent
Chlorophyll_mg_per_l	Amount of chlorophyll-a in the reef seawater. "Not applicable" = data is not relevant; "Not collected" = data not collected	milligrams per liter
Phaeophytin_mg_per_l	Amount of phaeophytin in the reef seawater. "Not applicable" = data is not relevant; "Not collected" = data not collected	milligrams per liter
coral	Four-letter AGGRA code indicating the coral species from which the tissue or near-coral seawater sample was collected. "Not applicable" = data is not relevant	unitless

healthbinary	binary indicator of apparent condition of the coral colony with H = healthy and D = stony coral tissue loss disease. A third category of BS = black spot for any Siderastrea spp. corals that appeared to have a different disease. "Not applicable" = data is not relevant	unitless
libraryID	Letter indicating the sequencing library the sample was added to. Multiple sequencing libraries were used to accommodate all the samples so we aimed to record this in case of any sequencing run bias. "Not applicable" = data is not relevant	unitless
sampleID	Another sample ID specific to the near-coral seawater and coral microbiome dataset within the project. "Not applicable" = data is not relevant	unitless
coralspecies	Genus species of corals sampled. "Not applicable" = data is not relevant	unitless
sworcoral	Description of whether the sample was from Coral = slurry of mucus, tissue, skeleton, and seawater, or Near-coral seawater = 50 ml syringe sample from less than 5 cm from the coral surface. "Not applicable" = data is not relevant	unitless
healthlong	For samples in the coral and near-coral seawater microbiome dataset, description of the apparent health state of the coral, or whether the sample was a laboratory control. "Not applicable" = data is not relevant	unitless

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Instruments

Dataset-specific Instrument Name	Illumina MiSeq
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	Illumina MiSeq using paired-end 250 bp sequencing - for 16S rRNA gene sequencing libraries of bulk reef seawater
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	Illumina NextSeq 2000
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	Illumina NextSeq 2000 with the P3 flow cell and paired-end 150 bp sequencing for shotgun metagenomics
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	Illumina iSeq 100 System
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	Illumina iSeq 100 System (Illumina) with the i1 cartridge, and 2x150 bp sequencing for 16S rRNA libraries of coral and near-coral seawater
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	Beckman-Coulter Altra flow cytometer
Generic Instrument Name	Flow Cytometer
Dataset-specific Description	Beckman-Coulter Altra flow cytometer (Beckman Coulter Life Sciences) for enumerating cell abundances
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	Accela Open Autosampler
Generic Instrument Name	Laboratory Autosampler
Dataset-specific Description	Triple stage quadrupole mass spectrometer (TSQ Vantage, Thermo Fisher ScientificTM) using UPLC (Accela Open Autosampler and Accela 1250 Pump, Thermo ScientificTM) coupled to a heated electrospray ionization source (H-ESI) for targeted metabolite analysis
Generic Instrument Description	Laboratory apparatus that automatically introduces one or more samples with a predetermined volume or mass into an analytical instrument.

Dataset-specific Instrument Name	Orbitrap Fusion Lumos Tribid mass spectrometer
Generic Instrument Name	Mass Spectrometer
Dataset-specific Description	Ultrahigh performance liquid chromatography system (Vanquish UHPLC, Thermo ScientificTM) coupled with an Orbitrap Fusion Lumos Tribid mass spectrometer (Thermo ScientificTM) for untargeted metabolomics
Generic Instrument Description	General term for instruments used to measure the mass-to-charge ratio of ions; generally used to find the composition of a sample by generating a mass spectrum representing the masses of sample components.

Dataset-specific Instrument Name	Niskin bottles
Generic Instrument Name	Niskin bottle
Dataset-specific Description	1.7 Liter Niskin bottles - seawater sampling
Generic Instrument Description	A Niskin bottle (a next generation water sampler based on the Nansen bottle) is a cylindrical, non-metallic water collection device with stoppers at both ends. The bottles can be attached individually on a hydrowire or deployed in 12, 24, or 36 bottle Rosette systems mounted on a frame and combined with a CTD. Niskin bottles are used to collect discrete water samples for a range of measurements including pigments, nutrients, plankton, etc.

Dataset-specific Instrument Name	groundwater pump
Generic Instrument Name	Pump
Dataset-specific Description	Mini-Monsoon 12V, Proactive Environmental Products, Bradenton, Florida, USA - to pump seawater from just above the reef benthos
Generic Instrument Description	A pump is a device that moves fluids (liquids or gases), or sometimes slurries, by mechanical action. Pumps can be classified into three major groups according to the method they use to move the fluid: direct lift, displacement, and gravity pumps

Dataset-specific Instrument Name	TSQ Vantage, Thermo Fisher ScientificTM
Generic Instrument Name	Quadrupole Mass Spectrometer
Dataset-specific Description	Triple stage quadrupole mass spectrometer (TSQ Vantage, Thermo Fisher ScientificTM) using UPLC (Accela Open Autosampler and Accela 1250 Pump, Thermo ScientificTM) coupled to a heated electrospray ionization source (H-ESI) for targeted metabolite analysis
Generic Instrument Description	A piece of apparatus that consists of an ion source, a mass-to-charge analyser, a detector and a vacuum system and is used to measure mass spectra. The detector is a quadrupole mass-to-charge analyser, which holds the ions in a stable orbit by an electric field generated by four parallel electrodes.

Dataset-specific Instrument Name	luer slip and luer lock syringes
Generic Instrument Name	syringe
Dataset-specific Description	10 mL luer slip and 60 mL luer lock syringes to collect coral tissue and near coral seawater, respectively
Generic Instrument Description	A device used to inject fluids into or withdraw them from something; consists of a hollow barrel fitted with a plunger and a hollow needle.

Dataset-specific Instrument Name	Technicon AutoAnalyzer II
Generic Instrument Name	Technicon AutoAnalyzer II
Dataset-specific Description	Technicon AutoAnalyzer II (SEAL Analytical) and an Alpkem RFA 300 Rapid Flow Analyzer to generate nutrient concentrations (µM)
Generic Instrument Description	A rapid flow analyzer that may be used to measure nutrient concentrations in seawater. It is a continuous segmented flow instrument consisting of a sampler, peristaltic pump, analytical cartridge, heating bath, and colorimeter. See more information about this instrument from the manufacturer.

Dataset-specific Instrument Name	Shimadzu TOC-VCSH TOC analyzer
Generic Instrument Name	Total Organic Carbon Analyzer
Dataset-specific Description	Shimadzu TOC-VCSH TOC analyzer (6) using a TNM-1 module for TOC and TN analysis
Generic Instrument Description	A unit that accurately determines the carbon concentrations of organic compounds typically by detecting and measuring its combustion product (CO2). See description document at: http://bcodata.whoi.edu/LaurentianGreatLakes_Chemistry/bs116.pdf

Dataset-specific Instrument Name	AquaFluor fluorometer
Generic Instrument Name	Turner Designs Aquafluor 8000
Dataset-specific Description	AquaFluor fluorometer (Turner Designs handheld 800446) fitted with a red sensitive photomultiplier for Chlorophyll analysis
Generic Instrument Description	The Turner Designs Aquafluor 8000 is a lightweight, handheld fluorometer/turbidimeter ideal for field use. It can be configured with one or two channels to measure turbidity, chlorophyll, algae, dyes, ammonium, CDOM, and more. Detailed description at https://www.turnerdesigns.com/aquafluor-handheld-fluorometer

Dataset-specific Instrument Name	Vanquish UHPLC, Thermo ScientificTM
Generic Instrument Name	Ultra high-performance liquid chromatography
Dataset-specific Description	Ultrahigh performance liquid chromatography system (Vanquish UHPLC, Thermo ScientificTM) coupled with an Orbitrap Fusion Lumos Tribid mass spectrometer (Thermo ScientificTM) for untargeted metabolomics
Generic Instrument Description	Ultra high-performance liquid chromatography: Column chromatography where the mobile phase is a liquid, the stationary phase consists of very small (< 2 microm) particles and the inlet pressure is relatively high.

Dataset-specific Instrument Name	Waters Vacuum Manifold (Agilent)
Generic Instrument Name	vacuum manifold
Dataset-specific Description	Waters vacuum manifold and 1 g/6 cc SPE cartridges (Bond Elut PPL; Agilent, Santa Clara, CA, United States) for concentrating seawater dissolved metabolites
Generic Instrument Description	A device that is used for the vacuum-driven processing of multiwell strips or plates, or spin columns.

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Deployments

OceanX

Website	https://www.bco-dmo.org/deployment/891192
Platform	Alucia
Start Date	2019-06-03
End Date	2019-06-20
Description	M/V Alucia OceanX Cruise June 3 to 20, 2019 Main Scientists: Amy Apprill (aapprill@whoi.edu), Erinn Muller (emuller@mote.org), Stuart Sandin (ssandin@ucsd.edu) Left from Miami, FL, and sampled reefs from near Miami, FL to Dry Tortugas National Park

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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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