Microbial abundances and DOC drawdown from algal substrate remineralization cultures in the FlavoPH experiment collected on cruise HIMB15-1 at Kaneohe Bay, Oahu, Nov. 2015

Website: https://www.bco-dmo.org/dataset/751530
Data Type: experimental, Cruise Results
Version: 0
Version Date: 2018-12-26

Project
» Collaborative Research: Dissolved organic matter feedbacks in coral reef resilience: The genomic & geochemical basis for microbial modulation of algal phase shifts (Coral DOM2)

Contributors	Affiliation	Role
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Abstract
This dataset includes microbial abundances and DOC drawdown from algal substrate remineralization cultures during the FlavoPH experiment. Samples were collected on cruise HIMB15-1 at Kaneohe Bay, Oahu in November 2015.

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Coverage

Spatial Extent: **Lat**:21.4339 **Lon**:-157.7881
Temporal Extent: 2015-11-10 - 2015-11-11

Dataset Description

This dataset includes microbial abundances and DOC drawdown from algal substrate remineralization cultures during the FlavoPH experiment. Samples were collected on cruise HIMB15-1 at Kaneohe Bay, Oahu in November 2015.

Methods & Sampling

DRAFT!

The data collected by the microbial partner will provide added value to the assessment and monitoring of coral reefs by combining the microbial taxonomic and functional composition and the fluxes of matter and energy they facilitate with the data on benthic and pelagic macro-biota. This will allow for characterization of coral reef

ecosystems from a molecular to an ecosystem scale across the entire US pacific.

We worked for about a year in 2015/2106 to improve our collection protocols for HARAMP based on criticisms we received from CRED. While we can see that this is still a work in progress, please note that we accomplished reducing seawater collections from about 100 liters to 15 liters and that we eliminated the task of delivering the microbial diver back to the ship early. We will continue to prioritize improvements to our protocols in order to strengthen the partnership between our team and CRED.

Collection of water chemistry using Minidon Niskin bottles

Primary goal: Collect water from reef benthos (light reef), reef matrix (dark reef), reef water column (5 meters) and offshore water column (5 meters) for DOC and inorganic nutrient concentrations, microbial abundances, and microbial DNA.

This provides (A) Most of the long term monitoring samples (water chemistry: organic carbon, inorganic nutrients; microbial activity: abundances and biomass, autotroph:heterotroph) and (B) On this round of cruises we have paired our sampling (reef, surface, offshore) with the OCC team's water samples for inorganic measurements (DIC/TA).

Procedural overview: The minidons will replace the standard 2 liter Niskin water chemistry set. The minidons allow for filtration of seawater through filter apparatus into the analytical vials during the dive. At every reef site, samples will be collected from 1) the reef benthos, 2) the reef matrix, and 3) the reef water column. Offshore samples will be collected opportunistically whenever the OCC team samples there. Each pair of minidons will produce the following analytes:

- DOC in a 60 ml plastic vial (via a 25mm GF/F) (1)
- fDOM in a 20 ml plastic vial (via a 0.2 um polycarbonate filter) (1)
- inorganic nutrients in a 20 ml plastic vial (via a 0.2 um polycarbonate filter) (1)
- microbial sizes and abundances (epi-tubes with 1 ml fixed seawater) (2)
- flow cytometry (cryovials with seawater fixed in PFA or glutaraldehyde) (3)
- 0.2um Sterivex filter for extraction of microbial DNA (1)

Two minidons will be deployed at each collection site (as listed above). When the paired minidons are taken apart for labeling and storage, each analyte will be pooled into one vial (detailed below).

Collection of water samples using Mega-Niskin (10 liters water) for SPE-DOM (Solid Phase Eluted Dissolved Organic Matter) and benthic community sample (2 liters water collected via bilge pump)

Primary goal: Isolation of DOM from seawater with low salt contamination for downstream analysis by HPLC, LCMS, NMR, or FTICRMS. These analyses will yield information concerning both the quality and quantity of DOM in benthic-associated seawater. Benthic associated samples will also be collected and processed to yield viromes via serial filtration and PEG precipitation. These should be collected along with the minidons as much as possible.

This provides (A) deeper characterization of the organic matter pool on reefs and (B) other long term monitoring samples (benthic microbiomes and viromes for metagenomic sequencing).

Procedural overview SPE-DOM: Large volumes of water (10L) are collected in inert polycarbonate Niskin-type bottles and pressure-filtered through 0.2 um filter to remove particles. The water is acidified to pH 2 with concentrated HCl to increase extraction efficiency. The acidified water is passed over the PPL sorbent (pre-cleaned with methanol) using a peristaltic pump at a flow rate < 40 mL/min to bind SPE-DOM, requiring roughly 4h. The cartridges are rinsed twice with 1 volume 0.01 M HCl made in low-DOC water to remove salts. The cartridges are dried with 5 minutes of airflow and immediately eluted with 1 volume methanol at a flow rate < 2 mL/min into borosilicate vials with crimp-seal teflon-lined silicone lids and stored –20°C.

Benthic metagenomes: This protocol is to replace the 80 liter water collections in cubies and TFF concentration methods. Instead, 1 cubie will be filled about ¼ full with the bilge pump (vacuum the reef as usual benthic metagenome collection process). On surface, pour water from the cubie into a 2 liter Niskin. Microbial metagenomes will be collected onto 0.45um Sterivex filters. The 0.45 um filtrate will be collected into Nalgene bottles (600 mls, PEG precipitates) and onto 0.03 um PES filters (the remaining 1400ml). PEG precipitates will be combined by island to generate "pooled viromes" via CsCl. Site level comparisons will be made using "total viral DNA" collected onto 0.03um filters.

Collection of coral:algal interaction tissue biopsies

Primary goal: Microbiologist will collect 1 coral:algal biopsy transects across coral-algal interaction interfaces per site (goal: 2-4 punch transects per island depending on island size. Biopsies will be processed to yield coral and algal metagenomes, metatranscriptomes, viromes, and metabolomes. These samples are to be collected at reef sites, but do not need to be at NCRMP sites.

These collections (A) replace the collections of rubble and algae (i.e., smashed reef) that we have collected on previous cruises and (B) provide a spatial dataset for investigating mechanisms of coral resistance to algal competition at coral:algal interaction interfaces.

Procedural overview: Find a coral-algal interaction with enough surface area to take 11 punches. For coral species, we are aiming for Porites lobata or Pocillopora meandrina. Use underwater drill to collect 1 cm diameter "biopsies" from coral algal interactions. Once back on ship, remove samples from drill bit into their respective vials: (a) virome samples (n = 3) into cryovial, then into dewer; (b) metatranscriptome (n = 5) into cryovial with 1 ml RNA later (c) metabolome samples (n = 3) into 20 ml amber vials with 5 ml 70% methanol.

Measurement of coral geometry

Primary goal: Microbiologists will take high resolution images of single coral colonies to create 3D coral models, which will allow for the calculation of precise surface area, perimeter, and rugosity measurements.

Procedural overview: Once the coral to image has been chosen, lay a chain link on the coral interface and try to get the link as close to the perimeter as possible without blocking the camera's view of the interface. Start imaging the perimeter from about 25 cm working distance. Try to achieve a 90% overlap with each picture and keep the same camera orientation as you move around the coral. Make sure the chain link is in at least one of the images. Also, if there are other interactions within the colony, repeat the process for those interactions and include at least one chain link for calibration.

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Remove the chain after you're done.From MCR_HIGH RES_DOC methods - delete as needed:

Procedural overview:

- All samples were collected via boat, using 1L square Polycarbonate bottles (surface grabs).
- Bottles were gravity filtered (combusted 47mm GF/F) into glass vials.
- Samples were returned to the on-shore laboratory, acidified and stored at Room Temperature.
- Samples were shipped to Craig Carlson's Laboratory at UCSB for analysis using the HTCO Method (Carlson, et al. 2010 DSRII).

DOC analysis methodology (from Carlson et al (2010)).

All samples were analyzed via high-temperature combustion on Shimadzu TOC-V analyzers that were slightly modified from the manufacturer's model system. The condensation coil was removed and the head space of an internal water trap was reduced to minimize system dead space. The combustion tube contained 0.5 cm Pt pillows placed on top of Pt alumina beads to improve peak shape and to reduce alteration of the combustion matrix throughout the analytical run. CO2-free carrier gas was delivered to the TOC-V systems via commercial ultra high purity gas cylinders or a Whatmans gas generator. Three milliliters of sample were drawn into a 5 ml injection syringe, acidified with 2 M HCL (1.5%), and sparged for 1.5 min with CO2-free gas. Three to five replicate 100 ml of sample were injected into the combustion tube heated to 680 1C. A magnesium perchlorate trap was added to the existing water and halide traps to ensure removal of water vapor from the gas line prior to entering a nondispersive infrared detector. The resulting peak area was integrated with Shimadzu chromatographic software.
Extensive conditioning of the combustion tube with repeated injections of low carbon water (LCW) and deep seawater was essential to minimize the machine blanks. The system response was standardized daily with a four-point calibration curve of potassium hydrogen phthalate solution in LCW. Sample and reference swapping and intercalibration exercises were conducted periodically between the UCSB and University of Miami to ensure comparability between sample sets. All samples were systematically referenced against low carbon water, deep Sargasso Sea reference waters (2600 m), and surface Sargasso Sea water every 6-8 analyses (Hansell and Carlson, 1998; Carlson et al., 2004). Daily reference waters were calibrated with DOC Consensus Reference Waters (Hansell, 2005). The standard deviation of the deep and surface references analyzed throughout a run generally had a coefficient of variation ranging between 1-2% over the 3-7 independent analyses (number of

references depended on the size of the run), allowing resolution of approximately 1 mmol/kg in the deep waters.

Data 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
- corrected ISO_DateTime to include reported time rather than 00:00:00; removed separate Time column
- rearranged columns for database best practices
- re-formatted date from m/d/yyyy to yyyy-mm-dd
- added ISO Date format generated from date and time values
- replaced blank cells with ‘nd’, no data
- reduced number of significant digits of latitude and longitude to reflect sampling precision methods

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Related Publications

Carlson, C. A., Hansell, D. A., Nelson, N. B., Siegel, D. A., Smethie, W. M., Khatiwala, S., Meyers, M. M., Halewood, E. (2010). Dissolved organic carbon export and subsequent remineralization in the mesopelagic and bathypelagic realms of the North Atlantic basin. Deep Sea Research Part II: Topical Studies in Oceanography, 57(16), 1433–1445. doi:10.1016/j.dsr2.2010.02.013
Methods

Nelson, C. E., Goldberg, S. J., Wegley Kelly, L., Haas, A. F., Smith, J. E., Rohwer, F., & Carlson, C. A. (2013). Coral and macroalgal exudates vary in neutral sugar composition and differentially enrich reef bacterioplankton lineages. The ISME Journal, 7(5), 962–979. doi:10.1038/ismej.2012.161
Related Research

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Parameters

Parameter	Description	Units
Cruise	cruise name	unitless
Campaign	Experiment or cruise event during which sample was collected	unitless
Experiment	experiment identifier	unitless
Station name	station identifier	unitless
Latitude	Latitude in decimal degrees north	decimal degrees
Longitude	Longitude in decimal degrees east	decimal degrees
Date	collection date formatted as yyyy-mm-dd	unitless
Time (local)	Local time formatted as hh:mm:ss (local Moorea = UTC-10)	unitless
SampleID	Unique Identified Code for each sample collected	unitless
Bottle	Internal reference code	unitless
Timepoint	Internal reference code	unitless
ISO_DateTime_Local	Local date and time formatted in based on ISO 8601:2004(E) formatted as YYYY-MM-DDTHH:MM:SS[.xx]	unitless
BACT [E8/L]	Bacterioplankton abundance in 10^8 cells per liter. Methodological reference is Nelson et al. 2011; The ISME Journal.	10^8 cells/liter
DOC [µM]	Dissolved organic carbon concentration by HTCO in micromolar units. Glass fiber filtrate type GF/F (Whatman). Methodological reference is Carlson et al. 2010 DSRII	micromol

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Instruments

Dataset-specific Instrument Name	Minidon Niskin bottles
Generic Instrument Name	Niskin bottle
Dataset-specific Description	Used to collect water samples from reef areas for DOC and inorganic nutrient concentrations, microbial abundances, and microbial DNA.
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	Shimadzu TOC-V analyzers, modified
Generic Instrument Name	Total Organic Carbon Analyzer
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

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Project Information

Collaborative Research: Dissolved organic matter feedbacks in coral reef resilience: The genomic & geochemical basis for microbial modulation of algal phase shifts (Coral DOM2)

Coverage: Pacific Coral Reefs

NSF award abstract:
Coral reef degradation, whether driven by overfishing, nutrient pollution, declining water quality, or other anthropogenic factors, is associated with a phase shift towards a reefs dominated by fleshy algae. In many cases managing and ameliorating these stressors does not lead to a return to coral dominance, and reefs languish in an algal-dominated state for years. Nearly a decade of research has demonstrated that trajectories toward increasing algal dominance are restructuring microbial community composition and metabolism; the investigators hypothesize that microbial processes facilitate the maintenance of algal dominance by metabolizing organic compounds released by algae thereby stressing corals through hypoxia and disease. The resilience of reefs to these phase shifts is a critical question in coral reef ecology, and managing reefs undergoing these community shifts requires developing an understanding of the role of microbial interactions in facilitating algal overgrowth and altering reef ecosystem function. The research proposed here will investigate the organics produced by algae, the microbes that metabolize the organics, and the impacts of these processes on coral health and growth. This research has implications for managing reef resilience to algal phase shifts by testing the differential resistance of coral-associated microbial communities to algae and defining thresholds of algal species cover which alter ecosystem biogeochemistry. This project provides mentoring across multiple career levels, linking underrepresented undergraduates, two graduate students, a postdoctoral researcher, and a beginning and established investigators.

This project will integrate dissolved organic matter (DOM) geochemistry, microbial genomics and ecosystem process measurements at ecologically-relevant spatial and temporal scales to test hypothetical mechanisms by

which microbially-mediated feedbacks may facilitate the spread of fleshy algae on Pacific reef ecosystems. A key product of this research will be understanding how the composition of corals and algae on reefs interact synergistically with complex microbial communities to influence reef ecosystem resilience to algal phase shifts. Emerging molecular and biogeochemical methods will be use to investigate mechanisms of microbial-DOM interactions at multiple spatial and temporal scales. This project will leverage the background environmental data, laboratory facilities and field logistical resources of the Mo'orea Coral Reef Long Term Ecological Research Project in French Polynesia and contribute to the mission of that program of investigating coral reef resilience in the face of global change. The investigators will quantify bulk diel patterns of DOM production and characterize the composition of chromophoric components and both free and acid-hydrolyzable neutral monosaccharides and amino acids from varying benthic algae sources. The team will also characterize planktonic and coral-associated microbial community changes in taxonomic composition and gene expression caused by algal DOM amendments in on-site controlled environmental chambers using phylogenetics and metatranscriptomics, including tracking algal exudate utilization by specific microbial lineages. Field-deployed 100 liter tent mesocosms will be used to examine in situ diel patterns of coupled DOM production and consumption, microbial community genomics and ecosystem metabolism over representative benthic communities comprising combinations of algal and coral species. Together these experimental results will guide interpretation of field surveys of centimeter-scale spatial dynamics of planktonic and coral-associated microbial genomics and metabolism at zones of coral-algal interaction, including boundary layer dynamics of oxygen, bacteria and DOM using planar optodes, high-throughput flow cytometry and fluorescence spectroscopy.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1538567
NSF Division of Ocean Sciences (NSF OCE)	OCE-1538393
NSF Division of Ocean Sciences (NSF OCE)	OCE-1538428

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