Fluorescent characteristics of the dissolved organic exudates of two species of crustose coralline algae in two water treatments and their effect on the microbial community cell count

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Version: 1
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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

Fluorescent characteristics of the dissolved organic exudates of two species of crustose coralline algae (Hydrolithon reinboldii and Porolithon onkodes) in two water treatments (pre-filtered and unfiltered) and their effect on the microbial community cell count.

Table of Contents

Coverage

Spatial Extent: **Lat**:21.4785 **Lon**:-157.8281
Temporal Extent: 2017-05-04 - 2017-05-09

Dataset Description

Fluorescent characteristics of the dissolved organic exudates of two species of crustose coralline algae (*Hydrolithon reinboldii* and *Porolithon onkodes*) in two water treatments (pre-filtered and unfiltered) and their effect on the microbial community cell count.

This dataset is published in the accepted manuscript Quinlan et. al (2019).

Acquisition Description

The following sections contain methodology excerpts from Quinlain et al. (2019) relevant to this dataset.

Crustose Coraline Algae Collection and Identification
Both Hydrolithon reinboldii and Porolithon onkodes were collected from Patch Reef 42 (21.4785˚, -157.8281˚) in Kāne'ohe Bay, O'ahu, Hawai'i on 4 May 2017. Porolithon onkodes is a common CCA species in the Pacific Ocean that is often used for larval settlement experiments with coral species in Australia (Heyward and Negri 1999). It is typically found in high light and high flow environments, such as at the top of the patch reefs in Kāneʻohe Bay. This species is characterized by its smooth surface texture, and diagnostic depressions of trichosite fields. While there is a recent paper showing that this species is a species complex globally (Gabrielson et al., 2018), we retain the use of the name P. onkodes here to be consistent with the published taxonomic monograph for CCA in Hawaiʻi (Adey et al., 1982). Hydrolithon reinboldii is also a common CCA species that is found throughout the Pacific Ocean. It is known to facilitate coral larval settlement (Harrington et al., 2004). This species often lives cryptically in cracks in the reef or on the bottom of small pieces of calcium carbonate rubble. It is characterized by slightly raised hemispherical single pore conceptacles (400-600 µm in diameter), and a patchy surface texture referred to as tessellate (Adey et al., 1982).

Fragments of both species of CCA were trimmed using bone cutters to ensure only a single plant was on each fragment. Each fragment still retained bare calcium carbonate along with the individual species of CCA. To control for the bare calcium carbonate, encrusted fragments of calcium carbonate were similarly trimmed to remove any small CCA plants and epiphytes leaving only the calcium carbonate rubble and endophytes. After fragmentation the specimens

were haphazardly placed into six containers and randomized within a 1300 L flow through seawater bath to maintain all treatments at a stable temperature, which was the same as those found in Kāneʻohe Bay. As there are currently no studies on the effect of fragmentation on exudate production we allowed the fragmented algae to recover for five days before starting the exudation experiment. Flow through seawater baths were covered by shade cloth to reduce natural irradiance to levels similar to those found at depth in Kāneʻohe Bay where both species are naturally found. Both species were exposed to the same light levels as to not bias by variation of abiotic parameters.

Incubations and sample collection

Twenty-four 250 mL glass beakers were washed with 10% volumetric HCl, rinsed with milliq-water and air-dried. At 07:30 on 9 May, 3 L of seawater (sand filtered and collected from the Hawaiʻi Institute of Marine Biology flow-through seawater system in Kāne'ohe Bay) was vacuum pre-filtered through 0.2µm polyethersulfone filters (47 mm; Sterlitech) in a 500 mL polysulfone graduated filter holder. Before water was aliquoted into the beakers, samples for fluorescent DOM (fDOM), dissolved organic carbon (DOC), and flow cytometry (FCM) were collected from the 500 mL polysulfone graduated filter holder. Each beaker was filled and randomized within a 1300L flow through seawater bath to maintain stable temperature between the treatments. Each organism treatment beaker (water control, calcium carbonate control, Hydrolithon reinboldii, or Porolithon onkodes) was filled with seawater (filtered or unfiltered) and replicated (n = 3) for a total of 24 beakers (4 organismal treatments * 2 water treatments * 3 replicates). Filtered and unfiltered treatments were designed to capture differences in sloughing behavior between species. A Multiple trimmed fragments of each organism were placed within their respective beakers so that the total surface area within each replicate beaker was standardized to 20-30 square cm (25.57 ± 4.13 cm2). The incubation began at 9:00 and was halted at 17:00 to maintain only exudates produced during the daylight hours. Surface area was digitally determined at the end of the experiment by analyzing images to scale with image-J (Schindelin, Arganda-Carreras, & Frise et al, 2012).

DOM samples were collected at the beginning of the experiment before aliquoting the water at 9:00 and from each beaker at 17:00. DOM samples were immediately filtered through a 0.2 µm polyethersulfone filter (47 mm; Sterlitech) in a 500 mL polysulfone graduated filter holder. Filtrate was poured directly from polysulfone graduated filter holder into its respective sample vial, Filtrate for fDOM samples were collected in acid washed, combusted, triple sample-rinsed amber borosilicate vials with Teflon septa caps and stored dark at 4˚C until analysis for fDOM within 24 hours. DOC was collected in acid washed, combusted, triple sample rinsed clear borosilicate vials with Teflon septa caps and measured as non-purgeable organic carbon via acidification, sparging and high temperature platinum catalytic oxidation on a Shimadzu TOC-V at the UCSB DOM Analytical Lab following the methods outlined by Carlson et al. (2010). Samples for flow cytometry were collected by pipet (1 ml amended to a final concentration of 0.5% paraformaldehyde, mixed by inversion, snap frozen -80ºC) at 9:00, 13:00, and 17:00.

Sample analysis

Flow Cytometry: Flow cytometry was used to measure total nucleic acid-stained cell concentrations. Samples were thawed and 200 µL were aliquoted into u-bottomed 96-well autosampler plates and stained with 2 µL of 100X SYBR Green I stain (final concentration of 0.5X). Samples were analyzed on an Attune Acoustic Focusing Cytometer with Autosampler Attachment (Life Technologies, Eugene, OR, USA). Samples were run at a flow rate of 100 µL min-1 on standard sensitivity; 150 µL of sample was aspirated, 75 µL was counted and data was collected only from the last 50 µL (event rates were empirically determined to be steady only after 25 µL of continuous sample injection per Nelson et al., 2015).

Fluorescence spectroscopy: Samples for fluorescence spectroscopy were measured using an Horiba Aqualog scanning fluorometer following the methods of Nelson et al. (2015), including scan time and resolution, spectral data processing, inner filter correction, Raman unit standardization, blank subtraction and PARAFAC modeling (Stedmon and Bro 2008; Lawaetz and Stedmon 2009; Kothawala et al. 2013). Scans were processed using a Matlab (v2007b) script written and specified by Nelson et al. (2015) and Quinlan et al., (2018; most recent version available at DOI: 10.5281/zenodo/3479841), modified to additionally capture the peak present at Excitation 240 nm and Emission 300 nm (phenylalanine-like: Lakowicz 2010). Six modeled components were validated using split half validation and outlier analysis (Quinlan et. al., 2018). All PARAFAC components had similar excitation-emission maxima and strong covariation among samples with previously identified fluorophores (Quinlan, et. al., 2018); for subsequent analyses we examined established fluorescence maxima from the literature (Coble 1996; Stedmon et al. 2003; Lakowicz 2010).

Processing Description

Data Processing:

R, R-studio (with packages: tidyverse, DescTools, data.table,Vegan, Mass, factoextra, ape)

BCO-DMO Processing:

- deleted blank rows;
- modified parameter names (removed units; replaced symbols; replaced spaces and hyphens with underscores);
- replaced missing data with 'nd' (no data).

[table of contents | back to top]

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

Coble, P. G. (1996). Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. Marine Chemistry, 51(4), 325–346. doi:10.1016/0304-4203(95)00062-3

Lakowicz, J. R. (Ed.). (2010). Principles of Fluorescence Spectroscopy. 3. ed., [4. corr. print.] New York, NY: Springer.

Quinlan, Z. A., Remple, K., Fox, M. D., Silbiger, N. J., Oliver, T. A., Putnam, H. M., … Nelson, C. E. (2018). Fluorescent organic exudates of corals and algae in tropical reefs are compositionally distinct and increase with nutrient enrichment. Limnology and Oceanography Letters, 3(4), 331–340. doi:10.1002/lol2.10074

Quinlan, Z. A., Ritson-Williams, R., Carroll, B. J., Carlson, C. A., & Nelson, C. E. (2019). Species-Specific Differences in the Microbiomes and Organic Exudates of Crustose Coralline Algae Influence Bacterioplankton Communities. Frontiers in Microbiology, 10. doi:10.3389/fmicb.2019.02397

Stedmon, C. A., Markager, S., & Bro, R. (2003). Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy. Marine Chemistry, 82(3-4), 239–254. doi:10.1016/S0304-4203(03)00072-0

[table of contents | back to top]

Parameters

Parameter	Description	Units
Water	Water treatment	unitless
Inhabitant	Organism or control treatment	unitless
Replicate	Replicate beaker	unitless
Timepoint	Time-lapse of data collection	unitless
Hours	Hours of incubation	hours
Cells	number of cells measure by FCM	cells per microliter (cells uL-1)
delta_Cells	change in cells from T0:TF	cells per microliter (cells uL-1)
Surface_Area	sum surface area of cca in treatment	square centimeters (cm^2)
Ultra_Violet_Humic_like	Coble Peak A (Ultra Violet Humic-like)	Raman units of water (RU)
Marine_Humic_like	Coble Peak M (Marine Humic-like)	Raman units of water (RU)
Visible_Humic_like	Coble Peak C (Visible Humic-like)	Raman units of water (RU)
Tryptophan_like	Coble Peak T (Tryptophan-like)	Raman units of water (RU)
Tyrosine_like	Coble Peak B (Tyrosine-like)	Raman units of water (RU)
Phenylalanine_like	Coble Peak F (Phenylalanine-like)	Raman units of water (RU)
Fulvic_Acid_like	Stedmon peak D (Fulvic acid like)	Raman units of water (RU)
DOC	Dissolved Organic Carbon (DOC)	micromoles per liter (umol L-1)

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	Horiba Aqualog scanning fluorometer
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	Shimadzu TOC-V Analyzer
Generic Instrument Name	Shimadzu TOC-V Analyzer
Generic Instrument Description	A Shimadzu TOC-V Analyzer measures DOC by high temperature combustion method.

Dataset-specific Instrument Name	Seal Analytical Segmented Flow Injection AutoAnalyzer AA3HR
Generic Instrument Name	Flow Injection Analyzer
Generic Instrument Description	An instrument that performs flow injection analysis. Flow injection analysis (FIA) is an approach to chemical analysis that is accomplished by injecting a plug of sample into a flowing carrier stream. FIA is an automated method in which a sample is injected into a continuous flow of a carrier solution that mixes with other continuously flowing solutions before reaching a detector. Precision is dramatically increased when FIA is used instead of manual injections and as a result very specific FIA systems have been developed for a wide array of analytical techniques.

[table of contents | back to top]

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.

[table of contents | back to top]

Funding

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

[table of contents | back to top]