Microbial ESV counts for Acropora millepora corals exposed to Sargassum seaweed

Website: https://www.bco-dmo.org/dataset/818317
Data Type: experimental
Version: 0
Version Date: 2020-07-13

Project
» Killer Seaweeds: Allelopathy against Fijian Corals (Killer Seaweeds)

Contributors	Affiliation	Role
Hay, Mark	Georgia Institute of Technology (GA Tech)	Principal Investigator
Clements, Cody	Georgia Institute of Technology (GA Tech)	Co-Principal Investigator, Contact
Copley, Nancy	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
Microbial ESV counts for Acropora millepora corals exposed to Sargassum

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Coverage

Spatial Extent: **Lat**:-18.2164722 **Lon**:177.7173056

Dataset Description

Microbial ESV counts for Acropora millepora corals exposed to Sargassum. These results are published in Figure 2 of Clements et al (2020). See 'Master ID Sheet.xlsx' in Supplemental Files for the treatment descriptions.

Because this data table is extremely wide, with 2368 columns and 42 data rows, it is only available as a downloadable file. See the Data Files section.

Columns in the data table:
Label: The ID for each sample
*All subsequent columns are counts for each exact sequence variant (ESV)

Methods & Sampling

Methodology:

Acropora millepora corals were subjected to one of nine experimental treatments for 33 days: (1) direct contact with four thalli of Galaxaura rugosa (live seaweed), (2) close proximity (i.e. ~1.5cm away, no contact) to four thalli of Galaxaura, (3) direct contact with four Galaxaura mimics (microfiber dust cloth), (4) close proximity to four Galaxaura mimics, (5) direct contact with four Sargassum polycystum thalli (live seaweed), (6) close proximity to four Sargassum thalli, (7) direct contact with four Sargassum mimics (plastic aquarium plants), (8) close proximity to four Sargassum mimics, or (9) no seaweed or mimic exposure (control) (n = 9-13 per treatment). Analyses of microbiome data were conducted separately to compare control corals and corals in direct contact or close proximity with Galaxaura or its mimics or Sargassum or its mimics.

Sampling and analytical procedures:

Total DNA was extracted from each coral sample by placing the clipping directly into a PowerBead tube from the PowerSoil DNA isolation kit (MO BIO Laboratories) and proceeding according to the manufacturer's instructions. The V3-V4 variable region of the 16S rRNA gene was amplified from 1 µl (25 µl total reaction volume) using the Platinum PCR SuperMix (Life Technologies) and the universal 16S rRNA gene primers 515F (Parada) (5'-GTGYCAGCMGCCGCGGTAA-3') and 806R (Apprill) (5'-GGACTACNVGGGTWTCTAAT-3'). Primers were modified with sample-specific barcodes and Illumina sequencing adapters according to Kozich et al. (2013) to allow for multiplexing of samples. Primers were added to each PCR reaction at a final concentration of 0.2 µM and PCR cycling conditions were: initial denaturation at 94°C for 3 min, followed by 30 cycles of denaturation for 45 s (94°C), primer annealing for 60 s (55°C), extension for 90 s (72°C), and a final 10 min extension step (72°C). PCR products were run on a 1% agarose/1X TAE gel to verify amplicon size and lack of contamination. PCR products were purified using Diffinity RapidTips (Sigma Aldrich) and quantified using the Qubit 2.0 fluorometer. Equimolar concentrations of all samples were pooled and sequenced on a MiSeq sequencer using a 500-cycle paired-end MiSeq Reagent V2 Kit (Illumina).

After sequencing and de-multiplexing, barcoded sequences were trimmed and filtered using Trim Galore! (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/, minimum Phred score > 25, minimum sequence length >100 nt). Exact sequence variants (ESVs) were determined from filtered sequences using DADA2 in the QIIME2 pipeline (Caporaso et al. 2010, Callahan et al. 2016). Taxonomy was assigned to ESVs by comparison to the SILVA ribosomal RNA database (Release 132). Singletons and ESVs assigned to chloroplast or mitochondrial sequences were removed from the analysis. All samples were normalized to a standard read count (n = 4273) for further analyses.

Analyses of microbiome data were conducted separately to compare control corals and corals in direct contact or close proximity with Galaxaura or its mimics or Sargassum or its mimics. In each case, principal coordinate analysis (PCO) and corresponding tests for differences in microbiome composition (permutational multivariate analysis of variance, PERMANOVA) and variability (PERMADISP) were implemented in Primer E (Clark 1993). Alpha diversity (ESV richness, Shannon diversity) of relevant datasets was calculated using QIIME2 (Caporaso et al. 2010). Differences among treatments were analyzed using LME models in the R package nlme (Pinheiro et al. 2017) with coral colony as a random factor. When necessary, the varIdent argument was used to control for heteroscedasticity. For each analysis, significance levels were adjusted to correct for multiple comparisons of relevant datasets (? = 0.025).

Data Processing Description

BCO-DMO Processing Notes:
- Because this data table is extremely wide, with 2368 columns and 42 data rows, it is only available as a downloadable file. See the Data Files section.

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Data Files

File
Microbial ESV counts for corals exposed to Sargassum filename: Coral_ESVs_Sargassum.xlsx (Microsoft Excel, 345.07 KB) MD5:7d9d277b745cb2d165f85bec3348b5a6 This is a very wide table, with 2367 columns and 42 data rows.

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Supplemental Files

File
Master list of sample id's, coral colony genotype, treatment, and treatment description filename: Master_ID_Sheet.xlsx (Microsoft Excel, 14.13 KB) MD5:62b3f2cb3afc25e8b36aae12af3261b2

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Related Publications

CLARKE, K. R. (1993). Non-parametric multivariate analyses of changes in community structure. Austral Ecology, 18(1), 117–143. doi:10.1111/j.1442-9993.1993.tb00438.x
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

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., … Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. Nature Methods, 7(5), 335–336. doi:10.1038/nmeth.f.303
Methods

Clements, C. S., Burns, A. S., Stewart, F. J., & Hay, M. E. (2020). Seaweed-coral competition in the field: effects on coral growth, photosynthesis and microbiomes require direct contact. Proceedings of the Royal Society B: Biological Sciences, 287(1927), 20200366. doi:10.1098/rspb.2020.0366
Results

Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., & Schloss, P. D. (2013). Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform. Applied and Environmental Microbiology, 79(17), 5112–5120. doi:10.1128/aem.01043-13
Methods

Pinheiro, J.D., Bates, D., DebRoy, S., Sarkar, D. and the R Core Team (2014) nlme: linear and nonlinear mixed effects models. R package version 3.1–131. http://CRAN.R-project.org package=nlme
Methods

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Parameters

Parameters for this dataset have not yet been identified

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Instruments

Dataset-specific Instrument Name	MiSeq sequencer
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	500-cycle paired-end MiSeq Reagent V2 Kit (Illumina).
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	Qubit 2.0 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	
Generic Instrument Name	qPCR Thermal Cycler
Generic Instrument Description	An instrument for quantitative polymerase chain reaction (qPCR), also known as real-time polymerase chain reaction (Real-Time PCR).

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Project Information

Killer Seaweeds: Allelopathy against Fijian Corals (Killer Seaweeds)

Coverage: Viti Levu, Fiji (18º13.049’S, 177º42.968’E)

Extracted from the NSF award abstract:

Coral reefs are in dramatic global decline, with reefs commonly converting from species-rich and topographically-complex communities dominated by corals to species- poor and topographically-simplified communities dominated by seaweeds. These phase-shifts result in fundamental loss of ecosystem function. Despite debate about whether coral-to-algal transitions are commonly a primary cause, or simply a consequence, of coral mortality, rigorous field investigation of seaweed-coral competition has received limited attention. There is limited information on how the outcome of seaweed-coral competition varies among species or the relative importance of different competitive mechanisms in facilitating seaweed dominance. In an effort

to address this topic, the PI will conduct field experiments in the tropical South Pacific (Fiji) to determine the effects of seaweeds on corals when in direct contact, which seaweeds are most damaging to corals, the role allelopathic lipids that are transferred via contact in producing these effects, the identity and surface concentrations of these metabolites, and the dynamic nature of seaweed metabolite production and coral response following contact. The herbivorous fishes most responsible for controlling allelopathic seaweeds will be identified, the roles of seaweed metabolites in allelopathy vs herbivore deterrence will be studied, and the potential for better managing and conserving critical reef herbivores so as to slow or reverse conversion of coral reef to seaweed meadows will be examined.

Preliminary results indicate that seaweeds may commonly damage corals via lipid- soluble allelochemicals. Such chemically-mediated interactions could kill or damage adult corals and produce the suppression of coral fecundity and recruitment noted by previous investigators and could precipitate positive feedback mechanisms making reef recovery increasingly unlikely as seaweed abundance increases. Chemically-mediated seaweed-coral competition may play a critical role in the degradation of present-day coral reefs. Increasing information on which seaweeds are most aggressive to corals and which herbivores best limit these seaweeds may prove useful in better managing reefs to facilitate resilience and possible recovery despite threats of global-scale stresses. Fiji is well positioned to rapidly use findings from this project for better management of reef resources because it has already erected >260 MPAs, Fijian villagers have already bought-in to the value of MPAs, and the Fiji Locally-Managed Marine Area (FLMMA) Network is well organized to get information to villagers in a culturally sensitive and useful manner.

The broader impacts of this project are far reaching. The project provides training opportunities for 2-2.5 Ph.D students and 1 undergraduate student each year in the interdisciplinary areas of marine ecology, marine conservation, and marine chemical ecology. Findings from this project will be immediately integrated into classes at Ga Tech and made available throughout Fiji via a foundation and web site that have already set-up to support marine conservation efforts in Fiji and marine education efforts both within Fiji and internationally. Business and community leaders from Atlanta (via Rotary International Service efforts) have been recruited to help organize and fund community service and outreach projects in Fiji -- several of which are likely to involve marine conservation and education based in part on these efforts there. Media outlets (National Geographic, NPR, Animal Planet, Audubon Magazine, etc.) and local Rotary clubs will be used to better disseminate these discoveries to the public.

PUBLICATIONS PRODUCED AS A RESULT OF THIS RESEARCH

Rasher DB, Stout EP, Engel S, Kubanek J, and ME Hay. "Macroalgal terpenes function as allelopathic agents against reef corals", Proceedings of the National Academy of Sciences, v. 108, 2011, p. 17726.

Beattie AJ, ME Hay, B Magnusson, R de Nys, J Smeathers, JFV Vincent. "Ecology and bioprospecting," Austral Ecology, v.36, 2011, p. 341.

Rasher DB and ME Hay. "Seaweed allelopathy degrades the resilience and function of coral reefs," Communicative and Integrative Biology, v.3, 2010.

Hay ME, Rasher DB. "Corals in crisis," The Scientist, v.24, 2010, p. 42.

Hay ME and DB Rasher. "Coral reefs in crisis: reversing the biotic death spiral," Faculty 1000 Biology Reports 2010, v.2, 2010.

Rasher DB and ME Hay. "Chemically rich seaweeds poison corals when not controlled by herbivores", Proceedings of the National Academy of Sciences, v.107, 2010, p. 9683.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-0929119

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