18S rRNA amplicon sequencing of microbial eukaryotes from the Mid-Cayman Rise acquired Jan-Feb, 2020

Website: https://www.bco-dmo.org/dataset/914399
Data Type: Other Field Results
Version: 1
Version Date: 2023-11-06

Project
» Probing subseafloor microbial interactions via hydrothermal vent fluids: A focus on protists (Microbial eukaryotes at hydrothermal vents)

Program
» Center for Dark Energy Biosphere Investigations (C-DEBI)

Contributors	Affiliation	Role
Hu, Sarah K.	Woods Hole Oceanographic Institution (WHOI)	Principal Investigator, Contact
Huber, Julie	Woods Hole Oceanographic Institution (WHOI)	Principal Investigator
Soenen, Karen	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
Single-celled microbial eukaryotes inhabit deep-sea hydrothermal vent environments and play critical ecological roles in the vent-associated microbial food web. 18S rRNA amplicon sequencing of diffuse venting fluids from two geochemically-distinct hydrothermal vent fields was applied to investigate community diversity patterns among protistan assemblages. Piccard and Von Damm vent fields are situated 20 km apart at the Mid-Cayman Rise in the Caribbean Sea. We describe species diversity patterns with respect to hydrothermal vent field and sample type, identify putative vent endemic microbial eukaryotes, and test how vent fluid geochemistry may influence microbial community diversity. Individual vent fields supported distinct and highly diverse assemblages of protists that included potentially endemic or novel vent-associated strains. This data adds to our growing knowledge of the biogeography of deep-sea microbial eukaryotes.

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Coverage

Spatial Extent: **Lat**:18 **Lon**:-82
Temporal Extent: 2020-01-14 - 2020-02-05

Methods & Sampling

Samples and experiments were collected and executed during cruise AT42-22 aboard the RV Atlantis with ROV Jason in January-February 2020 at the Von Damm (2300 m; 18°23’N, 81°48’W) and Piccard (5000 m; 18°33’N, 81°43’W) hydrothermal fields located along the Mid-Cayman Rise.

Fluids for shipboard grazing experiments and biogeochemistry were obtained in 10L volume bags (Kynar, Keika Ventures; polyvinylidene fluoride) using the Hydrothermal Organic Geochemistry (HOG) sampler mounted on ROV Jason. Between 4-10 L of vent fluid was collected and filtered through a 47 mm polyethersulphone (PES) filter (Millipore) with a pore size of 0.2 µm and preserved with RNAlater (Ambion) at the seafloor for molecular analysis of microbial communities.

Non-vent samples were collected from within the overlying non-buoyant hydrothermal plume at each site and from background seawater via CTD-mounted Niskin bottles. Plume samples were identified using in situ CTD sensors to detect the presence of hydrothermal influence in real-time (back-scatter and temperature) above each vent field. Background seawater samples were collected outside of the influence of the hydrothermal vent at approximately the same depth as the vent sites (~2350 m and ~4950 m).

Data Processing Description

Filters retrieved from ROV Jason and from the final time point of each grazing assay were processed identically. RNA was extracted from frozen filters (stored in RNAlater) as amplicon sequences originating from extracted RNA are more likely to represent metabolically active cells, rather than inactive cellular material that may have sunk from above. The filter was first separated from the RNAlater and distributed into tubes with a lysis buffer (Qiagen 1053393). The RNAlater was centrifuged for 15 minutes at 16,000 x g, and the supernatant was removed. Lysis buffer was added on top of any cellular materials collected, vortexed, and then combined with the filter. The filter and lysis buffer solution was vortexed thoroughly with RNAase-free silica beads. The lysis buffer was then separated from beads and filter material with a syringe and processed using the Qiagen RNeasy extract kit (Qiagen 74104), which included an inline RNAse-free DNase removal step (Qiagen 79256). Total RNA was reverse transcribed to cDNA and amplified with V4-specific primers. MiSeq 2 x 300 bp PE sequencing was performed at the Marine Biological Laboratory Bay Paul Centre Keck sequencing facility. Amplicon sequences were processed using QIIME2 (version 2021.4).

Following quality control, Amplicon Sequence Variants (or ASVs) were determined from the sequences and taxonomic assignment was done using the PR2 database (v 4.14). For this analysis, we focused primarily on the microeukaryotic population, removing sequences assigned to prokaryotes or Metazoa. ASVs were categorized as vent-only or cosmopolitan, based on their presence in only vent samples or throughout vent, plume, and background samples, respectively.

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Data Files

File
914399_v1_sra.csv (Comma Separated Values (.csv), 8.55 KB) MD5:94da19ff23d7d76fd508df4ab9f77223
Primary data file for dataset ID 914399, version 1

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Related Publications

Hu, S. K., Anderson, R. E., Pachiadaki, M. G., Edgcomb, V. P., Serres, M. H., Sylva, S. P., German, C. R., Seewald, J. S., Lang, S. Q., & Huber, J. A. (2023). Microbial eukaryotic predation pressure and biomass at deep-sea hydrothermal vents: Implications for deep-sea carbon cycling. https://doi.org/10.1101/2023.08.11.552852
Results

Hu, S. K., Herrera, E. L., Smith, A. R., Pachiadaki, M. G., Edgcomb, V. P., Sylva, S. P., Chan, E. W., Seewald, J. S., German, C. R., & Huber, J. A. (2021). Protistan grazing impacts microbial communities and carbon cycling at deep-sea hydrothermal vents. Proceedings of the National Academy of Sciences, 118(29). https://doi.org/10.1073/pnas.2102674118
Related Research

Hu, S. K., Smith, A. R., Anderson, R. E., Sylva, S. P., Setzer, M., Steadmon, M., Frank, K. L., Chan, E. W., Lim, D.

Parameters

Parameter	Description	Units
Experiment_Accession	Accession ID in SRA	unitless
Experiment_Title	Overall sample title	unitless
Organism_Name	Type of sample	unitless
Instrument	Sequencing platform used	unitless
Study_Accession	Accession ID for study in SRA	unitless
Study_Title	Microbial eukaryotic biodiversity (18S rRNA gene) from the Mid-Cayman Rise deep-sea hydrothermal vent field	unitless
Sample_Accession	Individual sample accession IDs for SRA	unitless
Total_Size_Mb	Size of data file	Mb
Total_Bases	Total number of base pairs	bp
Library_Name	Unique library ID	unitless
Library_Strategy	Type of library preparation	unitless
Library_Source	Source genetic material for library preparation	unitless
Library_Selection	Mode of amplification or library prep	unitless
Vent_field	Hydrothermal vent field	unitless
Collection	Origin of fluid collection	unitless
Vent_name	Name of vent site	unitless
DiveID	ID for ROV Dive or CTD cast for sample	unitless

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Instruments

Dataset-specific Instrument Name	Illumina MiSeq
Generic Instrument Name	Automated DNA Sequencer
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.

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Deployments

AT42-22

Website	https://www.bco-dmo.org/deployment/914418
Platform	R/V Atlantis
Start Date	2020-01-14
End Date	2020-05-20

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Project Information

Probing subseafloor microbial interactions via hydrothermal vent fluids: A focus on protists (Microbial eukaryotes at hydrothermal vents)

Website: https://www.darkenergybiosphere.org/award/probing-subseafloor-microbial-interactions-via-hydrothermal-vent-fluids-a-focus-on-protists/

Coverage: Axial Seamount Juan de Fuca Ridge NE Pacific 46 N 130 W, Gorda Ridge NE Pacific 41 N 127 W, Mid-Cayman Rise Caribbean Sea 18 N 82 W

Adjusted C-DEBI Award Description:

Highly reduced and thermally charged venting fluids from the subseafloor mix with surrounding seawater, creating a sharp geochemical gradient which promotes a hub of biological diversity at hydrothermal vent ecosystems. While studies of prokaryotic diversity at hydrothermal vent sites have highlighted the important roles microorganisms play in deep sea carbon cycling and offered a unique window into subseafloor microbial communities, depictions of deep-sea marine ecology and food webs are incomplete without characterization of single-celled microbial eukaryotes (protists). I propose to use culture-independent techniques (tag-sequencing and metatranscriptomics) to provide a thorough understanding of protistan biogeography in and near venting fluids, focusing on the vent fluid-seawater interface. Additionally, these qualitative analyses will be paired with quantitative experiments that measure protistan grazing pressure. Understanding trophic interactions within the protistan community is incredibly important, as these processes form the foundation of deep-sea marine food webs and mediate a significant amount of carbon transferred to higher trophic levels.

C-DEBI project link: https://www.darkenergybiosphere.org/award/probing-subseafloor-microbial-interactions-via-hydrothermal-vent-fluids-a-focus-on-protists/

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Program Information

Center for Dark Energy Biosphere Investigations (C-DEBI)

Website: http://www.darkenergybiosphere.org

Coverage: Global

The mission of the Center for Dark Energy Biosphere Investigations (C-DEBI) is to explore life beneath the

seafloor and make transformative discoveries that advance science, benefit society, and inspire people of all ages and origins.

C-DEBI provides a framework for a large, multi-disciplinary group of scientists to pursue fundamental questions about life deep in the sub-surface environment of Earth. The fundamental science questions of C-DEBI involve exploration and discovery, uncovering the processes that constrain the sub-surface biosphere below the oceans, and implications to the Earth system. What type of life exists in this deep biosphere, how much, and how is it distributed and dispersed? What are the physical-chemical conditions that promote or limit life? What are the important oxidation-reduction processes and are they unique or important to humankind? How does this biosphere influence global energy and material cycles, particularly the carbon cycle? Finally, can we discern how such life evolved in geological settings beneath the ocean floor, and how this might relate to ideas about the origin of life on our planet?

C-DEBI's scientific goals are pursued with a combination of approaches:
(1) coordinate, integrate, support, and extend the research associated with four major programs—Juan de Fuca Ridge flank (JdF), South Pacific Gyre (SPG), North Pond (NP), and Dorado Outcrop (DO)—and other field sites;
(2) make substantial investments of resources to support field, laboratory, analytical, and modeling studies of the deep subseafloor ecosystems;
(3) facilitate and encourage synthesis and thematic understanding of submarine microbiological processes, through funding of scientific and technical activities, coordination and hosting of meetings and workshops, and support of (mostly junior) researchers and graduate students; and
(4) entrain, educate, inspire, and mentor an interdisciplinary community of researchers and educators, with an emphasis on undergraduate and graduate students and early-career scientists.

Note: Katrina Edwards was a former PI of C-DEBI; James Cowen is a former co-PI.

Data Management:
C-DEBI is committed to ensuring all the data generated are publically available and deposited in a data repository for long-term storage as stated in their Data Management Plan (PDF) and in compliance with the NSF Ocean Sciences Sample and Data Policy. The data types and products resulting from C-DEBI-supported research include a wide variety of geophysical, geological, geochemical, and biological information, in addition to education and outreach materials, technical documents, and samples. All data and information generated by C-DEBI-supported research projects are required to be made publically available either following publication of research results or within two (2) years of data generation.

To ensure preservation and dissemination of the diverse data-types generated, C-DEBI researchers are working with BCO-DMO Data Managers make data publicly available online. The partnership with BCO-DMO helps ensure that the C-DEBI data are discoverable and available for reuse. Some C-DEBI data is better served by specialized repositories (NCBI's GenBank for sequence data, for example) and, in those cases, BCO-DMO provides dataset documentation (metadata) that includes links to those external repositories.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-0939564

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