High throughput tag-sequencing data from Gorda Ridge Hydrothermal vent field, including 16S and 18S rRNA gene sequences, and environmental metadata from Gorda Ridge Seamount, May/June 2019

Website: https://www.bco-dmo.org/dataset/828392
Data Type: Cruise Results
Version: 1
Version Date: 2020-11-09

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
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Abstract
High throughput tag-sequencing data from Gorda Ridge Hydrothermal vent field, including 16S and 18S rRNA gene sequences, and environmental metadata from Gorda Ridge Seamount, May/June 2019.

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Coverage

Spatial Extent: **N**:42.7613 **E**:-126.7048 **S**:42.7495 **W**:-126.743
Temporal Extent: 2019-05-30 - 2019-06-07

Dataset Description

See SRA BioProject accession: PRJNA637089 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA637089) and BioSample accession IDs: SAMN15341559-SAMN15341619. Sequences are available via SRA (NCBI).

Acquisition Description

Sample collection:

The Gorda Ridge spreading center, ~200 km off the coast of southern Oregon was visited in May-June 2019 with the EV Nautilus (cruise NA108). Cruise operations were a collaborative effort with OET, NASA-SUBSEA, NOAA, URI, and WHOI. The ROV Hercules was positioned next to sites visually identified to have active hydrothermal flow. Temperatures at sites of active diffuse flow were obtained using a temperature probe controlled by the ROV Hercules arm. The SUPR sampler intake value then pumped this fluid into air-tight bags for grazing experiments and whole vent fluid water collection or pumped this fluid across 0.2 um PES Millipore filters (size, manufacturer). Fluid was also collected within the plume above each vent site by situating the ROV ~5 m above an active venting site and firing Niskin bottles. Background seawater from the water column at ~2,100 m was obtained by Niskin bottle. Samples from the plume and background collected by emptying Niskin bottles into acid-washed cubitainers and processed in the lab as described below. Upon recovery of ROV Hercules from each dive, bags filled with hydrothermal vent fluid were processed immediately. Filters were placed in RNAlater and kept frozen until return to the lab and fluid collected into bags was processed for grazing experiments (see 18S rRNA gene sequences) or filtered onto sterivex filters (both 18S and 16S sequences).

16S sequences

DNA was extracted from 142 mm filters or Sterivex filters. The 142 mm filters were snap-frozen after folding and then rolling them into 50 mL tubes. To extract DNA, sterile forceps were used to pull the filter out of the tube, ethanol-flamed scissors were used to cut approximately 1/3 of the filter off and into a sterile 15 mL tube, then the rest of the filter was replaced at – 80oC in RNA Later. For the Sterivex filters, the filter housing was cracked open to expose the filter, the filter was cut off with sterile razor blade, and finally placed into a sterile tube with forceps. Approximately ½ of the filter was used, then the rest was saved at - 80oC covered in RNA Later.

All filters were washed in 2 mL 1x sterile PBS by swishing them up and down in the PBS in the 15 mL tubes using sterile forceps. The PBS was transferred to 2 mL tubes and centrifuged at 14,000 rpm for 5 minutes to pellet cells. Filters were then placed in the pellet tubes for digestion. We added 900 uL DNA extraction buffer (50 mL, 100mM Tris, 100mM EDTA, 100mM NaH_2PO_4, 1.5M NaCl, 1% CTAB), 20 uL proteinase K (10mg/mL), and 40 uL of lysozyme (50mg/mL) to each tube. We proceeded to do freeze-thaw 3 times at -80oC for 15 minutes and thaw at 37oC for 15 minutes. Tubes were then incubated in a 37oC heat block for 20 minutes. Filter-sterile SDS (50 mL of 20% solution in water) was added to teach tube and inverted several times to mix. Tubes were incubated for 2 hours in a 65oC water bath

For the extraction steps, we worked in a fume hood and began by filling each tube to the top with phenol:chloroform:isoamyl alcohol (25:24:1, pH 8.0), and vortexing. Next, we centrifuged at low speed (3000 rpm) for 5 minutes and transferred the top layer to a new 2 mL tube, and repeated (total 2 washes). We added 0.6 volumes of room temperature 100% Isopropanol and inverted gently to mix. Tubes were incubated at room temperature overnight in a dark drawer and then centrifuged at top speed for 30 minutes at 4 oC. We then carefully pipetted off the buffer + isopropanol and saved this fraction in a new 2 mL tube. Ice cold 70% EtOH (1mL) was added to each 2 mL tube, inverted several times, and centrifuged at max speed for 10 min. EtOH was pipetted off and rinsing was repeated two times before allowing the tubes to air dry with a Kim wipe draped over the top. The dried DNA pellet was then dissolved into 50 uL nuclease-free sterile water and quantified using the PicoGreen Assay.

DNA was PCR amplified using the 16S V4 primers 515F (Parada et al. 2016) and 806R (Apprill et al. 2015). Amplicons were sequenced with Illumina MiSeq sequencing.

18S sequences

Samples collected for molecular analyses included in situ filters from the SUPR sampler, shipboard Sterivex

filters, or time points from grazing experiments (all collected into 0.2-um pore size filters). Some of the 18S rRNA gene tag sequence samples are associated with grazing experiments, where vent fluid was incubated with analog prey (Fluorescently-labeled bacterial prey) to track grazing rates among single-celled microbial eukaryotes. These samples were taken at the start of the experiments (T0) or at various time points (T1, T2). For all samples, RNA was extracted and amplified similarly to the protocol described in Hu et al. 2018 (https://dx.doi.org/10.17504/protocols.io.hk3b4yn). Frozen filters were thawed and placed into sterile 15-ml falcon tubes with sterile forceps, 1-2 mL of RLT+ buffer (with β-Mercaptoethanol, Qiagen, Valencia, CA, USA), and RNase-free silica beads was added to each tube. Falcon tubes were bead-beaten by vortexing vigorously for 5 minutes. The original sample collection tubes with RNAlater were centrifuged to pellet any cellular material left in the RNAlater; the RNAlater was removed and replaced with 500-ul of RLT+ buffer (with β-Mercaptoethanol). This was vortexed and added to the 15-ml falcon tube. RNA was extracted with the RNAeasy kit (Qiagen #74104) with the in-line genomic DNA removal step (RNase-free DNase reagents, Qiagen #79254). RNA concentrations were determined using the Ribogreen protocol. Extracted RNA was reverse transcribed into cDNA using a cDNA synthesis kit (iScript Select cDNA Synthesis, BioRad, #1708896, Hercules, CA); the concentration of RNA was normalized for the cDNA synthesis reaction (input –ng of RNA). Primers targeting the V4 hypervariable region of the 18S rRNA gene (Stoeck et al. 2010; Hu et al. 2015) were used in PCR reactions, which consisted of a final concentration of 1X Q5 High Fidelity Master Mix (NEB #M0492S, Ipswich, MA), 0.5 μM each of forward and reverse primers, and 1 ng of genetic material. The PCR thermal protocol started with an initial activation step (Q5 specific) of 98°C for 2 min, followed with 10 cycles of 98°C for 10 s, 53°C for 30 s, 72°C for 30 s, and 15 cycles of 98°C for 10 s, 48°C for 30 s, and 72°C for 30 s, and a final extension of 72°C for 2 min (modified from Rodriquez Martinez et al. 2012). The original extract total RNA was also PCR amplified to ensure no genomic DNA was present in the sample. PCR products were checked by confirming the presence of an ~400 bp product on an agarose gel. In cases with no amplification, the PCR reaction was repeated with a higher concentration of cDNA (1.5-2 ng). If this did not yield the expected PCR product, the reaction was repeated with an additional 5 cycles. Three shipboard blanks (MilliQ water) and one extraction blank were also extracted and PCR amplified; while no PCR product was observed in these control samples they were processed similarly to all true samples and sequenced. All PCR products were cleaned using the AMPure bead clean up (Beckman Coulter #A63881, Brea, CA). Samples were multiplexed, pooled at equimolar concentrations and sequenced using the MiSeq 300 x 300 bp PE sequencing at Marine Biological Laboratory Bay Paul Center sequencing facility.

Processing Description

BCO-DMO Processing Notes:
- data submitted in Excel files "Axial-Metagenome.environmental.1.0_JH.xlsx" sheet "tmp" and file "Axial-SRA_metadata_acc.xlsx" sheet "SRA_data" and extracted to csv
- added conventional header with dataset name, PI name, version date
- renamed columns to conform with BCO-DMO naming conventions (removed spaces)
- combined environmental and SRA tables into one table; joined on sample_name and library_ID
- removed unpopulated columns (ref_biomaterial, rel_to_oxygen, samp_collect_device, samp_mat_process, samp_size, host)
- split lat_lon into lat and lon columns; changed sign of lon to negative to signify degrees west; reduced precision from variable to 4 decimal places
- replaced commas with semi-colons

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Related Publications

Hu, S. (2017). RNA (and optional DNA) extraction from environmental samples (filters) v2 (protocols.io.hk3b4yn). Protocols.io. doi:10.17504/protocols.io.hk3b4yn
Methods

Parameters

Parameter	Description	Units
biosample_accession	NCBI BioSample accession number	unitless
library_ID	Short unique identifier for the sequencing library.	unitless
title	Short description that will identify the dataset on public pages.	unitless
library_strategy	Amplicon = Sequencing of overlapping or distinct PCR or RT-PCR products	unitless
library_source	Metagenomic = Mixed material from metagenome	unitless
library_selection	PCR = Source material was selected by designed primers	unitless
library_layout	Paired-end or Single	unitless
platform	Sequencing platforms [Illumina]	unitless
instrument_model	Illumina instrument and model used for sequencing	unitless
design_description	Free-form description of the methods used to create the sequencing library; a brief materials and methods section.	unitless
filetype	file type: fastq	unitless
filename_R1	NCBI R1 filename	unitless
filename_R2	NCBI R2 filename	unitless
sample_title	NCBI sample title	unitless
bioproject_accession	NCBI BioProject identifier	unitless
organism	Description of sample organism(s)	unitless
isolation_source	Type of source: environmental	unitless
collection_date	Collection date(s)	unitless
geo_loc_name	Geographic location of sample source	unitless
lat	Latitude; north is positive	decimal degrees
lon	Longitude; east is positive	decimal degrees
samp_collect_device	Sampling device used for collection	unitless
source_material_id	Description of methodology	unitless
bacterial_or_eukaryotic_community	Whether the sample is bacterial or eukaryotic	unitless
Grazing_experiment_or_in_situ	Whether sample is from grazing experiment or in situ collection	unitless
Site_name	Name of sampling site	unitless
Replicate_information	Replicate information	unitless
Time_point	Time point for experiments or 'in situ' for collections	unitless
sample_id	Sample identifier for EV Nautilus cruise NA108	unitless
sample_name	sample name with further description	unitless

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Instruments

Dataset-specific Instrument Name	Niskin bottle
Generic Instrument Name	Niskin bottle
Dataset-specific Description	Used to collect background seawater sample.
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	MiSeq 300 x 300 bp PE
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.

Dataset-specific Instrument Name	
Generic Instrument Name	PCR Thermal Cycler
Generic Instrument Description	General term for a laboratory apparatus commonly used for performing polymerase chain reaction (PCR). The device has a thermal block with holes where tubes with the PCR reaction mixtures can be inserted. The cycler then raises and lowers the temperature of the block in discrete, pre-programmed steps. (adapted from http://serc.carleton.edu/microbelife/research_methods/genomics/pcr.html)

Dataset-specific Instrument Name	Beckman Coulter #A63881, Brea, CA
Generic Instrument Name	Coulter Counter
Dataset-specific Description	Used to clean PCR products.
Generic Instrument Description	An apparatus for counting and sizing particles suspended in electrolytes. It is used for cells, bacteria, prokaryotic cells and virus particles. A typical Coulter counter has one or more microchannels that separate two chambers containing electrolyte solutions. from https://en.wikipedia.org/wiki/Coulter_counter

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Deployments

NA108

Website	https://www.bco-dmo.org/deployment/828612
Platform	E/V Nautilus
Start Date	2019-05-24
End Date	2019-06-09
Description	See also https://www.rvdata.us/search/cruise/NA108

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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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