Metabarcoding data from microbial diversity survey at Axial Seamount aboard the R/V Thompson cruise TN420 in Jul 2022 and Jul 2023

Website: https://www.bco-dmo.org/dataset/969102
Data Type: Cruise Results
Version: 1
Version Date: 2025-07-29

Project
» Characterizing and quantifying the impact of phagotrophic protists at hot spots of primary production at Axial Seamount (PROT-ATAX)

Contributors	Affiliation	Role
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Abstract
Microorganisms are the ancestral forms of life on our planet and have been instrumental in shaping all of Earth's environments into what they are today. In the ocean, microbial prokaryotes and eukaryotes form the foundation of marine food webs through their activity and interactions. Single-celled microbial eukaryotes (or protists) are some of the most important species on the planet, yet our understanding of how their activities influence and regulate the ocean ecosystem is poorly constrained. At deep-sea hydrothermal vents in particular, our understanding of microbial food web dynamics is incomplete without including the role of microbial eukaryotes. In July 2023, we completed an ROV expedition to Axial Seamount (NE Pacific), where we used ROV Jason to collect low temperature diffuse vent fluid and surrounding plume and background seawater. These samples were filtered in situ or shipboard to collect the microbial communities. Data provided are the product of amplicon tag-sequencing using both 18S rRNA and 16S rRNA gene primers.

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Coverage

Location: NE Pacific Ocean at Axial Seamount between 1300-1540 m depth
Spatial Extent: **N**:45.933592 **E**:-129.979065 **S**:45.916228 **W**:-130.0495
Temporal Extent: 2022-07-09 - 2023-07-25

Methods & Sampling

Collection

A subset of samples from a prior year's expedition to Axial Seamount (TN405) was also included in the analysis. During TN405 (July 2022), one ROV Jason dive collected diffuse vent fluid using the Hydrothermal Fluid and Particle Sampler (labeled "HFS" in the dataset; designed by David Butterfield) and seawater from the plume was collected via Niskin bottles during CTD casts. TN405 was interrupted and TN420 represents the majority of samples associated with this project.

Collection of diffuse vent fluid and nearby seawater (background) was conducted using the Universal Fluid Obtainer (UFO) and the SUPR sampler. The UFO was the primary device used for fluid sampling for 3 ROV dives (J2-1500 - J2-1502), while the SUPR was the primary mode of fluid collection for 4 ROV dives (J2-1503 - J2-1506). No fluid sampler was operational during the first dive (J2-1499). For the first 4 sites (J2-1499, J2-1501, J2-1502, and J2-1503), 3 Niskin bottles mounted on ROV Jason were fired a few meters above active venting fluid.

The UFO has 8 total ports for fluid collection. For each dive, 4 L bags were attached to 6 ports and sterivex filters (0.22 µm pore size) were connected to the final 2 ports. ROV Jason temperature probe would identify a suitable spot, based on consistent fluid flow and temperature (we targeted 20-80°C fluid), and then would use an intake wand connected to the UFO in the other manipulator. For sterivex filters, once they were recovered from the UFO manifold, they were preserved with RNAlater and stored at -80°C.

The SUPR sampler was typically deployed with 10 2 L bottles and 4 filter holders, where the in situ filters used were either 0.2 µm PES or 0.7 µm nominal GFF filters. The intake for the SUPR sampler includes a built in temperature probe, in order to simultaneously monitor fluid temperature while collecting vent fluid. Each filter holder used on the SUPR included a built-in reservoir for RNAlater; RNAlater would ‘weep’ onto the filter as fluid was pulled through the filter and holder.

Sample processing

For all samples, RNA was extracted and amplified similarly to the protocol described in Hu et al. (2018). 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. PCR products were sent to Georgia Genomics and Bioinformatics Core (GGBC) and sequencings using the Illumina NextSeq2000 platform.

BCO-DMO Processing Description

- Imported "AxialSeamount_2023_metadata_BCO-DMO.xlsx" into the BCO-DMO system
- Converted date to ISO format YYYY-MM-DD
- Renamed fields to remove spaces and special characters
- Renamed "sample_name" to "site_name" after consulting with the submitter
- Replaced "6202024" and "7162024" values with "not applicable" in the "depth_category" parameter after consulting with submitter
- Removed "SPUID" and "sample_identifier" to reduce duplication after consulting with submitter
- Exported file as "969102_v1_microbial_diversity_axial_seamount.csv"

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Data Files

File
969102_v1_microbial_diversity_axial_seamount.csv (Comma Separated Values (.csv), 22.73 KB) MD5:1dde81233ff55023a23826d7e960a15b Primary data file for dataset ID 969102, version 1

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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

Rodriguez-Martinez, R., Rocap, G., Logares, R., Romac, S., & Massana, R. (2012). Low Evolutionary Diversification in a Widespread and Abundant Uncultured Protist (MAST-4). Molecular Biology and Evolution, 29(5), 1393–1406. https://doi.org/10.1093/molbev/msr303
Methods

STOECK, T., BASS, D., NEBEL, M., CHRISTEN, R., JONES, M. D. M., BREINER, H., & RICHARDS, T. A. (2010). Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. Molecular Ecology, 19(s1), 21–31. Portico. https://doi.org/10.1111/j.1365-294x.2009.04480.x https://doi.org/10.1111/j.1365-294X.2009.04480.x
Methods

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Related Datasets

References

Texas A&M University. Microbial populations from Axial Seamount Raw sequence reads. 2025/05. In: BioProject [Internet]. Bethesda, MD: National Library of Medicine (US), National Center for Biotechnology Information; 2011-. Available from: http://www.ncbi.nlm.nih.gov/bioproject/PRJNA1259551. NCBI:BioProject: PRJNA1259551.

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Parameters

Parameter	Description	Units
Accession	Unique identifier as cataloged in NCBI	unitless
Sample_Name	Complete sample name, also appears as fastq file name, as well as in the SRA database	unitless
NCBI_Organism_Name	NCBI code for sample origin	unitless
NCBI_Tax_ID	NCBI taxonomy code	unitless

BioProject	Identifier for project submitted to NCBI	unitless
dive_ctd	Identifier for the ROV Jason dive number or CTD cast number. "Control" and "HUBERLAB" used for control samples	unitless
site_name	Name of site, abbrivated	unitless
collection_method	Method of fluid collection; CTD, HFS, Lab, SUPR, UFO	unitless
sample_type	Sample type within deep-sea vent survey	unitless
filter	Type of filter fluid was collected onto	unitless
processing	Collection in situ in the deep sea or processed on board after fluid recovery	unitless
primer	18S or 16S primers were used	unitless
target_population	Intended target for microbial diversity	unitless
location_name	Full site name	unitless
depth_category	Type of sample with respect to depth	unitless
depth	Depth of sample	m
fluid_origin	Origin of fluid collection	unitless
AXIAL_YEAR	Axial Seamount cruise year	unitless
lat	Latitude of collection, N is positive	decimal degrees
long	Longitude of collection, W is negative	decimal degrees
DATE	Date of collection, PST	unitless

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Instruments

Dataset-specific Instrument Name	Illumina NextSeq2000
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	PCR products were sent to Georgia Genomics and Bioinformatics Core (GGBC) and sequencings using the Illumina NextSeq2000 platform.
Generic Instrument Description	A DNA sequencer is an instrument that determines the order of deoxynucleotides in deoxyribonucleic acid sequences.

Dataset-specific Instrument Name	Universal Fluid Obtainer (UFO) and the SUPR sampler
Generic Instrument Name	Discrete water sampler
Dataset-specific Description	Collection of diffuse vent fluid and nearby seawater (background) was conducted using the Universal Fluid Obtainer (UFO) and the SUPR sampler.
Generic Instrument Description	A device that collects an in-situ discrete water sample from any depth and returns it to the surface without contamination by the waters through which it passes, such as a water bottle.

Dataset-specific Instrument Name	Hydrothermal Fluid and Particle Sampler
Generic Instrument Name	Discrete water sampler
Dataset-specific Description	A subset of samples from a prior year's expedition to Axial Seamount (TN405) was also included in the analysis. During TN405 (July 2022), one ROV Jason dive collected diffuse vent fluid using the Hydrothermal Fluid and Particle Sampler (labeled "HFS" in the dataset; designed by David Butterfield) and seawater from the plume was collected via Niskin bottles during CTD casts.
Generic Instrument Description	A device that collects an in-situ discrete water sample from any depth and returns it to the surface without contamination by the waters through which it passes, such as a water bottle.

Dataset-specific Instrument Name	Niskin bottle
Generic Instrument Name	Niskin bottle
Dataset-specific Description	No fluid sampler was operational during the first dive (J2-1499). For the first 4 sites (J2-1499, J2-1501, J2-1502, and J2-1503), 3 Niskin bottles mounted on ROV Jason were fired a few meters above active venting fluid.
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	ROV Jason
Generic Instrument Name	ROV Jason
Dataset-specific Description	In 2023, we completed an ROV expedition to Axial Seamount (NE Pacific), where we used ROV Jason to collect low temperature diffuse vent fluid and surrounding plume and background seawater.
Generic Instrument Description	The Remotely Operated Vehicle (ROV) Jason is operated by the Deep Submergence Laboratory (DSL) at Woods Hole Oceanographic Institution (WHOI). WHOI engineers and scientists designed and built the ROV Jason to give scientists access to the seafloor that didn't require them leaving the deck of the ship. Jason is a two-body ROV system. A 10-kilometer (6-mile) fiber-optic cable delivers electrical power and commands from the ship through Medea and down to Jason, which then returns data and live video imagery. Medea serves as a shock absorber, buffering Jason from the movements of the ship, while providing lighting and a bird's eye view of the ROV during seafloor operations. During each dive (deployment of the ROV), Jason pilots and scientists work from a control room on the ship to monitor Jason's instruments and video while maneuvering the vehicle and optionally performing a variety of sampling activities. Jason is equipped with sonar imagers, water samplers, video and still cameras, and lighting gear. Jason's manipulator arms collect samples of rock, sediment, or marine life and place them in the vehicle's basket or on "elevator" platforms that float heavier loads to the surface. More information is available from the operator site at URL. https://ndsf.whoi.edu/jason/

Dataset-specific Instrument Name	Vortex
Generic Instrument Name	Shaker
Dataset-specific Description	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.
Generic Instrument Description	A Shaker is a piece of lab equipment used to mix, blend, or to agitate substances in tube(s) or flask(s) by shaking them, which is mainly used in the fields of chemistry and biology. A shaker contains an oscillating board which is used to place the flasks, beakers, test tubes, etc.

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Deployments

TN420

Website	https://www.bco-dmo.org/deployment/969109
Platform	R/V Thomas G. Thompson
Start Date	2023-07-17
End Date	2023-07-27
Description	Characterizing and quantifying the impact of phagotrophic protists at hot spots of primary production

TN405

Website	https://www.bco-dmo.org/deployment/938749
Platform	R/V Thomas G. Thompson
Start Date	2022-07-08
End Date	2022-07-12

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Project Information

Characterizing and quantifying the impact of phagotrophic protists at hot spots of primary production at Axial Seamount (PROT-ATAX)

Coverage: Axial Seamount

NSF Award Abstract:

Microorganisms are the ancestral forms of life on our planet and have been instrumental in shaping all of Earth?s environments into what they are today. In the ocean, microbial prokaryotes and eukaryotes form the foundation of marine food webs through their activity and interactions. Single-celled microbial eukaryotes (or protists) are some of the most important species on the planet, yet our understanding of how their activities influence and regulate the ocean ecosystem is poorly constrained. At deep-sea hydrothermal vents in particular, our understanding of microbial food web dynamics is incomplete without including the role of microbial eukaryotes. This project provides quantification of phagotrophic protistan grazing on the microbial communities inhabiting the highly productive diffuse vent mixing zone at hydrothermal vents, where the vent fluid-seawater interface promotes an increase in biological activity compared to the surrounding deep seawater. The results are contributing novel insights into the diversity and metabolic activities of the microbial eukaryotic community at vent fluid-seawater interfaces, establish the extent to which microbial eukaryotes impact primary production in the deep ocean by quantifying predation pressure, and estimate the amount of carbon transferred from primary producers to larger organisms. The investigators are training community college students from Cape Cod Community College by involving them in laboratory research through summer internships. The goal is to promote science, technology, engineering and math literacy among community college students through hands-on research experiences, peer-to-peer mentoring, and professional development opportunities, while also encouraging students to transfer to a four-year university, obtain a degree in a STEM subject, and continue on in a STEM field.

Grazing by microbial eukaryotes is a significant source of mortality for microbes in the oceans, thus influencing the composition of communities and serving as a major route for remineralization of organic material to all organisms. This project is quantifying the in situ rates of eukaryotic grazing on prokaryotic communities at hot spots of primary productivity in the deep sea and characterize the diversity of microbial eukaryotes, their abundance, and metabolic activities at the seafloor. The focus of the effort is at the underwater volcano Axial Seamount, home of the Ocean Observatories Initiative (OOI) Regional Cabled Array with well-characterized low-temperature diffusely venting fluids.

The two objectives of the work are to:

1. Quantify the rates and impact of phagotrophic microbial eukaryote grazing on prokaryotic communities at the seafloor in low-temperature diffuse fluid mixing zones.

2. Characterize microbial eukaryotic diversity, abundance, and metabolic gene expression at the seafloor in low-temperature diffuse fluid mixing zones.

The modular Microbial Sampler-Submersible Incubation Device (MS-SID) is being used for in situ seafloor tracer incubations and compared to shipboard incubations for the proposed grazing studies. This combination of technology provides detailed and quantitative assessments of protistan communities and establishes the extent to which microbial eukaryotes impact primary production in the deep ocean. Expected outcomes include the quantification of protistan grazing rates on bacterial and archaeal communities within the seafloor mixing zone, comparison of predation pressure at the vent-seawater interface and surrounding deep ocean

water, as well as identification of key bacteriovores, fungi, and other protists and associated major active metabolic pathways within the hydrothermal mixing zones. The project is providing a significant advance in the understanding of the microbial loop in the deep ocean as current depictions of microbial ecology at hydrothermal vent sites do not typically include the role of microbial eukaryotes.

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1947776

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