Gastropod counts by species collected from inactive sulfides on the East Pacific Rise during R/V Roger Revelle cruise RR2102 in April 2021 and R/V Atlantis cruise AT50-06 in December 2022

Website: https://www.bco-dmo.org/dataset/946438 Data Type: Cruise Results Version: 2 Version Date: 2024-12-20

Project

 » <u>RUI: Collaborative: The Predictive Nature of Microbial Biofilms for Cuing Larval Settlement at Deep-Sea</u> <u>Hydrothermal Vents</u> (Vent Settlement Cues)
» <u>Collaborative Research: Life after Death: Do Inactive Sulfides Fuel a Unique Ecosystem at the Deep Seafloor?</u> (Inactive Sulfides)

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Abstract

This dataset provides counts by species for gastropods collected mainly from rock grabs at two inactive sulfide mounds (Lucky's Mound and Sentry Spire) near 9°47'N on the East Pacific Rise. Sampling was conducted on dives with HOV Alvin during R/V Atlantis cruise AT50-06 in December 2022 and with ROV Jason during R/V Roger Revelle cruise RR2102 in April 2021. Specimens were preserved in ethanol or fixed in formalin. Gastropods were identified morphologically to species (with genetic sequences obtained for a subset); two of the species were new to science as described by Chen et al. (2024). Counts are not comprehensive thus cannot be used for quantitative analyses. This dataset is provided in a single compiled table formatted as a Darwin Core Occurrence table for provision to the Ocean Biodiversity Information System.

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Coverage

Location: East Pacific Rise 9 N 104 W depth 2500m Spatial Extent: N:9.79032 E:-104.2870238 S:9.7722685 W:-104.2874059 Temporal Extent: 2021-04-09 - 2022-12-27

Methods & Sampling

Location: East Pacific Rise 9 N 104 W depth 2500m

Collection at the seafloor:

Sampling was conducted near 9°47'N on the East Pacific Rise (EPR) on dives with the Human Occupied Vehicle (HOV) Alvin during R/V Atlantis cruise AT50-06 (cruise DOI: 10.7284/909880); this dataset includes collections from HOV Alvin Dives 5134 and 5135 at Lucky's Mound on 18 and 19 December 2022 and from Dive 5142 at Sentry Spire on 27 December 2022. This dataset also includes one sampling event from the Remotely Operated Vehicle (ROV) Jason II Dive 1311 at Sentry Spire on 9 April 2021 during R/V Revelle cruise RR2102 (cruise DOI: 10.7284/909120). Sampling events are specified in the dataset as "rock grab" or "slurp." For rock grabs - the manipulators were used to break off a rock then place it into an individual compartment of a closable, vehicle-mounted container (biobox). For slurps - the substratum (but not the rock targeted for sampling) was vacuumed with a five-chambered hydraulic slurp sampler. The sampling event log was associated by time to renavigated vehicle data including latitude, longitude, and depth (for AT50-06 used renavigated vehicle data version June 2023). We thank the captain, crew, Alvin and Jason teams, and scientists on-board both cruises for enabling sample collection.

Shipboard sample processing:

After recovery on deck, animals were either rinsed or gently scraped off the surfaces of sulfides or siphoned out of the biobox compartment for the collected rock. Siphoned biobox water and slurps were poured over a sieve (60 micrometer on AT50-06). The animal samples were placed into 4°C filtered seawater, sorted in petri dishes to high rank taxonomic groups (e.g., gastropods), and placed into 2.5-mL cryotubes. On cruise AT50-06 gastropods were either preserved in 80% ethanol and stored at -20°C or fixed in 10% buffered formalin; the formalin material was transferred to 80% ethanol after 1-2 days, refreshed with 80% ethanol after 1 day, and again after 1 day, then accidentally placed in a -80°C freezer on-board the research vessel. On cruise RR2102 gastropods were preserved in 95% ethanol and stored at room temperature.

Laboratory sorting and morphological identification:

Cryotubes containing gastropod specimens from a total of 8 rock grabs and 2 slurps from cruise AT50-06 were thawed and poured into a dish for sorting by morphotype. A subset of sorted specimens was photographed and placed into new 1-mL tubes [recorded as "samples and images" metadata tables for the sorted tubes (1 for ethanol, 1 for formalin)]. The new tubes were shipped at room temperature from WHOI to JAMSTEC.

Specimens were identified morphologically, recorded into ID tables for cruise AT50-06 (1 for ethanol, 1 for formalin); specimens from 1 rock grab also were identified for cruise RR2102. Gastropod specimens were gently cleaned using small brushes, and observed under a stereo dissecting microscope (Olympus SZX9). To obtain the operculum and radula for scanning electron microscopy, dissections were done under the microscope using fine forceps and tungsten needles. As listed in the compiled data table column otherCatalogNumbers, a subset of specimens was deposited in Senckenberg Natural History Museum, Frankfurt (SMF), National Museum of Nature and Science, Tsukuba (NSMT), or Muséum National d'Histoire Naturelle, Paris (MNHN). For more information about gastropod morphological identification, please see Chen et al. (2024).

Genetic evidence for identification:

DNA was extracted from one whole specimen of each of the three *Melanodrymia* species using the DNeasy Blood & Tissue kit (Qiagen) following the manufacturer's protocols. The same specimen's DNA extraction was used for both mitochondrial COI and mitogenome sequencing, with sequences deposited in National Center for

Biotechnology Information (NCBI) GenBank under accession numbers OR828527-OR828529 (Chen et al. 2024) and under BioProject number PRJNA1048888 (Zhang et al. 2024), respectively.

Data Processing Description

Dataset compilation:

The compiled data table is a single table formatted as a Darwin Core Occurrence table. The table was created using a script to join the three types of input data files for cruise AT50-06 [sampling event log, sorted tube tables, and ID tables), standardize taxa to the World Register of Marine Species (WoRMS), and add a few Darwin Core terms required by the Ocean Biodiversity Information System (OBIS). The script AT50-06_snails_compilation.R is attached to this dataset as a supplemental file (the author contributed this file from https://github.com/sbeaulieu/EPR-inactive/blob/main/AT50-06_snails_compilation.R (access date 2024-10-28, commit:af07a9e). One row in the data table was manually added for the one sampling event included from cruise RR2102. A Darwin Core Event table can be created from the right-most columns of the compiled data table.

BCO-DMO Processing Description

* Data from source file gastropods_inactive_AT50-06_RR2102_BCO-DMO_20241022.csv were imported into the BCO-DMO data system as the primary data table for the dataset.

* character "é" in dataset values replaced with "e" to comply with BCO-DMO conventions.

* The data submitter indicated "The script is available online at <u>https://github.com/sbeaulieu/EPR-</u> <u>inactive/blob/main/AT50-06_snails_compi...</u> (access date 2024-10-28). " and that they would prefer it be attached to the BCO-DMO dataset as a supplemental file.

The corresponding commit SHA:af07a9e for AT50-06_snails_compilation.R at that time (access date 2024-10-28) was downloaded from the github repository and attached a supplemental file.

Problem Description

The data table identifies only 7 (of the expected 8) rock sampling events for AT50-06 because for the specimens fixed in formalin, it was not possible to exactly match each individual to its source (host) rock. Instead, the matching was by the greatest number of that species in photographs taken during the sorting into new tubes, and 1 (AL5134-LuckysM-R003) did not have the greatest number (in this case, M. galeronae formalin_C2_1 occurred on both AL5134-LuckysM-R001 and AL5134-LuckysM-R003).

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Data Files

File
946438_v1_gastropods_inactive_at50-06_rr2102.csv(Comma Separated Values (.csv), 19.98 KB) MD5:51d4f801215ae347bcbb12cafd7892b8
Primary data file for dataset ID 946438, version 1

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Supplemental Files

File

R Script to compile AT50-06 snail identifications data table

filename: AT50-06_snails_compilation.R

(R Script, 8.67 KB) MD5:d796d55e8d08c59223ec4b5e131a8cf4

R script to compile AT50-06 snail identifications data table for BCO-DMO dataset (946438 version 1).

Script provided to BCO-DMO by author Stace Beaulieu from https://github.com/sbeaulieu/EPR-inactive/blob/main/AT50-06_snails_compilation.R (commit: af07a9e)

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Related Publications

Beaulieu, S. (2024). GitHub - sbeaulieu/EPR-inactive/AT50-06_snails_compilation.R: script to compile data table for BCO-DMO (Commit:af07a9e). GitHub. <u>https://github.com/sbeaulieu/EPR-</u> <u>inactive/blob/af07a9e24230e898824997c72620fc889c0ba41f/AT50-06_snails_compilation.R</u> *Software*

Beaulieu, S. (n.d.). GitHub - sbeaulieu/EPR-inactive: scripts for EPR inactive sulfides project. GitHub. https://github.com/sbeaulieu/EPR-inactive Software

Chen, C., Li, Y., Sun, J., Beaulieu, S. E., & Mullineaux, L. S. (2024). Two new melanodrymiid snails from the East Pacific Rise indicate the potential role of inactive vents as evolutionary stepping-stones. Systematics and Biodiversity, 22(1). https://doi.org/<u>10.1080/14772000.2023.2294014</u> *Results*

Zhang, L., Gu, X., Chen, C., He, X., Qi, Y., & Sun, J. (2024). Mitogenome-based phylogeny of the gastropod order Neomphalida points to multiple habitat shifts and a Pacific origin. Frontiers in Marine Science, 10. https://doi.org/<u>10.3389/fmars.2023.1341869</u> *Results*

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Related Datasets

IsRelatedTo

Chen,C., Zhang,L. and Sun,J. (2024). Melanodrymia galeronae isolate AL5142 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial. The National Center for Biotechnology Information GenBank: OR828527.1 Available from https://www.ncbi.nlm.nih.gov/nuccore/OR828527

Chen,C., Zhang,L. and Sun,J. (2024). Melanodrymia sp. I CC-2023 isolate AL5135_moonglow cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial. The National Center for Biotechnology Information GenBank: OR828527.1 Available from https://www.ncbi.nlm.nih.gov/nuccore/OR828529

Chen,C., Zhang,L. and Sun,J. (2024). Melanodrymia sp. t CC-2023 isolate AL5135_starburst cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial. The National Center for Biotechnology Information GenBank: OR828527.1 Available from https://www.ncbi.nlm.nih.gov/nuccore/OR828528

Ocean University of China (2023). Illumina sequence of Melanodrymiidae snails. 2023/12. In: NCBI:BioProject: PRJNA1048888 [Internet]. Bethesda, MD: National Library of Medicine (US), National Center for Biotechnology Information. Available from: <u>http://www.ncbi.nlm.nih.gov/bioproject/PRJNA1048888</u>

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Parameters

Parameter	Description	Units

occurrencelD	Unique identifier for the occurrence with eventID, preservative (ethanol or formalin), and shipped tube position (letter and number) http://rs.tdwg.org/dwc/terms/occurrencelD	unitless
kingdom	scientific name of the kingdom in which the taxon is classified http://rs.tdwg.org/dwc/terms/kingdom	unitless
verbatimIdentification	Name initially used to identify the specimens not necessarily a scientific name <u>http://rs.tdwg.org/dwc/terms/verbatimIdentification</u>	unitless
scientificName	Name from World Register of Marine Species (WoRMS) at the lowest taxonomic rank that matches the verbatimIdentification <u>http://rs.tdwg.org/dwc/terms/scientificName</u>	unitless
scientificNameID	Life Science Identifier (LSID) containing the AphiaID from WoRMS that matches to scientificName <u>http://rs.tdwg.org/dwc/terms/scientificNameID</u>	unitless
taxonRank	taxonomic rank of the scientificName http://rs.tdwg.org/dwc/terms/taxonRank	unitless
identifiedBy	person who identified the specimens for this occurrence <u>http://rs.tdwg.org/dwc/terms/identifiedBy</u>	unitless
identificationRemarks	Comments or notes about the verbatimIdentification <u>http://rs.tdwg.org/dwc/terms/identificationRemarks</u>	unitless
individualCount	The number of individuals with this scientificName in the shipped tube <u>http://rs.tdwg.org/dwc/terms/individualCount</u>	integer count
associatedSequences	A subset of the individuals with this scientificName in this shipped tube has genetic sequence(s) with NCBI accession number(s) <u>http://rs.tdwg.org/dwc/terms/associatedSequences</u>	unitless
otherCatalogNumbers	A subset of the individuals with this scientificName in this shipped tube has been provided to Senckenberg Natural History Museum, Frankfurt (SMF), National Museum of Nature and Science, Tsukuba (NSMT), or Museum National d'Histoire Naturelle, Paris (MNHN) http://rs.tdwg.org/dwc/terms/otherCatalogNumbers	unitless
occurrenceStatus	Darwin Core controlled vocabulary term present http://rs.tdwg.org/dwc/iri/occurrenceStatus	unitless
basisOfRecord	Darwin Core controlled vocabulary term PreservedSpecimen http://rs.tdwg.org/dwc/terms/basisOfRecord	unitless

verbatimLabel	shipped tube label <u>http://rs.tdwg.org/dwc/terms/verbatimLabel</u>	unitless
occurrenceRemarks	Comments or notes about the occurrence http://rs.tdwg.org/dwc/terms/occurrenceRemarks	
eventID	Unique identifier for the sampling event with dive number, locality, and rock number (or slurp chamber color) http://rs.tdwg.org/dwc/terms/eventID	unitless
eventDate	Date time for the sampling event. DateTime with timezeone (UTC) in ISO 8601 format. <u>http://rs.tdwg.org/dwc/terms/eventDate</u>	unitless
locality	inactive sulfide mound (Lucky's Mound or Sentry Spire) http://rs.tdwg.org/dwc/terms/locality	unitless
decimalLatitude	geographic latitude north positive for the sampling event at the seafloor <u>http://rs.tdwg.org/dwc/terms/decimalLatitude</u>	decimal degrees
decimalLongitude	geographic longitude east positive for the sampling event at the seafloor <u>http://rs.tdwg.org/dwc/terms/decimalLongitude</u>	decimal degrees
geodeticDatum	geodetic datum upon which the geographic coordinates are based http://rs.tdwg.org/dwc/terms/geodeticDatum	unitless
coordinateUncertaintyInMeters	uncertainty in horizontal distance from the given geographic coordinates for the sampling event at the seafloor <u>http://rs.tdwg.org/dwc/terms/coordinateUncertaintyInMeters</u>	meters (m)
minimumDepthInMeters	Depth of Alvin or Jason below the water surface minus 5 m http://rs.tdwg.org/dwc/terms/minimumDepthInMeters	meters (m)
maximumDepthInMeters	Depth of Alvin or Jason below the water surface plus 5 m http://rs.tdwg.org/dwc/terms/maximumDepthInMeters	meters (m)
relatedResourceID	International Generic Sample Number (IGSN) for the rock in this sampling event <u>http://rs.tdwg.org/dwc/terms/relatedResourceID</u>	unitless
samplingProtocol	collection method for the sampling event (rock grab or slurp) http://rs.tdwg.org/dwc/terms/samplingProtocol	unitless

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Instruments

Dataset- specific Instrument Name	
Generic Instrument Name	HOV Alvin
Dataset- specific Description	HOV Alvin Dives AL5134, Al5135, AL5142
Generic Instrument Description	
Dataset-	

Dataset- specific Instrument Name	
Generic Instrument Name	Multi-chamber Suction Sampler
Generic	An underwater device that collects animals and other samples under gentle suction and deposits them into a collection chamber. Also known as an oceanographic 'slurp' sampler. The primary components are an electrical or hydraulic pump, outlet hose connected to collection chamber, and inlet hose.

Dataset- specific Instrument Name	ROV Jason II
Generic Instrument Name	ROV Jason
Dataset- specific Description	Dive 1311
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/

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Deployments

RR2102

Website	https://www.bco-dmo.org/deployment/879915
Platform R/V Roger Revelle	
Start Date	2021-03-24
End Date	2021-04-25

AT50-06

Website	https://www.bco-dmo.org/deployment/933990
Platform	R/V Atlantis
Start Date	2022-12-03
End Date	2023-01-01
Description	Project: RUI: Collaborative: The Predictive Nature of Microbial Biofilms for Cuing Larval Settlement at Deep-Sea Hydrothermal Vents START/END PORT: Puntarenas, Costa Rica

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Project Information

RUI: Collaborative: The Predictive Nature of Microbial Biofilms for Cuing Larval Settlement at Deep-Sea Hydrothermal Vents (Vent Settlement Cues)

NSF Award Abstract:

Over four decades of research have shown that tiny free-swimming offspring of the unique inhabitants of hydrothermal vents can disperse effectively between their specialized habitats. Yet, we know almost nothing about how these larval animals complete the journey by locating and settling down in suitable locations. This question remains one of the key unresolved puzzles in the ecology of the deep sea and is becoming increasingly important to solve as hydrothermal vents are becoming threatened by human impacts. The investigators suggest that the films of bacteria that first form at vents are good signposts for settlement of larvae because they indicate that the hydrothermal vents are suitable for life. This project uses a combined program of field experiments, cutting-edge molecular biology techniques, and shipboard experiments with hydrothermal-vent larvae and cultured bacterial films. The project also connects undergraduate research interns at a primarily undergraduate institution (Western Washington University) with undergraduate research interns at two research institutions (Rutgers and Woods Hole Oceanographic Institution) while working on the project at sea together. Finally, the team is producing a science-in-action documentary filled with ocean science and exploration intended for television distribution and museum screenings. The investigators are using footage of the deep-sea vents, shipboard and diving operations, and laboratory work to create a documentary that highlights the foundation of scientific research—hypothesis-driven research, the application of the scientific method, and the importance of critical thinking—all in the framework of the study of an exciting, but threatened, ecosystem.

Hydrothermal vents are particularly tractable systems in which to study questions about the roles of biofilms in larval settlement because biofilms at vents are relatively low-complexity; vent animals are strictly dependent on vent microbes, often through symbiotic partnerships acquired after settlement; and environmental variations are present within the range of a common larval pool. Moreover, decades of research on settlement in model organisms give us good insight into biofilm cues; there is solid foundational understanding about colonization patterns at vents; we now have excellent tools to collect, identify, and culture vent larvae and microbes; and modern environmental "-omics" techniques are a good tool to characterize biological cues produced by biofilms. The project provides an unprecedented, quantitative look into the role of microbial biofilms in structuring larval settlement at hydrothermal vents, achieved only through the close collaboration of microbial and larval ecologists. The combined field program of short-term settlement experiments, microbial "-omics" work, and subsequent shipboard settlement experiments allows the investigative team to use field experiments to statistically model the factors that best predict larval settlement in the field, then test those predictions with shipboard experiments that decouple covarying conditions. This extensive characterization of putative larval settlement cues and their relationship to colonization success in heterogeneous vent habitat niches will contribute to a broader understanding of colonization success across diverse marine ecosystems. Understanding the role that the initial settlement of larvae plays in the recovery and resilience of hydrothermalvent ecosystems is critical to developing informed management plans for deep-sea mining.

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.

Collaborative Research: Life after Death: Do Inactive Sulfides Fuel a Unique Ecosystem at the Deep Seafloor? (Inactive Sulfides)

Coverage: East Pacific Rise near 10 N

NSF Award Abstract:

This project is investigating a newly discovered community of animals and microbes near deep-sea hydrothermal vents that appears to inhabit only cool, inactive sulfide features. The main objectives are to determine what species live on these features, whether they are new to science, and how they function in the community. The discovery of this novel community, which may be fueled by production of resident microbes, is likely to change the way we think about inactive vents and their contribution to deep-sea biodiversity and productivity. This project has broad impact in four different areas: 1) Informing policy for sustainable use (mining) of inactive sulfides; 2) Contributing to global data systems and the NSF-funded repository at BCO-DMO to make our data available for research use at other temporal, spatial, and taxonomic scales; 3) Increasing public scientific literacy by enhancing K-12 education in the sciences at Memorial Junior High in Eagle

Pass TX with about 98% Hispanic and 2% Native American students and a high number of English Language Learners and migrants; and 4) Developing a diverse workforce by engaging students from under-represented and marginalized groups into undergraduate intern programs.

Hydrothermal venting of heated, reduced fluids from the seafloor occurs globally at plate tectonic boundaries and mid-plate hotspots and has been the subject of vigorous geological, chemical and biological research. However, this venting is ultimately transient, leaving behind only the sulfide mineral-rich deposits after the fluid flow stops. This project investigates the organisms living on these lesser studied inactive sulfide features in order to understand their ecology and associations with the mineral substratum. Recent discoveries indicate that some microbial and animal species inhabiting inactive sulfides are not found elsewhere in the marine environment, suggesting the sulfides serve as a unique habitat, distinct from other seafloor topographic features. The main project objectives are to characterize the species and functional diversity of the inactive sulfide ecosystem across all three domains of life (eukaryotic, bacterial, and archaeal), determine which animal species are endemic or predominantly associated with inactive sulfides, and explore the biological and geological characteristics governing those associations. The investigators are conducting field studies between 9-10 degrees N on the East Pacific Rise at sites within the axial summit trough as well as at recently discovered off-axis sites away from modern day venting features. The discovery of this novel community of organisms inhabiting inactive sulfide features at hydrothermal vent fields, fueled by resident chemolithotrophic microorganisms, is likely to change the way we think about the role of these ecosystems in deep-sea biodiversity and productivity.

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)	<u>OCE-2152453</u>

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