## Sample log from Jason-II dives for Sievert collected from the R/V Atlantis (AT26-10) in the East Pacific Rise, Pacific Ocean (Microbial Communities at Deep-Sea Vents project)

Website: https://www.bco-dmo.org/dataset/559780 Data Type: Cruise Results Version: 1 Version Date: 2015-05-26

### Project

» An Integrated Study of Energy Metabolism, Carbon Fixation, and Colonization Mechanisms in Chemosynthetic Microbial Communities at Deep-Sea Vents (Microbial Communities at Deep-Sea Vents)

#### Programs

» Dimensions of Biodiversity (Dimensions of Biodiversity)

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

Contributors	Affiliation	Role
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### Abstract

This dataset is a sample log of samples collected by ROV Jason II at the 9<sup>o</sup>N deep-sea hydrothermal vent field on the East Pacific Rise, Pacific Ocean, January 2014.

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## Coverage

**Spatial Extent**: N:11.8398 **E**:-104.279 **S**:9.7708 **W**:-106.2915 **Temporal Extent**: 2014-01-03 - 2014-01-19

#### Methods & Sampling

The taxonomic composition of the microbial community present in fluid samples and shipboard incubations conducted in gas-tight isobaric samplers (IGT) was investigated by sequencing 16S rRNA amplicons using 454 technology. Further, samples from shipboard incubations were taken to obtain cell counts, activity measurements using nano-scale secondary ion mass spectrometry (nanoSIMS), to determine bulk 13C-incoproration into biomass, and measure pH and the concentration of select chemicals (nitrate, ammonium, sulfide, hydrogen, methane, oxygen). On select samples obtained with the large volume pump (LVP), metagenomic, metaproteomic, and lipid biomarker analyses will be performed.

Samples were collected at several sites at the 9°N deep-sea hydrothermal vent field on the East Pacific Rise. They included ROV Jason-II deployments J2-758, J2-759, J2-760, J2-761, J2-762. DNA was extracted following established protocols. We were able to successfully sequence 16S rRNA amplicons for Bacteria and Archaea from a total of 17 shipbaord incubations, 10 LVP samples, and one basalt rock sample. Sequence data are currently being analyzed and will be deposited in GenBank prior to publication and will be made available to the scientific community. From the incubations, the following analyses have been completed: total cell numbers, NanoSIMS analyses, 13C-bulk organic carbon analysis, and chemical measurements. These data are currently being prepared for a manuscript and data will be publically released with the publication. Metagenomic sequencing, metaproteomic analyses, and lipid biomarker analysis of the LVP samples are currently underway. Data will be made available to the scientific community once the data processing is complete and data are published. This is expected to be the case in the first half of 2016.

16S rRNA amplicons for Bacteria and Archaea were generated using 454 sequence technology. Obtained sequences are currently being analyzed using the QIIME pipeline. The reads are being dereplicated, denoised, screened for chimeric sequences and taxonomically classified using the RDP and GreenGenes databases. Multivariate ordination techniques are being used to discriminate among samples with similar community structures. Total sulfide was determined by combining a 2mL sample with sulfide antioxidant buffer and measuring voltage with a sulfide-selective electrode. The electrode was calibrated daily with a serial dilution of a standard sodium sulfide solution. To account for oxidation of the sulfide solution, the solution was titrated daily with lead nitrate to determine the actual sulfide concentration. pH was measured using an electrode, which was calibrated daily. Methane and hydrogen were determined by quantitative headspace extraction of a known volume of sample and measured on a GC-FID (for methane and concentrations of hydrogen  $> 5\mu$ M) or GC-TCD (for concentrations of hydrogen  $<5\mu$ M). Oxygen was determined by passing hydrothermal fluid through a specially designed flow-through cell fitted with a commercially available oxygen optode (Pts3, Presens, Germany). Fluorescence lifetime decay was measured every second using a computerized system corrected for temperature effects (Fibox 3, Presens, Germany), and the most stable final oxygen value was used. The oxygen optode spot was calibrated once with oxygen-free water (treated with sodium dithionate) and airsaturated water to make a two-point calibration. Prior to measurements, the optode flow-through cell was flushed with N2-purged FBSW to remove air bubbles and connected to the IGT sample valve while both were dripping liquid to avoid entrainment of air bubbles in the sample chamber. For subsequent nutrient analysis, fluids were filtered through a 0.2µm GTTP membrane and stored frozen at -20oC. Total nitrate+nitrite was determined by conversion to NO and chemiluminescent determination using the NoxBox instrument (Teledyne. San Diego CA, USA) following the original protocol (Garside 1982). Similarly, filtered samples were analyzed shipboard for total ammonium+ammonia by flow injection as previously described. All standards were pure chemicals made up in distilled water. All analytes, with the exception of oxygen, were measured in analytical duplicates. Cells were prepared for counting by preserving 1.5mL of fluids with 40µL of borate-buffered formalin, and subsequently adding 200µL of 0.1% acridine orange solution. The fixed and stained sample was then filtered under gentle vacuum onto a black 0.2µm polycarbonate filter, and enumerated aboard the ship by fluorescence microscopy. 10 grids were counted per sample and extrapolated using the area filtered to determine cell concentrations. All counts were done in analytical duplicates.

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## **Data Files**



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## **Related Datasets**

#### IsSupplementTo

**project).** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2017-01-13 http://lod.bco-dmo.org/id/dataset/674781 [view at BCO-DMO]

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## **Parameters**

Parameter	Description	Units
sample_num	sequential sample number	unitless
cruise_id	cruise identification	unitless
dive	Jason-II dive number	unitless
lat	latitude; north is positive	decimal degrees
lon	longitude; east is positive	decimal degrees
depth	sample depth	meters
date	sampling date, UTC	yyyy-mm-dd
sample_type	sample type: fluid or rock	unitless
site	sampling site	unitless
sample	sampe id	unitless
collection_method	collection method: IGT=Isobaric Gastight Sampler ; LVP=Large Volume Pump	unitless
sample_description	description of the sample taken	unitless
analysis	types of analysis performed on the sample	unitless

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## Instruments

Dataset- specific Instrument Name	
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	NoxBox instrument
Generic Instrument Name	Chemiluminescence NOx Analyzer
Dataset- specific Description	For nitrate+nitrite measurement. Teledyne, San Diego CA, USA
Generic Instrument Description	The chemiluminescence method for gas analysis of oxides of nitrogen relies on the measurement of light produced by the gas-phase titration of nitric oxide and ozone. A chemiluminescence analyzer can measure the concentration of NO/NO2/NOX. One example is the Teledyne Model T200: <u>https://www.teledyne-api.com/products/nitrogen-compound-instruments/t200</u>

Dataset-specific Instrument Name	Oxygen Optode
Generic Instrument Name	Optode
Dataset-specific Description	Fibox 3/Pts3, Presens, Germany
Generic Instrument Description	An optode or optrode is an optical sensor device that optically measures a specific substance usually with the aid of a chemical transducer.

Dataset- specific Instrument Name	Jason-II
Generic Instrument Name	ROV Jason
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.

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# Deployments

AT26-10

Website	https://www.bco-dmo.org/deployment/529031
Platform	R/V Atlantis
Report	http://dmoserv3.bco-dmo.org/data_docs/Microbe_Vent_Communities/AT26- 10_Cruise_Report_v2_2015-07-09.pdf
Start Date	2013-12-29
End Date	2014-01-27
Description	Samples were collected by ROV Jason II at the 9N deep-sea hydrothermal vent field on the East Pacific Rise, Pacific Ocean

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## **Project Information**

An Integrated Study of Energy Metabolism, Carbon Fixation, and Colonization Mechanisms in Chemosynthetic Microbial Communities at Deep-Sea Vents (Microbial Communities at Deep-Sea Vents)

Deep-sea hydrothermal vents, first discovered in 1977, are poster child ecosystems where microbial chemosynthesis rather than photosynthesis is the primary source of organic carbon. Significant gaps remain in our understanding of the underlying microbiology and biogeochemistry of these fascinating ecosystems. Missing are the identification of specific microorganisms mediating critical reactions in various geothermal systems, metabolic pathways used by the microbes, rates of the catalyzed reactions, amounts of organic carbon being produced, and the larger role of these ecosystems in global biogeochemical cycles. To fill these gaps, the investigators will conduct a 3-year interdisciplinary, international hypothesis-driven research program to understand microbial processes and their quantitative importance at deep-sea vents. Specifically, the investigators will address the following objectives: 1. Determine key relationships between the taxonomic, genetic and functional diversity, as well as the mechanisms of energy and carbon transfer, in deep-sea hydrothermal vent microbial communities. 2. Identify the predominant metabolic pathways and thus the main energy sources driving chemoautotrophic production in high and low temperature diffuse flow vents. 3. Determine energy conservation efficiency and rates of aerobic and anaerobic chemosynthetic primary productivity in high and low temperature diffuse flow vents. 4. Determine gene expression patterns in diffuse-flow vent microbial communities during attachment to substrates and the development of biofilms.

Integration: To address these objectives and to characterize the complexity of microbially-catalyzed processes at deep-sea vents at a qualitatively new level, we will pursue an integrated approach that couples an assessment of taxonomic diversity using cultivation-dependent and -independent approaches with methodologies that address genetic diversity, including a) metagenomics (genetic potential and diversity of community), b) single cell genomics (genetic potential and diversity of uncultured single cells), c) meta-transcriptomics and -proteomics (identification and function of active community members, realized potential of the community). To assess function and response to the environment, these approaches will be combined with 1) measurement of in situ rates of chemoautotrophic production, 2) geochemical characterization of microbial habitats, and 3) shipboard incubations under simulated in situ conditions (hypothesis testing under controlled physicochemical conditions). Network approaches and mathematical simulation will be used to reconstruct the metabolic network of the natural communities. A 3-day long project meeting towards the end of the second year will take place in Woods Hole. This Data Integration and Synthesis meeting will allow for progress reports and presentations from each PI, postdoc, and/or student, with the aim of synthesizing data generated to facilitate the preparation of manuscripts.

Intellectual Merit. Combining the community expression profile with diversity and metagenomic analyses as well as process and habitat characterization will be unique to hydrothermal vent microbiology. The approach will provide new insights into the functioning of deep-sea vent microbial communities and the constraints regulating the interactions between the microbes and their abiotic and biotic environment, ultimately enabling us to put these systems into a quantitative framework and thus a larger global context.

Broader Impacts. This is an interdisciplinary and collaborative effort between 4 US and 4 foreign institutions, creating unique opportunities for networking and fostering international collaborations. This will also benefit the

involved students (2 graduate, several undergraduate) and 2 postdoctoral associates. This project will directly contribute to many educational and public outreach activities of the involved PIs, including the WHOI Dive & Discover program; single cell genomics workshops and Cafe Scientifique (Bigelow); REU (WHOI, Bigelow, CIW); COSEE and RIOS (Rutgers), and others. The proposed research fits with the focus of a number of multidisciplinary and international initiatives, in which PIs are active members (SCOR working group on Hydrothermal energy and the ocean carbon cycle, <u>http://www.scorint</u>. org/Working\_Groups/wg135.htm; Deep Carbon Observatory at CIW, <u>https://dco.gl.ciw.edu/;</u> Global Biogeochemical Flux (GBF) component of the Ocean Observatories Initiative (OOI), <u>http://www.whoi.edu/GBF-OOI/page.do?pid=41475</u>)

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## **Program Information**

#### Dimensions of Biodiversity (Dimensions of Biodiversity)

Website: <u>http://www.nsf.gov/funding/pgm\_summ.jsp?pims\_id=503446</u>

Coverage: global

(adapted from the NSF Synopsis of Program)

Dimensions of Biodiversity is a program solicitation from the NSF Directorate for Biological Sciences. FY 2010 was year one of the program. [MORE from NSF]

The NSF Dimensions of Biodiversity program seeks to characterize biodiversity on Earth by using integrative, innovative approaches to fill rapidly the most substantial gaps in our understanding. The program will take a broad view of biodiversity, and in its initial phase will focus on the integration of genetic, taxonomic, and functional dimensions of biodiversity. Project investigators are encouraged to integrate these three dimensions to understand the interactions and feedbacks among them. While this focus complements several core NSF programs, it differs by requiring that multiple dimensions of biodiversity be addressed simultaneously, to understand the roles of biodiversity in critical ecological and evolutionary processes.

#### 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 <u>Data Management Plan (PDF)</u> and in compliance with the <u>NSF Ocean Sciences Sample and Data Policy</u>. 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)	<u>OCE-1136727</u>

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