Lost City hydrothermal fluids sequence data from R/V Atlantis AT42-01 in the Lost City hydrothermal field from September to October 2018 (Lost City Limits to Life project)

Website: https://www.bco-dmo.org/dataset/897962
Data Type: Cruise Results
Version: 1
Version Date: 2023-06-27

Project
» Collaborative Research: Investigating the Lost City as an ultramafic urban center of the subseafloor, fueled by energy and carbon from the mantle (Lost City Limits to Life)

Contributors	Affiliation	Role
Brazelton, William	University of Utah	Principal Investigator, Contact
Newman, Sawyer	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
Hydrothermal fluid samples were collected from actively venting chimneys at the Lost City hydrothermal field using ROV Jason during the 2018 Lost City expedition aboard R/V Atlantis (AT42-01). This study includes 39 samples of hydrothermal fluids that were dedicated to DNA and RNA sequencing, including analyses of amplicon sequence variants (ASVs), metagenomes, and metatranscriptomes. The present study included hydrothermal fluid samples from seven chimney locations: Camel Humps, Sombrero, Marker 3, Marker C, Calypso, Marker 2, and Marker 8. Several fluid samples collected from the Beehive chimney, where the highest fluid temperatures have been measured, yielded only low-quality DNA sequences and are not included in this study. The Sombrero site was sampled on two different ROV Jason dives, and samples from the separate dives are labeled as Sombrero1 or Sombrero2 when appropriate. Fluid samples collected from Markers C, 2, 3, and 8 were included in an early microbial diversity study (Brazelton et al., 2006), but microbial diversity data from the other chimneys are reported here for the first time. The fluid samples ranged from those that were barely distinguishable from ambient seawater (~11 ºC, pH 8) to warm and highly alkaline hydrothermal fluids (~80 ºC, pH 10). Direct counts of visible cells showed little variability among fluids, with densities approximately 2-8 × 104 mL-1 in all samples, although the two samples with the highest temperatures had the least number of cells.

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Coverage

Spatial Extent: **N**:30.2708 **E**:30.2708 **S**:-42.1246 **W**:30.0054
Temporal Extent: 2018-09-08 - 2018-10-01

Methods & Sampling

Methods and Sampling

On the seafloor, venting fluids were slowly pumped through 0.2 µm Millipore Sterivex cartridge filters or into

acid-washed Kynar bags with the HOG sampler. Samples intended for RNA extractions were collected into 2 L Kynar bags containing 67 mL of a stop solution (97.5% 200 proof ethanol, 2.5% Trizol LS). Fluid temperatures were monitored in real-time during sampling with a probe embedded into the sampler intake. Immediately upon shipboard recovery of ROV Jason, all Sterivex filters were stored at -80 ºC.

Extraction of DNA from all Sterivex filters (including in situ-filtered fluids and fluids collected in Kynar bags and filtered shipboard) was conducted as described in the full protocol available via the Zenodo-archived GitHub repository (DOI: 10.5281/zenodo.5798015) and on protocols.io (DOI: dx.doi.org/10.17504/protocols.io.bykqpuvw). Total RNA was extracted from the Sterivex filters with a modification of the DNA extraction protocol optimized for RNA. The full protocol is available in the Zenodo-archived GitHub repository (DOI: 10.5281/zenodo.5798015) and on protocols.io (DOI: dx.doi.org/10.17504/protocols.io.bykspuwe). First-strand synthesis of cDNA was performed with SuperScript IV Reverse Transcriptase and random hexamers (Thermo Fisher).

Sequencing of amplicons generated from 16S rRNA genes and cDNA was performed at the Genomics Core Facility at Michigan State University on an Illumina MiSeq instrument using dual-indexed Illumina fusion primers targeting the V4 region of the 16S rRNA gene. Metagenome libraries were constructed with size-selected, sonicated DNA fragments of 500-700 bp with the NEBnext Ultra DNA II library kit for Illumina (E7645S). Paired-end sequencing (2 x 125 bp) of metagenomic libraries was conducted at the University of Utah High-Throughput Genomics Core Facility at the Huntsman Cancer Institute with an Illumina HiSeq2500 platform. The two metatranscriptome libraries were constructed and sequenced by the University of Utah High-Throughput Genomics Core Facility at the Huntsman Cancer Institute. Total RNA was hybridized with NEBNext rRNA Depletion Solution Bacteria (E7850L) to substantially diminish rRNA from the samples. Stranded RNA sequencing libraries were prepared using the NEBNext Ultra II RNA Library Prep Kit for Illumina (E7770L). Following the transfer of the flowcell to an Illumina NovaSeq 6000 instrument, a 150 cycle paired-end sequence run was performed using a NovaSeq 6000 S4 reagent Kit v1.5 (20028312).

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Related Publications

Brazelton, W. J., McGonigle, J. M., Motamedi, S., Pendleton, H. L., Twing, K. I., Miller, B. C., Lowe, W. J., Hoffman, A. M., Prator, C. A., Chadwick, G. L., Anderson, R. E., Thomas, E., Butterfield, D. A., Aquino, K. A., Früh-Green, G. L., Schrenk, M. O., & Lang, S. Q. (2022). Metabolic Strategies Shared by Basement Residents of the Lost City Hydrothermal Field. Applied and Environmental Microbiology, 88(17). https://doi.org/10.1128/aem.00929-22
Results

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Parameters

Parameters for this dataset have not yet been identified

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Instruments

Dataset-specific Instrument Name	HOG (hydrothermal organic geochemistry) sampler
Generic Instrument Name	Hydrothermal Organic Geochemistry Sampler
Dataset-specific Description	On the seafloor, venting fluids were slowly pumped through 0.2 µm Millipore Sterivex cartridge filters or into acid-washed Kynar bags with the HOG sampler. Samples intended for RNA extractions were collected into 2 L Kynar bags containing 67 mL of a stop solution (97.5% 200 proof ethanol, 2.5% Trizol LS). Fluid temperatures were monitored in real-time during sampling with a probe embedded into the sampler intake.
Generic Instrument Description	The Hydrothermal Organic Geochemistry (HOG) sampler is designed to collect large volume (2–9 L) fluid samples with minimal introduction of organic or microbial contamination, and to be powered and deployed in real time from a submersible. Additional design constraints include utilizing materials appropriate for sampling fluids with elevated temperatures, fitting the sampler into the space available on the submersible, and minimizing the time needed to remove samples and prepare the sampler for re-deployment between dives.

Dataset-specific Instrument Name	R/V Atlantis Niskin rosette
Generic Instrument Name	Niskin bottle
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	Hydrothermal fluid samples were collected from actively venting chimneys at the Lost City hydrothermal field using ROV Jason during the 2018 Lost City expedition aboard R/V Atlantis (AT42-01). This study includes 39 samples of hydrothermal fluids that were dedicated to DNA and RNA sequencing, including analyses of amplicon sequence variants (ASVs), metagenomes, and metatranscriptomes.
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

AT42-01

Website	https://www.bco-dmo.org/deployment/782074
Platform	R/V Atlantis
Report	https://datadocs.bco-dmo.org/docs/Lost_City_Limits_to_Life/data_docs/AT42-01_Cruise%20Report_reduced.pdf
Start Date	2018-09-08
End Date	2018-10-01

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Project Information

Collaborative Research: Investigating the Lost City as an ultramafic urban center of the subseafloor, fueled by energy and carbon from the mantle (Lost City Limits to Life)

Coverage: Lost City Hydrothermal Field

NSF Award Abstract:
The vast majority of deep seafloor sediments are inhabited by microbial communities that survive under extreme energy limitation, with apparent generation times of centuries to millennia. Hydrothermal systems are a stark contrast to these energy-starved environments and may represent important, high-activity, 'population centers' in the oceanic subsurface. When rocks from the Earth's mantle are uplifted and exposed to water, the resulting reactions lead to acidic fluids with high concentrations of hydrogen. Under certain circumstances, small organic molecules such as methane can also form in the absence of biology. These compounds can provide energy to subseafloor microbial communities and, given the ubiquity of mantle rocks, such reactions may fuel a significant proportion of the active subsurface biosphere. The current project will characterize the microbial communities inhabiting an iconic example of this type of system, the Lost City Hydrothermal Field, using a remotely operated vehicle. The ghostly spires of Lost City are highly telegenic and have been featured in professional documentaries. The high definition underwater video footage collected during the expedition will provide the raw material for an 8 week educational training program in digital media focused on kindergarten through 12th grade high school students and undergraduate students. The resulting short documentaries will be published on YouTube and the Utah Education Network.

Mantle rocks comprise significant portions of the seafloor, and microbial communities hosted within them may be important mediators of carbon and energy exchange between the deep Earth and the surface biosphere. Upon tectonic uplift and exposure to water, the serpentinization of these materials releases potential energy in the form of hydrogen, methane, and heat, and further reaction of these products can sustain the abiogenic synthesis of small organic molecules. Recent studies have highlighted, however, the lack of alkalithermophiles that are capable of survival at the high pH (9-11) and elevated temperatures found in these systems. The almost complete lack of carbon dioxide (CO2) represents a second, and possibly more significant, limitation to growth. To better understand the extent of the serpentinite subsurface, this project will address the question: What limits biological activity in the serpentinite subsurface? Specifically, the proposed work will test the hypotheses: (1) microbial diversity spans a wider range of temperature-pH conditions than currently recognized and (2) the scarcity of CO2 is a key biological limitation to serpentinization-driven ecosystems that can be overcome by the metabolic activity of one or a few foundation species. These hypotheses will be tested during a 20 day (10 days on site) expedition to the Lost City Hydrothermal Field, focusing on fluids as windows to the subsurface biosphere. The sampling approach will capitalize on the differences in temperature, carbon availability, and microbial activity across the field. The analytical approach will integrate multidisciplinary

techniques performed on replicate subsamples and feature the application of next-generation sequencing technologies to these marine serpentinizing fluids for the first time. This study will generate extensive sequence data from environmental DNA, environmental mRNA, and single-cell genomes, allowing us to identify the in situ expression of metabolic pathways and the genomics of active single cells. These efforts will be closely linked with a thorough characterization of carbon in these fluids that will focus on identifying available substrates (e.g. methane, CO2, organic acids) and on characterizing biomarkers that reflect specific metabolic pathways (e.g. lipids, amino acids).

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1536702
NSF Division of Ocean Sciences (NSF OCE)	OCE-1536405
NSF Division of Ocean Sciences (NSF OCE)	OCE-1535962

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