Direct counts (flow cytometry) on microbes obtained by Niskin bottle and pressure-retaining sampler from the Leggo Lander on R/V Falkor cruise FK141215 in the Challenger Deep, Mariana Trench in December 2014

Website: https://www.bco-dmo.org/dataset/684323
Data Type: Cruise Results
Version: 1
Version Date: 2017-03-13

Project

» Patterns of Microbial Community Structure Within and Between Hadal Environments (Mariana Perspectives)

Contributors	Affiliation	Role
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Abstract

Direct counts (flow cytometry) on the microbes obtained in the Leggo drop 1 Niskin bottle and the Leg drop 1 pressure-retaining sampler.

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Coverage

Spatial Extent: **Lat**:11.36639 **Lon**:142.432555
Temporal Extent: 2014-12-16

Dataset Description

Direct counts (flow cytometry) on the microbes obtained in the Leggo drop 1 Niskin bottle and the Leg drop 1 pressure-retaining sampler.

Acquisition Description

This data set is associated with PI Douglas Bartlett (NSF OCE-1536776) and Schmidt Ocean Institute R/V Falkor cruise FK141215. The cruise occurred December 15-21, 2014 in the Challenger Deep within the territorial waters of the Federated States of Micronesia. During this cruise the Leggo lander was deployed multiple times and drops 1 and 3 recovered seawater samples that were analyzed. Additional details can be found at: https://schmidtocean.org/cruise/expanding-mariana-trench-perspectives/ and https://scripps.ucsd.edu/labs/dbartlett/contact/challenger-deep-cruise-2014/

Leggo Lander Drop 1:
Time (in Guam) deployed/recovered: December 16, 9:00/19:26.
Position at deployment: 11° 21.9836 N 142° 25.9533 E, middle section of the Challenger Deep.
Greatest depth of dive: approximately ~10,900 m.
In situ temperature on seafloor: 2.6°C.
Notes: This drop recovered seawater samples from about a meter off the seafloor. This included a 3 L Niskin bottle of seawater and ~ 150 mls of seawater collected in a pressure-retaining seawater sampler. The PRS sampler held more than 81% of the in situ pressure.

Flow Cytometry:
Direct counts (flow cytometry) on the microbes obtained in the Leggo drop 1 Niskin bottle and the Leg drop 1 pressure-retaining sampler. Samples were fixed with ~1% PFA and frozen. Later samples were removed from the -80 freezer and thawed in the dark.

The Attune was started and a Performance Test was run with the "Performance Tracking Beads" to check that all lasers and filters were working, and that voltages were correct. Cleaning and decontamination was also done using the Attune Wash solution and MilliQ water. Random samples selected from Logan's samples were run using Instrument Settings in the Attune software. This allows for real-time adjustments of voltages and thresholds in the different channels to get the best resolution for that day’s run as well as allows for quantification of instrument noise for a given day.

Once samples were thawed, 300 uL of each sample was loaded into a 96-well U-bottom plate. 3 uL of Invitrogen Sybr-green stain (diluted to 100x in MilliQ water) was then added to each well. The plate then incubated in the dark for 30 minutes before being run. The Sybr-green stain stains any DNA within a cell which then fluoresces when passing the BL1 channel laser, allowing for counts of cells.

Once instrument settings were determined for the day, the plate was loaded into the NxT autosampler and the run was started. Samples were run at "Standard" sensitivity at 100 uL/min for a total volume of 250 uL. Counts were delayed for 15 seconds to avoid any noise or dilution that can occur when sample starts being sipped. Once entire plate was run, I used the Attune software to correct for noise and gate various populations within the samples.

Note: Leggo1 is the first drop of the Leggo Lander and the values reflect the flow cytometric cell counts from its 2 liter Niskin bottle. Leggo1_PRS_day_2 is the flow cytometric cell count obtained from the pressure retaining sampler used on the first Leggo drop, following incubation in ice water for about 36 hours after recovery.

Colony Identification:
Data from the identification of bacteria cultured from the Leggo drop 1 and 3 Niskin bottles are available as a supplemental file (.txt). These identifications were performed using standard methods associated with PCR amplification of the 16S rRNA gene followed by dideoxy sequencing at Retrogen Inc.

Processing Description

BCO-DMO Processing:
-modified parameter names to conform with BCO-DMO naming conventions;
-replaced spaces with underscores;
-replaced '-' with 'nd' (no data);
-added dates, times, locations from metadata form.

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Parameters

Parameter	Description	Units
drop_name	Name of the lander drop	unitless
cells_per_mL	Number of cells per milliliter (mL)	cells/mL
ISO_DateTime_deploy	Date and time (local Guam time zone) of lander deployment; formatted to ISO 8601 standard.	unitless
ISO_DateTime_recover	Date and time (local Guam time zone) of lander recovery; formatted to ISO 8601 standard.	unitless
lat	Latitude of lander deployment	decimal degrees
lon	Longitude of lander deployment	decimal degrees

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Instruments

Dataset-specific Instrument Name	Niskin bottle
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	
Generic Instrument Name	Flow Cytometer
Generic Instrument Description	Flow cytometers (FC or FCM) are automated instruments that quantitate properties of single cells, one cell at a time. They can measure cell size, cell granularity, the amounts of cell components such as total DNA, newly synthesized DNA, gene expression as the amount messenger RNA for a particular gene, amounts of specific surface receptors, amounts of intracellular proteins, or transient signalling events in living cells. (from: http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm)

Dataset-specific Instrument Name	
Generic Instrument Name	Leggo Lander
Generic Instrument Description	The "Leggo Lander" is a lander system that primarily relies on syntactic foam for buoyancy and uses iridium GPS, radio signal, strobe light and flag for surface recovery, and acoustics for underwater monitoring and instrument control. The lander has a timer with 5 control settings for various operations. It routinely measures pressure (depth) throughout its dive and temperature on the seafloor. The lander payloads include a pressure-retaining seawater sampler plus 2 liter Niskin bottle, and a camera/battery/light system that also includes a 30 liter Niskin bottle and a sea cucumber trap. With the camera payload it travels down or up the water column at about 39 meters per minute (~ 4.5 hours for a descent to the Challenger Deep at ~10,920 m). (Description obtained from the R/V Falkor FK141215 post-cruise report (PDF))

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Deployments

FK141215

Website	https://www.bco-dmo.org/deployment/684236
Platform	R/V Falkor
Report	http://dmoserv3.whoi.edu/data_docs/Mariana_Perspectives/Bartlett-final-FK141215-cruise-report.pdf
Start Date	2014-12-15
End Date	2014-12-21
Description	During this cruise the Leggo lander was deployed multiple times and drops 1 and 3 recovered seawater samples that were analyzed. Additional details can be found at: https://schmidtocean.org/cruise/expanding-mariana-trench-perspectives/ and https://scripps.ucsd.edu/labs/dbartlett/contact/challenger-deep-cruise-2.... More information is available in the post-cruise and final expedition reports (PDF). Original cruise data are available from the NSF R2R data catalog

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Project Information

Patterns of Microbial Community Structure Within and Between Hadal Environments (Mariana Perspectives)

Coverage: Challenger Deep, Mariana Trench

Award Abstract from NSF: The deepest portion of the ocean is present in ocean trenches, whose steep walls descend from approximately 4 miles down to depths that in some cases are close to 7 miles below the seawater surface. At these locations Earth's crust is recycled. Perhaps not surprisingly given their remoteness, deep ocean trenches are the least understood habitats in the ocean. The researchers participating in this project are working to characterize the microbes present in two of the deepest trenches present on Earth, both in the Pacific Ocean, the Kermadec Trench located north of New Zealand, and the Mariana Trench, located east and south of the island of Guam. Most of the Mariana Trench is located within the United States Mariana Trench Marine National Monument. Relatively little is known about the diversity and adaptations of the microorganisms in deep ocean trenches. An unknown fraction of the microbes present have descended from shallow waters above and are unlikely to participate in any nutrient cycles in the deep sea. Others are adapted to near freezing temperatures and up to

pressures greater than 10e7 kilograms per square meter (16,000 pounds per square inch). These latter microbes perform important roles recycling organic matter. But who are they? This project is contributing to the training of diverse undergraduate and graduate students participating in research, additional undergraduate students learning about microbes inhabiting extreme environments in a web-based class, and additional graduate students and postdoctoral scientists participating in an advanced training course being offered in Antarctica. Experiments being performed include direct counts of prokaryotes and viruses in seawater and sediments, analyses of the abundance and phylogenetic breadth of culturable heterotrophic bacteria at a range of pressures, measurements of bacterial community species diversity and richness both within and across seawater and sediment samples, as well as within and across the two trench systems, measurements of microbial activity as a function of pressure and the identification of high pressure-active cells. The data generated from these analyses are being integrated into the results of additional chemical, geological and biological measurements performed by others as a part of the National Science Foundation funded Hadal Ecosystems Studies Project. Two of the working hypotheses are that prokaryote numbers and diversity are generally positively correlated with surface productivity and proximity to the trench axis and that bacterial taxa exist which are endemic to specific trenches, present in multiple trenches and more widely distributed in deep-sea environments.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1536776

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