Data on microbial metatranscriptomics: Niskin vs. in situ sampler from the R/V Urania cruise in the Mediterranean Sea during 2012 (Pickled Protists project)

Website: https://www.bco-dmo.org/dataset/554005
Version: 2015-03-19

Project
» Pickled Protists or Community Uniquely Adapted to Hypersalinity? (Pickled Protists)

Contributors	Affiliation	Role
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Dataset Description

Metatranscriptome of whole microbial community from a deep Mediterranean normal salinity site comparing results for a Niskin sample vs. an in situ sampler

Related Reference:

Edgcomb, V.P., Taylor, C., Pachiadaki, M., Engstrom, I., Yakimov, M. 2014. Comparison of Niskin vs. *in situ* approaches for analysis of gene expression in deep Mediterranean Sea water samples. Deep Sea Res II, doi: 10.1016/j.dsr2.2014.10.020

Methods & Sampling

Water was collected using 12L Niskin bottles mounted on a General Oceanics rosette sampler equipped with conductivity-temperature and depth (CTD) and pressure sensors. After transferring water from Niskin bottles to a large sterile carboy, 30 liters of water were pumped through a 0.22µm Sterivex filter cartridge using a peristaltic pump operating around 125 ml/min containing a Durapore filter (Millipore, Millford, MA, USA), which was immediately filled with RNAlater (Life Technologies Inc., Grand Island, NY, USA) and frozen at -80°C until extraction. Water samples from the same depth and on the same day were also collected and preserved *in situ* using the MS-SID in situ microbial sampler equipped with a CTD, two turbidity sensors, and an oxygen optode. Three MS-SID samples were filtered *in situ* at 125 ml/min through a 47mm 0.2 µm Durapore (Millipore, USA) filter that, upon cessation of filtration was within 10-20 seconds flooded with the preservative RNAlater following filtration. The three filters collected 4L, 3L and 3.4L of water, respectively. Upon retrieval of the instrument to the ship's deck, the Fixation Filter Units were disassembled, and the filters and associated RNAlater solutions were transferred aseptically to three separate sterile cryovials, and frozen at -80°C until extraction. The Sterivex capsule (from Niskin bottle collection) was also stored at -80°C until extraction. RNA preps performed as in Edgcomb et al. 2014 DSR II.

Data Processing Description

Quality trimming of the reads (minimum quality score 28, minimum read length 94 bp and no ambiguous nucleotides) as well as read assembly into contigs and mapping of reads to contigs were performed using CLC Genomics Workbench 6.0, CLCBio, Cambridge, MA, USA). The Rapid Analysis of Multiple Metagenomes with a Clustering and Annotation Pipeline (RAMMCAP) was used to assign contigs to clusters of orthologous gene (COG) families, gene ontologies (GO), and protein families (Pfam). Taxonomic assignments of contigs were made using PhymmBL, incorporating all available fungal and protist genomes in public databases. The total number of annotated reads assigned to different COG families for each dataset was expressed as a percentage of the total annotated reads for each dataset.

BCO-DMO Processing:
original file: EdgcombKM3MetatranscriptomeData.xlsx
- added conventional header with dataset name, PI name, version date
- renamed parameters to BCO-DMO standard
- added cruise id, lat, lon
- added html links to GenBank BioProject

Data Files

File
KM3_metatranscriptomics.csv (Comma Separated Values (.csv), 1.02 KB)   MD5:12e9004eee3e6b305dbca369fabca361   Primary data file for dataset ID 554005

Parameters

Parameter	Description	Units
cruise_id	cruise identification	unitless
site	sampling location	unitless
lat	latitude; north is positive	decimal degrees
lon	longitude; east is positive	decimal degrees
sampling_method	sampling method	unitless
project_id	GenBank Bioproject number	unitless
accession_number	GenBank accession number	unitless
sample	sample identification	unitless
tax_id	GenBank Taxonomy ID number	unitless
filename	sequence result file name	unitless

Instruments

Dataset-specific Instrument Name	Sequencer
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	Illumina HiSeq system
Generic Instrument Description	A DNA sequencer is an instrument that determines the order of deoxynucleotides in deoxyribonucleic acid sequences.

Dataset-specific Instrument Name	Niskin bottle
Generic Instrument Name	Niskin bottle
Dataset-specific Description	12L Niskin bottles mounted on a General Oceanics rosette sampler equipped with conductivity-temperature and depth (CTD) and pressure sensors.
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	MS_SID
Generic Instrument Name	Submersible Incubation Device-In Situ Microbial Sampler
Dataset-specific Description	Microbial Sampler - In Situ Incubation Device (MS-SID)
Generic Instrument Description	The Submersible Incubation Device-In Situ Microbial Sampler (SID-ISMS) system was developed for the 2011 NSF funded DHAB Metazoans Mediterranean Brine research project and first used on cruise AT18-14. The system includes several integrated components including: a 2 liter incubation chamber; fixation filters and water sample bottles; a High Range CTD (Neil Brown Ocean Sensors, Inc., USA) equipped with two turbidity sensors (Wet Labs ECOView); an Aanderra 2808F oxygen optode; an SDSL-data link; and a sonardyne beacon, a pinger and a 24 volt deep-sea battery. The sensors and sampling devices are mounted on a frame that is attached to the hydro-wire. Lowering rate and recovery speed are controlled by a winch mounted on the surface vessel.

Deployments

Urania-2012-09

Website	https://www.bco-dmo.org/deployment/554001
Platform	R/V Urania
Start Date	2012-09-14
End Date	2012-10-01
Description	Microbial sample collection

Project Information

Pickled Protists or Community Uniquely Adapted to Hypersalinity? (Pickled Protists)

Coverage: Mediterranean Sea

Protists are an essential component of microbial food webs and play a central role in global biogeochemical cycles, and thus are key players in sustaining the healthy functioning of any ecosystem. Over the past few years a rich diversity of protists has been revealed in a range of extreme environments, indicating that the frontiers of eukaryotic life are still being explored. Only recently, one of the most extreme marine environments known to science was discovered in the eastern Mediterranean Sea at a depth of ~3500m, namely deep hypersaline anoxic basins (DHABs). These basins are characterized by extremely high salt concentrations (up to saturation) that have been considered anathema to life. Instead, highly diverse communities of bacteria exist in the waters of these basins. With the exception of a preliminary study to this proposal that indicated a diverse and active assemblage of protists in the water column along the halocline and below the halocline, these DHABs remain largely unexplored regarding eukaryotic life forms. The sediments of the DHABs have not been explored for protists at all.

The investigators will collect water column and sediment samples on a short cruise to two basins with different brine chemistries. An exciting combination of molecular, cultivation-independent and culture-based approaches will be used to study the microbial communities of two basins. Investigators will use those approaches to determine adaptive strategies of marine protist communities to hypersaline, anoxic environments and the degree of their potential impact on biogeochemical cycling as a result of their predation activities, the degree to which the dominant protists maintain bacterial or archaeal symbionts, and the identity of those symbionts. The original research proposal identified Bannock and Discovery Basins as the field study areas, however the 2009 cruise collected samples at Discovery and Urania Basin. Methods to be employed include RNA-based sequence analysis of diversity based on 18S rDNA genes, statistical analyses of community composition and phylotype richness, geochemical documentation of the water column and sediments using classical and microelectrode approaches, expression profiling using 3'-UTR fragments of mRNAs, sequencing of complete gene transcripts for proteins appearing to confer adaptation to hypersalinity, analysis of the proteome signatures, FISH-SEM to characterize novel extremophiles, CARD-FISH to identify eukaryote prey and putative symbionts, and TEM to assess morphology and endobiont presence in common benthic morphotypes.

Hypersaline environments rank highly in the list of extreme systems that have attracted increasing notice in science as well as by the lay public. For example, considering predictions of increasing temperatures and drought in certain regions of our planet, the number of hypersaline habitats may increase dramatically causing this ecosystem to gain importance on a global scale. Thus, an understanding of the ecosystem in these habitats will help predict future ecosystem functioning due to global change. From a different perspective, revealing the mechanisms of adaptation to high salinity has become a major objective, both for biological science and for potential commercial exploitation of natural products associated with those adaptations.

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-0849578