Microbial metatranscriptomics: upper and lower halocline water column data from the R/V Oceanus and R/V Urania (OC454-02) in the eastern Mediterranean Sea in 2009 (Pickled Protists project)

Website: https://www.bco-dmo.org/dataset/554055
Version: 2015-03-19

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

Contributors	Affiliation	Role
Edgcomb, Virginia P.	Woods Hole Oceanographic Institution (WHOI)	Principal Investigator
Bernhard, Joan M.	Woods Hole Oceanographic Institution (WHOI)	Co-Principal Investigator
Copley, Nancy	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Table of Contents

Dataset Description

Cloned SSU rRNA sequences of kinetoplastids detected in Discovery, Urania and Kryos Basin haloclines and brines.

Related Reference:

Edgcomb, V.P., W. Orsi, H.-W. Breiner, A. Stock, S. Filker, M.M. Yakimov, and T. Stoeck. 2011, Novel kinetoplastids associated with hypersaline anoxic lakes in the Eastern Mediterranean deep-sea. Deep-Sea Research I 58(10):1040-1048.

Methods & Sampling

The position of the halocline was determined during the R/V Oceanus cruise using a SBE9 CTD (Sea-Bird Electronics, USA) equipped with an SBE43 oxygen sensor (Sea-Bird Electronics, USA). Samples were collected from the halocline and brine of Discovery basin using a rosette equipped with Niskin bottles (12 L) on R/V Oceanus.

RNA was extracted as described in detail previously (Alexander et al., 2009) using bead beating of filters in an extraction buffer and E-Matrix tubes (QBiogene, MP Biomedicals, USA), followed by the RNA/DNA Allprep kit (Qiagen, Hildesheim, Germany) according to the manufacturer's instructions. Total RNA was transcribed into cDNA using the Two-Step Omniscript Reverse Transcription kit (Qiagen) according to the manufacturer's instructions. Subsequently, the small subunit ribosomal RNA gene (18S rRNA) was amplified using the kinetoplastid-specific primers 14F (5′-CTGCCAGTAGTCATATATGCTTGTTTCAAGGA-3′) and 2026R (5′-GATCCTCTGCAGGTTCACCTACAGCT-3′) (von der Heyden et al., 2004).

Data Processing Description

PCR products were cloned and Sanger sequenced (bidirectionally)

BCO-DMO Processing:
original file: EdgcombKinetoplastid.xlsx
- added conventional header with dataset name, PI name, version date
- renamed parameters to BCO-DMO standard
- converted lat/lon to decimal degrees from degrees-decimal minutes
- added cruise id's
- added html links to GenBank Popset

[table of contents | back to top]

Data Files

File
kinetoplastid.csv (Comma Separated Values (.csv), 1.45 KB) MD5:1cd23500771f53fa20071ab21606e376 Primary data file for dataset ID 554055

[table of contents | back to top]

Parameters

Parameter	Description	Units
cruise_id	cruise identification	unitless
lat	latitude; north is positive	decimal degrees
lon	longitude; east is positive	decimal degrees
sample_location	sampling location	unitless
sample_descrip	description of sample	unitless
depth	depth	meters
sal	total salinity	unitless
O2	dissolved oxygen	ml/L
cond	conductivity	S/m
accession_number	GenBank accession number	unitless
url	link to GenBank accession data	unitless

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	Sequencer
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	Roche 454 FLX Titanium platform
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	Niskin bottle
Generic Instrument Name	Niskin bottle
Dataset-specific Description	12L Niskin bottles mounted on a General Oceanics rosette sampler equipped with a SBE-911plus conductivity-temperature-depth (CTD) sensor (Sea- Bird Electronics)
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.

Dataset-specific Instrument Name	PCR
Generic Instrument Name	Thermal Cycler
Generic Instrument Description	A thermal cycler or "thermocycler" is a general term for a type of laboratory apparatus, commonly used for performing polymerase chain reaction (PCR), that is capable of repeatedly altering and maintaining specific temperatures for defined periods of time. The device has a thermal block with holes where tubes with the PCR reaction mixtures can be inserted. The cycler then raises and lowers the temperature of the block in discrete, pre-programmed steps. They can also be used to facilitate other temperature-sensitive reactions, including restriction enzyme digestion or rapid diagnostics. (adapted from http://serc.carleton.edu/microbelife/research_methods/genomics/pcr.html)

[table of contents | back to top]

Deployments

OC454-02

Website	https://www.bco-dmo.org/deployment/58164
Platform	R/V Oceanus
Report	http://data.bco-dmo.org/Protists_2009/OC454-02_cruise_report.pdf
Start Date	2009-07-28
End Date	2009-08-05

Description	This cruise aboard Oceanus from Piraievs, Greece did field work in the Mediterranean Sea for the NSF OCE funded project: "Pickled Protists or Community Uniquely Adapted to Hypersalinity?". The cruise dates are from the Final UNOLS schedule (ID #10808 Version #7 Date: 1/5/2010) and agree with the cruise dates specified in the UNOLS Post-Cruise Assessment Report filed by the Chief Scientist. The cruise dates, 7-25 - 8-05-2009, from the R2R cruise catalog include the previous transit cruise during which no sampling was done for this project. The original science objectives included collection of water column and sediment samples at 3 different hypersaline anoxic basins, L'Atalante, Discovery, and Urania. The plan was to collect Niskin samples and SID samples (a WHOI sampler that carries out in situ fixation) from above the halocline, within the halocline, and within the brine of each basin (3 depths, two types of samples per depth, Niskin and SID). Researchers also planned to collect multicores from the bottom sediments where the halocline impinged on the seafloor, a reference sample from above the halocline, and a sample within the brine (3 depths along a transect through the halocline) from two of the basins. Science activities conducted during the cruise included sampling in two of the three basins, Discovery and Urania Basin: (1) 11 CTD casts to various depths as needed to support sampling objectives as determined by the depth of the halocline (2) Water samples were collected from Niskin bottle from 3 designated depths from two basins (3) SID samples were collected from Discovery Basin, but the SID malfunctioned due to extreme depth before a complete sampling program could be completed in Urania Basin (4) Partially successful deployments of the multicorer at Discovery Basin yielded some multicores from within the brine and above the halocline, but attempts to collect a halocline sample were unsuccessful. A brine sediment sample was successfully collected at Urania Basin, but repeated attempts to collect multicores failed. Multicore sampling was negatively affected by several factors: the tubes failed to close properly, the multicorer didn't fire properly, and researchers had difficulty locating the halocline since neither the multicorer nor the CTD instrument package had a functioning camera system. Although the original cruise plan included sample collection in a third basin (L'Atalante Basin), only two basins were occupied. The cruise plan was adjusted when equipment malfunctions negatively impacted the success of the coring work. The decision was made that co-PI Bernhard should collect some sediment cores from the second basin, Urania Basin, on the last day of the cruise instead of conducting the water column sampling in the third basin. More information is included in the 4 August 2010 Cruise report prepared by K. Kormas, M. Pachiadaki and P. Sigala (cruise report PDF file). Funded by: NSF OCE-0849578 (see abstract from NSF site) Cruise information and original data are available from the NSF R2R data catalog

Urania-2009-09

Website	https://www.bco-dmo.org/deployment/554069
Platform	R/V Urania
Start Date	2009-09-08
End Date	2009-09-21
Description	microbial sampling

[table of contents | back to top]

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.

[table of contents | back to top]

Funding

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

[table of contents | back to top]