454 pyrotags for Eukaryote taxa in deep hypersaline anoxic basin (DHAB) halocline sediments collected on R/V Atlantis cruise AT18-14 in the Eastern Mediterranean (35.3 N, 21.7 E) in 2011

Website: https://www.bco-dmo.org/dataset/553959
Version: 2015-05-22

Project
» Pickled Protists or Community Uniquely Adapted to Hypersalinity? (Pickled Protists)
» Investigations into the Physiological State of DHAB Metazoans (DHAB Metazoans)

Contributors	Affiliation	Role
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Dataset Description

Pyrotags of eukaryotes obtained from Urania, Discovery, and L'Atalante DHAB halocline sediments.

Small subunit ribosomal RNA V4 hyper variable region eukaryotic pyrotags prepared from total RNA extracted from sediments below haloclines of 3 deep hypersaline anoxic basins in the Eastern Mediterranean Sea and from nearby control sites (normal saline deep marine sediments in close proximity to deep hypersaline anoxic basins). Total RNA was transcribed to cDNA, and eukaryotic V4 pyrotags amplified from cDNA.

Related References:

Bernhard, J.M., CM Morrison, E Pape, DJ Beaudoin, MA Todaro, MG Pachiadaki, K Ar Kormas, & VP Edgcomb. 2015 (submitted). Metazoans of redoxcline seidments in Mediterranean deep-sea hypersaline anoxic basins.

Bernhard, J.M., Kormas, K.A., Pachiadaki, M.G., Rocke, E., Beaudoin, D.J., Morrison, C., Visscher, P.T., Cobban, A., Starczak, V.R., and Edgcomb, V.P. 2014. Benthic protists and fungi of Mediterranean deep hypersaline anoxic basin redoxcline sediments. Frontiers in Microbiology, doi: 10.3389/fmicb.2014.00605.

Methods & Sampling

ROV Jason was used to collect push cores 6.35cm diameter obtained from the Deep Submergence Lab at Woods Hole Oceanographic Institution (www.whoi.edu/groups/DSL/) and configured with a seal to prevent contamination during ascent from the halocline sediments of L' Atalante, Urania and Discovery basins in the eastern Mediterranean Sea at a depth of about 3500-4000m. Sediments were profiled for oxygen and immediately frozen upon return to surface.

Data Processing Description

RNA from approximately 8 g of the top 2 cm from each frozen syringe subcore was extracted using an optimized protocol with the RNA Power Soil kit (MoBio, USA). Major modifications included introduction of 3 cycles of freeze-thaw (-80 degrees C, 5 min., 65 degrees C, 5 min.), bead beating with 2× 5 min. intervals on a horizontal vortexer, an overnight nucleic acid precipitation, and a one-hour centrifugation during the precipitation step. In addition, we introduced two DNAase treatments using TurboDNAase (Ambion, USA). Removal of DNA was confirmed by PCR using general eukaryotic primers. RNA was purified using the MEGAclear kit (Ambion, USA). Reverse transcription of the purified RNA samples was performed using the QuantiTect kit (Qiagen, USA). Tag-pyrosequencing of the eukaryotic small subunit rRNA (18S rRNA) gene was performed using PCR amplification of the V4 region of the 18S rRNA gene and the primer pair TAReuk454FWD1 (5′-CCAGCA(G/C)C(C/T)GCGGTAATTCC-3′) and TAReukREV3 (5′-ACTTTCGTTCTTGAT(C/T)(A/G)A-3′). Following PCR, all amplicon products (ca. 450 bp) from different samples were purified using the MinElute Reaction Cleanup kit (Qiagen, USA). Amplicon libraries were sequenced utilizing the Roche 454 FLX Titanium platform and reagents following manufacturer's guidelines at the MR DNA (Molecular Research LP, Shallowater, TX, USA) sequencing facility.

Denoising of the flowgrams was performed using Acacia. Processing of the resulting sequences, i.e. trimming and quality control, was performed using QIIME. Sequences with 380 bp or more and no ambiguous base calls and no homopolymers of 6 bp or more were included in further analysis. All sequences were binned into Operational Taxonomic Units (OTUs) and were clustered (average neighbor algorithm) at 97% sequence similarity identity. Taxonomic assignments were made using BLAST within QIIME.

BCO-DMO Processing:
- original file: EdgcombDHABeukaryoteTags.xlsx
- replaced positions for Discovery and Urania with those in event log for pushcores
- added html links to GenBank BioProject
- added cruise_id, lat, lon
- added html links to the GenBank BioSample (22 May 2015)

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Data Files

File
sediment_pyrotags_euk.csv (Comma Separated Values (.csv), 964 bytes) MD5:5406d6120b3df991f90897c6feada585 Primary data file for dataset ID 553959

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Parameters

Parameter	Description	Units
project_id	GenBank Project identification	unitless
seq_target	sequence target	unitless
BioSample_id	GenBank BioSample identification number and hyperlink.	unitless
SRA	GenBank Sequence Read Archive identification	unitless
sample_location	sampling location	unitless
cruise_id	cruise identification	unitless
lat	latitude; north is positive	decimal degrees
lon	longitude; east is positive	decimal degrees

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Instruments

Dataset-specific Instrument Name	Automated 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	ROV Jason
Generic Instrument Name	ROV Jason
Dataset-specific Description	Sampled with push cores of 6.35cm diameter and configured with a seal to prevent contamination during ascent.
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.

Dataset-specific Instrument Name	Thermal Cycler
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)

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Deployments

AT18-14

Website	https://www.bco-dmo.org/deployment/58732
Platform	R/V Atlantis
Start Date	2011-11-25
End Date	2011-12-08
Description	According to the pre-cruise plan, the two main science objectives are: (1) water column sampling at two basins: Discovery and Urania Basins, at 3 depths: brine (approx 3500-4000m depth), halocline (~3500m), and reference (~2000m) using a new sampler, the SID-ISMS (under construction), with the vessel CTD/Niskin rosette as backup and (2) sediment coring at both basins, using ROV Jason. Cores will be collected in 3 locations for each basin, the "bathtub ring" where the halocline impinges on the seafloor, the brine, and a reference core sample from above the halocline. Station "Discovery" (35° 19.213' N 21° 41.351' E) will be occupied for 6 days as will "Station 2" (35° 13.674' N 21° 28.58' E). The proposed science activities include: (1) water column sampling using the SID-ISMS to collect in situ filtered water (ship must hold position during deployment while instrument is working) and preserved in situ for molecular work; (2) water column sampling using the SID-ISMS to collect in situ filtered and preserved samples for FISH/microscopy experiments; (3) grazing experiment using SID-ISMS to collect water from halocline of each basin and measure the grazing rates of protozoa over a 6 hour period. The instrument must remain at depth during the 6 hour SID-ISMS grazing experiments. The sampler can be lifted to ~3000 m depth to get it away from the bottom, but the ship must maintain position to avoid dragging the sampler; (4) coring of "bathtub ring" at each basin using the ROV Jason that will be used to locate the bathtub ring and then collect cores at that location; (5) coring of brine at each basin (ROV Jason will reach into the brine from the bathtub ring area and will collect cores). Corers will be a combination of large Jason pushcores (property of co-PI Bernhard) and also some RNAlater samplers (similar to those used by Tim Shank (WHOI). The RNAlater samplers must be fabricated (and perhaps some borrowed from the Shank lab group); and (6) coring of a reference sample from outside the halocline (above) at each basin (normal seawater sediments). The research team aboard the R/V Atlantis, headed south on 25 November 2011 from Piraeus (port of Athens, Greece), to the study areas about 100 miles west of the island of Crete. No cruise report will be submitted for this cruise, but the science party did maintain a blog at the Dive and Discover site for Dive and Discover Expedition 14 - The Mediterranean Deep Brines (URL: http://www.divediscover.whoi.edu/expedition14/index.html). During the cruise, 5 ROV Jason dives, 10 SID-ISMS deployments, 1 multicorer (no samples recovered), and 3 Niskin rosette casts were completed. Image data from the ROV Jason dives for the AT18-14 cruise are available from the WHOI ROV Jason Virtual Van by clicking the year 2011 on the page's left side panel, and then clicking on the link for AT18-14. The cruise was supported by NSF-BIOLOGY awards:http://www.nsf.gov/awardsearch/showAward.do?AwardNumber=0849578 (Edgcomb)http://www.nsf.gov/awardsearch/showAward.do?AwardNumber=1061391 (Bernhard) Original cruise data are available from the NSF R2R data catalog

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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.

Investigations into the Physiological State of DHAB Metazoans (DHAB Metazoans)

Coverage: Eastern Mediterranean; 35.3 N, 21.7 E

Invasion of the Body Snatchers!

Description text from the NSF award abstract:
Although it has been known for many decades that metazoans inhabit anoxic habitats either on a periodic, transient, or semi permanent basis, none have been shown to complete an entire life cycle without access to oxygen. The remarkable recent observation that loriciferan metazoans complete a full life cycle without access to dissolved oxygen raises questions in the fields of physiology and evolution. The habitat from which the anaerobic animals were collected is sediment from a Deep Hypersaline Anoxic Brine (DHAB) in the eastern Mediterranean Sea at a water depth greater than 3 kilometers. DHABs are one of the most extreme marine environments known to science, with a water chemistry considered anathema to eukaryotic life. While the possibility of anaerobic metazoa is exciting, there are other potential explanations that warrant investigation before biology textbooks are rewritten. One alternative scenario is that remnant metazoa bodies were inhabited by anaerobic bacteria and/or archaea.

The overall goal of this project is to determine if the dominant loriciferan and nematode taxon in each of three DHABs represent living populations. Because remnant DNA can be preserved in anoxic settings for long periods of time, the project will include in situ preservation for RNA analysis. Further, because there is also some chance of RNA preservation in these anoxic sedimentary environments, the study will include analyses of the more ephemeral mRNA and also Transmission Electron Microscopy (TEM). On three ship days added to a funded cruise to sample DHABs for other purposes, an ROV will be used to preserve samples in situ. The specific aims are to: (1) Use RNA and DNA analysis to establish if metazoan ribosomal RNA and functional genes were active at the time of in situ preservation in the dominant two metazoan taxa from each DHAB. (2) Identify the prokaryotes associated with DHAB metazoans using RNA analysis and FISH/CARD FISH. (3) Assess the state of cellular ultrastructure in metazoans using TEM to determine the state of organelles (e.g., nuclei, Golgi, hydrogenosomes) and if DHAB metazoans have specialized cellular structures.

Regardless of results, significant information will be obtained. If the metazoans are not living in the DHABs, then a paradigm shift is unnecessary and physiology text books do not need to be rewritten. If the metazoans are living in the DHAB, then a paradigm shift is required.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1061391
NSF Division of Ocean Sciences (NSF OCE)	OCE-0849578

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