# Scientific sampling event log from R/V Atlantis cruise AT18-14 in the Eastern Mediterranean; 35.3 N 21.7 E (Pickled Protists project, DHAB Metazoans project)

Website: https://www.bco-dmo.org/dataset/3568

Version: 21 December 2011 Version Date: 2011-12-21

#### **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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#### **Table of Contents**

- Dataset Description
  - Methods & Sampling
  - Data Processing Description
- Data Files
- Parameters
- Instruments
- <u>Deployments</u>
- Project Information
- Funding

## **Dataset Description**

The science party used the Rolling Deck to Repository (R2R) event log application (ELOG with cruise-specific custom configuration files) to create a digital event log to record all instrument deployments during the cruise. The log includes information about all scientific sampling events recorded during the cruise including time, position, instrument name and additional comments regarding sampling details.

#### Methods & Sampling

According to the event log, 5 ROV Jason dives, 10 SID-ISMS deployments, 3 CTD/Niskin rosette casts and one XBT profile were completed during the cruise. A multi-corer (MC800) deployment was conducted as well, but was not successful in collecting any cores, and therefore no data will be reported. It is unlikely that the XBT profile data will be reported either as it was done simply to calibrate some shipboard instrumentation.

#### **Data Processing Description**

A preliminary version of the event log was generated by BCO-DMO after the cruise. A quality assurance review was done to ensure unique event numbers, and some cleanup was done to move comments from other fields into the comment field. Several comments had been entered in the notebook page and seafloor depth field. On December 20 and 21, 2011 updates were made to the event after iterative review and consultation with the Chief Scientist. About two dozen events had to be adjusted for the correct date and time (including all of the Deep-SID events). The few entries in the station column were entered as cast numbers instead, and the station column removed as it was no longer needed. The 21 December 2011 version includes all modifications requested by the Chief Scientist.

## **Data Files**

File

**event\_log.csv**(Comma Separated Values (.csv), 35.71 KB)

MD5:4a8556eefccd165c0f31aa8cfdce221c

Primary data file for dataset ID 3568

[ table of contents | back to top ]

### **Parameters**

Parameter	Description	Units
TZ	timezone (offset from UTC) in which sampling event was conducted	dimensionless
event	unique sampling event number derived from local YYYYMMDD.HHMM	dimensionless
date	date (UTC) as YYYYMMDD	dimensionless
time	time (UTC) using 24 hour clock HHMM format	dimensionless
latitude	latitude (North is positive; South is negative)	decimal degrees
longitude	longitude (East is positive; West is negative)	decimal degrees
instrument	name of instrument or sampling system used to collect data	dimensionless
action	activity performed with the instrument; e.g. deploy/recover, start/end, etc.	dimensionless
cast	cast number	dimensionless
seafloor	depth of water; seafloor depth from the shipboard 12 kHz Knudsen echosounder	meters
author	name of person entering the event	dimensionless
comment	free text comment	dimensionless
dive	unique dive identification or number	dimensionless
sample_type	Sample type is a custom field created for this cruise, used to describe the types of samples taken. See the dataset documentation for a detailed explanation.	dimensionless
basin	A term describing the basin location where sampling occurred. For this cruise it one of three possible terms: L'Atalante, Urania or Discovery.	dimensionless
habitat	Habitat is a custom field added to the event log for this cruise. It is one of three terms: control, halocline or brine.	dimensionless

## Instruments

Dataset- specific Instrument Name	CTD Sea-Bird SBE 911plus
Generic Instrument Name	CTD Sea-Bird SBE 911plus
Dataset- specific Description	For this cruise aboard R/V Atlantis, the Seabird CTD 9 included dual conductivity and temperature sensors, a SBE 43 oxygen sensor and a Wet Labs C*Star transmissometer (660nm wavelength). The CTD was deployed in a frame with a 24-position, 10-liter bottle Rosette system.
Generic Instrument Description	The Sea-Bird SBE 911 plus is a type of CTD instrument package for continuous measurement of conductivity, temperature and pressure. The SBE 911 plus includes the SBE 9plus Underwater Unit and the SBE 11plus Deck Unit (for real-time readout using conductive wire) for deployment from a vessel. The combination of the SBE 9 plus and SBE 11 plus is called a SBE 911 plus. The SBE 9 plus uses Sea-Bird's standard modular temperature and conductivity sensors (SBE 3 plus and SBE 4). The SBE 9 plus CTD can be configured with up to eight auxiliary sensors to measure other parameters including dissolved oxygen, pH, turbidity, fluorescence, light (PAR), light transmission, etc.). more information from Sea-Bird Electronics

Dataset- specific Instrument Name	Deep Submersible Incubation Device
Generic Instrument Name	Deep Submersible Incubation Device
Dataset- specific Description	The Deep-SID was used by Dr. Joan Bernhard to conduct timed, in situ grazing experiments during AT18-14.
Generic Instrument Description	The Deep Submersible Incubation Device (Deep-SID) is capable of collecting a 4-liter sample that can then be pushed to 8 subsample chambers that contain a fixative. The Deep-SID was used by Dr. Joan Bernhard to conduct in situ grazing experiments during AT18-14.

Dataset- specific Instrument Name	XBT
Generic Instrument Name	Expendable Bathythermograph
SDACITIC	According to the event log, one XBT probe was fired. The Chief Scientist did not know the type (T-5 or T-7).
	An XBT is an expendable free-fall temperature probe that provides a profile of measured temperature against depth calculated from a fall-rate model. For example, two popular XBT models are the T-5 and T-7 probes from Sippican. More information is available from Lockheed Martin Sippican at URL: <a href="http://www.sippican.com/">http://www.sippican.com/</a> .

Dataset- specific Instrument Name	ROV Jason
Generic Instrument Name	ROV Jason
Dataset- specific Description	During the ROV Jason dives for this cruise, two types of sampling were conducted: injector push cores or.sediment scooped into chamber pots each injected with one of three types of preservatives: RNAlater, formalin, or glutaraldehyde.
	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. <a href="https://ndsf.whoi.edu/jason/">https://ndsf.whoi.edu/jason/</a>

Dataset- specific Instrument Name	Submersible Incubation Device-In Situ Microbial Sampler
Generic Instrument Name	Submersible Incubation Device-In Situ Microbial Sampler
Dataset- specific Description	The SID-ISMS was developed for and first deployed during this cruise.
	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**

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 bas

## **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.

# Funding

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

[ table of contents | back to top ]