qPCR data for archaeal amoA ecotypes; samples collected on METZYME cruise.

Website: https://www.bco-dmo.org/dataset/647622
Data Type: Cruise Results
Version: 24 May 2016
Version Date: 2016-05-24

Project
» Connecting Trace Elements and Metalloenzymes Across Marine Biogeochemical Gradients (GPc03) (MetZyme)
» Gene content, gene expression, and physiology in mesopelagic ammonia-oxidizing archaea (AmoA Archaea)

Program
» U.S. GEOTRACES (U.S. GEOTRACES)

Contributors	Affiliation	Role
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Dataset Description

Abundance of ammonia monooxygenase subunit A (*amoA*) genes determined using TaqMan quantitative polymerase chain reaction (qPCR). Archaeal *amoA* genes were quantified using quantitative PCR using the assay described in Mosier and Francis 2011. Complete methods are described in Santoro et al., submitted.

Related references:

Mosier, A. C., and C. A. Francis. 2011. Determining the distribution of marine and coastal ammonia-oxidizing archaea and bacteria using a quantitative approach. Methods Enzymol. 486**:** 205-221. doi:10.1016/B978-0-12-381294-0.00009-2

Santoro, A.E., Saito, M.A., Goepfert, T.J., Lamborg, C.H., Dupont, C.L., G.R. DiTullio. Thaumarchaeal ecotype distributions across the equatorial Pacific and their potential roles in nitrification and flux attenuation. Submitted to *Limnology & Oceanography, April 2016*.

Methods & Sampling

Water samples were collected at discrete depths using either a standard 24-bottle rosette sampler equipped with an SBE9plus conductivity-temperature-depth (CTD) sensor package (SeaBird Electronics, Bellevue, WA) or a 12-bottle trace metal clean rosette equipped with an SBE19 CTD. Samples for nucleic acid extraction and qPCR were collected from the rosette in 2-4 L polycarbonate bottles. Cells were harvested by pressure filtration onto 25 mm diameter, 0.2 um pore-size polyethersulfone membrane filters (Supor-200, Pall Corporation, Port Washington, NY) housed in polypropylene filter holders (Whatman SwinLok, GE Healthcare,

Pittsburgh, PA) using a peristaltic pump and silicone tubing. For DNA extraction and analysis, 1-4 L sample volumes were filtered depending on the biomass present at each station and depth, and the filters were flash frozen in liquid nitrogen in 2 mL gasketed bead beating tubes (Fisher Scientific).

Data Processing Description

Nucleic acids (DNA) were extracted as described previously (Santoro et al. 2010), with slight modifications. Briefly, cells on the filters were lysed directly in the bead beating tubes with sucrose-ethylene diamine tetraacetic acid (EDTA) lysis buffer (0.75 M sucrose, 20 mM EDTA, 400 mM NaCl, 50 mM Tris) and 1% sodium dodecyl sulfate (SDS). Filter samples were subject to three freeze-thaw cycles of 5 min in liquid nitrogen and 5 min in a 65 degree C water bath. Tubes were then agitated in a bead beating machine (Biospec) for 1.5 min, and proteinase K (Invitrogen) was added to a final concentration of 0.5 mg mL-1. Filters were incubated at 55 degrees C for approximately 4 h and the resulting lysates were purified with the DNeasy kit (Qiagen) using a slightly modified protocol (Santoro et al. 2010). The purified nucleic acids were eluted in 200 uL of DNase, RNase-free water (Gibco) and quantified using a fluorometer (Qubit and Quanti-T BR reagent, Invitrogen Molecular Probes).

All qPCR assays were conducted using group-specific assays for the thaumarchaeal *amoA* gene for 'shallow' water column ecotype A (WCA) and 'deep' water column ecotype B (WCB) (Mosier and Francis 2011) with TaqMan Environmental Mastermix (Life Technologies) chemistry on a CFX96 qPCR machine (Bio-Rad, Inc., Hercules, CA). Detection limits for TaqMan assays were 1 copy mL-1 or better. All samples were run in triplicate against a standard curve spanning approximately 101 - 105 templates, run in duplicate. Plasmids containing cloned inserts of the target gene (TOPO pCR4 vector, Invitrogen or pGem vector, Promega) were used as standards. Standards were linearized with the restriction enzyme NotI (New England Biolabs), purified (DNeasy, Qiagen), quantified by fluorometry (Quanti-T HS reagent, Invitrogen), and stored at -80 degrees C. Fresh standard dilutions were made from frozen stocks for each day of analysis. qPCR was carried out using the following thermal profile: 95 degreesC for 10 min, followed by 40 cycles of 95 degrees C for 30 s and 55 degrees C for 30 s. A minimum of three negative control qPCR reactions to which no DNA template was added were run with every assay.

Gene copies per qPCR reaction were converted to volumetric concentrations (i.e. copies mL-1 seawater) using the volume of seawater filtered, the DNA elution volume, and the volume of purified DNA used per reaction, and are reported as the mean of triplicate analyses.

BCO-DMO Processing:
- re-formatted date and time; added ISO date/time field;
- copied station, date, time, lon, and lat from separate spreadsheet into dataset.

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Data Files

File
qPCR.csv (Comma Separated Values (.csv), 14.84 KB) MD5:2cb08000b4161a1b312758b6c9e61232 Primary data file for dataset ID 647622

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Parameters

Parameter	Description	Units
cruise	Cruise identifier.	unitless
cast	Cast number. TMR = Trace Metal Rosette.	unitless
station	Station number.	unitless
lon	Longitude east.	decimal degrees
lat	Latitude north.	decimal degrees
date	Month, day, and year; local time.	mm/dd/YYYY
time_local	Time in hours and minutes; local time.	HHMM
ISO_DateTime_Local	Date and time formatted to the ISO8601 standard.	YYYY-mm-ddTHH:MM:SS.xx
depth	Sample depth.	meters (m)
niskin	Niskin bottle number.	unitless
vol_filtered	Volume of water filtered.	liters (L)
WCB	WCB amoA abundance. WCB = water column ‘B’; assay for the deep ecotype of ammonia-oxidizing archaea.	copies per milliliter (copies/mL)
WCB_stdev	Standard deviation of WCB.	copies per milliliter (copies/mL)
WCA	WCA amoA abundance. WCA = water column ‘A’; assay for the shallow ecotype of ammonia-oxidizing archaea.	copies per milliliter (copies/mL)
WCA_stdev	Standard deviation of WCA.	copies per milliliter (copies/mL)
AOA	Total AOA amoA genes (calculated from WCA + WCB).	copies per milliliter (copies/mL)
pcnt_WCB	Percent WCB; calculated as WCB/totalAOA.	percent (%)

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Instruments

Dataset-specific Instrument Name	
Generic Instrument Name	CTD Sea-Bird SEACAT 19
Generic Instrument Description	The Sea-Bird SBE 19 SEACAT Recorder measures conductivity, temperature, and pressure (depth). The SEACAT is self-powered and self-contained and can be deployed in profiling or moored mode. The SBE 19 SEACAT was replaced in 2001 by the 19plus. more information from Sea-Bird Electronics

Dataset-specific Instrument Name	
Generic Instrument Name	Sea-Bird SBE 911plus CTD
Generic Instrument Description	[tethered, real-time] 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	
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)

Dataset-specific Instrument Name	
Generic Instrument Name	Trace Metal Bottle
Generic Instrument Description	Trace metal (TM) clean rosette bottle used for collecting trace metal clean seawater samples.

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Deployments

KM1128

Website	https://www.bco-dmo.org/deployment/59053
Platform	R/V Kilo Moana
Start Date	2011-10-01
End Date	2011-10-25
Description	This is a MetZyme project cruise. The original cruise data are available from the NSF R2R data catalog.

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Project Information

Connecting Trace Elements and Metalloenzymes Across Marine Biogeochemical Gradients (GPc03) (MetZyme)

Coverage: Tropical North Pacific along 150 degrees West from 18 degrees North to the equator

MetZyme project researchers will determine the role of enzymatic activity in the cycling of trace metals. Specifically the research will address the following questions: (1) degradation of sinking particulate organic material in the Tropical North Pacific can be influenced by the ability of microbes to synthesize zinc proteases, which in turn is controlled by the abundance or availability of zinc, and (2) methylation of mercury is controlled, in part, by the activity of cobalt-containing enzymes, and therefore the supply of labile cobalt to the corrinoid-containing enzymes or co-factors responsible for methylation. To attain their goal, they will collect dissolved and particulate samples for trace metals and metalloenzymes from three stations along a biogeochemical gradient in the Tropical North Pacific (along 150 degrees West from 18 degrees North to the equator). Sinking particles from metal clean sediment traps will also be obtained. The samples will also be used to carry out shipboard incubation experiments using amendments of metals, metal-chelators, B12, and proteases to examine the sensitivity and metal limitation of heterotrophic, enzymatic degradation of organic matter within the oceanic "Twilight Zone" (100-500 m). This study will result in a novel metaproteomic/metalloenzyme datasets that should provide insights into the biogeochemical cycling of metals, as well as co-limitation of primary productivity and controls on the export of carbon from the photic zone. In addition to the final data being contributed to BCO-DMO, an online metaproteomic data server will be created so the community has access to the raw data files generated by this research.

Gene content, gene expression, and physiology in mesopelagic ammonia-oxidizing archaea (AmoA Archaea)

Coverage: Epipelagic and mesopelagic, Equatorial Pacific

NSF award abstract:

Intellectual Merit. How organisms respond to their physical and chemical and environment is a central question in marine ecology. For microbes living in the mesopelagic - the ocean's "twilight zone" - an efficient response is particularly important to capitalize on the intermittent delivery of organic and inorganic compounds sinking from the surface ocean. These organisms must have a suite of metabolic and regulatory strategies used to cope with environmental variability, but these strategies are largely unknown. Understanding when and why metabolic genes are expressed is critical to our understanding of nutrient remineralization in the ocean. Marine group 1 (MG1) archaea are ubiquitous, abundant microbes in the meso- and bathypelagic and promising model organisms for investigating these questions. MG1 archaea are chemolithoautotrophs that oxidize ammonia for energy and fix carbon for biomass, and as such, play a central role in the ocean's coupled carbon and nitrogen cycles. Though MG1 have historically eluded cultivation, recent efforts have been successful at bringing representative MG1 archaea from the open ocean into culture and demonstrating their importance in the

production of the greenhouse gas nitrous oxide. This project takes advantage of unique MG1 cultures and the recently sequenced draft genome of one of the organisms - strain CN25 - to investigate the physiological and transcriptional responses of MG1 archaea to variations in their chemical environment, specifically:

1. Comparative transcriptomics of CN25 cells grown under a range of energy availability and nitrosative stress will identify select genes that can be used to diagnose the physiological state of natural populations

2. Improvements in the genomic and transcriptomic knowledge of MG1 archaea will facilitate a thorough reinterpretation of existing metagenomic and metatranscriptomic datasets, as well as provide a better contextual understanding in future studies

The investigators will conduct comparative transcriptomics of CN25 cells harvested in mid-exponential growth and stationary phase versus starved cells. Transcriptomes of cells grown at high nitrate concentrations and low pO2 with those grown in standard conditions will be characterized. A strand-specific, high-density RNAseq approach will be used to examine the expression of putative ORFs, polycistronic operons, and small RNAs, which, in addition to gene expression profiling, has the ancillary benefit of improving genome annotation. Finally, the investigators will sequence the genomes of two additional MG1 strains isolated from the open ocean, as well as single cells from environmental surveys, and leverage the combination with the CN25 genome to reanalyze available metagenomic and metatranscriptomic datasets. The results will define the transcriptional response of a model mesopelagic microbe to a range of chemical environments, and show how the physicochemical environment induces changes in gene expression and gene content that result in greenhouse gas production. This work will rapidly generate new knowledge of how some of the most ubiquitous, yet heretofore elusive, microorganisms respond to geochemical variability and shape our evolving understanding of the marine nitrogen cycle.

Broader Impacts. The scientific and societal impact of the project will be to elucidate the mechanisms of greenhouse gas production in a model marine organism that is of broad interest to biological and chemical oceanographers. Transcriptome sequencing will improve the assembly of the CN25 genome, the first genome of an MG1 archaeon from the open ocean. Both the genome and transcriptomes will be important references for researchers using metagenomics, metatranscriptomics, and metaproteomics in the ocean, as these techniques are reliant on a knowledgebase composed of both DNA sequence and physiology. Thus, the results add value to both existing and future studies. The proposed research will advance education, teaching, and training for the next generation of marine scientists by providing support for two early-career investigators, one postdoctoral researcher, and a secondary school teacher.

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Program Information

U.S. GEOTRACES (U.S. GEOTRACES)

Website: http://www.geotraces.org/

Coverage: Global

GEOTRACES is a SCOR sponsored program; and funding for program infrastructure development is provided by the U.S. National Science Foundation.

GEOTRACES gained momentum following a special symposium, S02: Biogeochemical cycling of trace elements and isotopes in the ocean and applications to constrain contemporary marine processes (GEOSECS II), at a 2003 Goldschmidt meeting convened in Japan. The GEOSECS II acronym referred to the Geochemical Ocean Section Studies To determine full water column distributions of selected trace elements and isotopes, including their concentration, chemical speciation, and physical form, along a sufficient number of sections in each ocean basin to establish the principal relationships between these distributions and with more traditional hydrographic parameters;

* To evaluate the sources, sinks, and internal cycling of these species and thereby characterize more completely the physical, chemical and biological processes regulating their distributions, and the sensitivity of these processes to global change; and

* To understand the processes that control the concentrations of geochemical species used for proxies of the past environment, both in the water column and in the substrates that reflect the water column.

GEOTRACES will be global in scope, consisting of ocean sections complemented by regional process studies. Sections and process studies will combine fieldwork, laboratory experiments and modelling. Beyond realizing the scientific objectives identified above, a natural outcome of this work will be to build a community of marine scientists who understand the processes regulating trace element cycles sufficiently well to exploit this knowledge reliably in future interdisciplinary studies.

Expand "Projects" below for information about and data resulting from individual US GEOTRACES research projects.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1031271
NSF Division of Ocean Sciences (NSF OCE)	OCE-1260006

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