Nitrite Oxidoreductase targeted metaproteomics from R/V Kilo Moana cruise KM1128 and R/V Falkor cruise FK160115 in the Central Pacific Ocean in 2011 and 2016

Website: https://www.bco-dmo.org/dataset/806510
Data Type: Cruise Results
Version: 1
Version Date: 2020-04-21

Project
» Connecting Trace Elements and Metalloenzymes Across Marine Biogeochemical Gradients (GPc03) (MetZyme)
» The ProteOMZ Expedition: Investigating Life Without Oxygen in the Pacific Ocean (ProteOMZ (Proteomics in an Oxygen Minimum Zone))
» Collaborative Research: Iron and phosphorus balanced limitation of nitrogen fixation in the oligotrophic ocean (TriCoLim)
» US GEOTRACES PMT: Cobalt Biogeochemical Cycling and Connections to Metalloenzymes in the Pacific Ocean (PMT Cobalt and Metalloenzymes)
» Marine Microbial Investigator Award: Investigator Mak Saito (MM Saito)
» Collaborative Research: Evolutionary, biochemical and biogeochemical responses of marine cyanobacteria to warming and iron limitation interactions (Cyanobacteria Warming Responses)
» Collaborative Research: Underexplored Connections between Nitrogen and Trace Metal Cycling in Oxygen Minimum Zones Mediated by Metalloenzyme Inventories (CliOMZ)

Programs
» U.S. GEOTRACES (U.S. GEOTRACES)
» Marine Microbiology Initiative (MMI)

Contributors	Affiliation	Role
Saito, Mak A.	Woods Hole Oceanographic Institution (WHOI)	Principal Investigator
York, Amber D.	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
Nitrite Oxidoreductase targeted metaproteomics from R/V Kilo Moana cruise KM1128 and R/V Falkor cruise FK160115 in the Central Pacific Ocean in 2011 and 2016. NxrA and NxrB peptide concentrations in fmol/L. Peptide names are using the GEOTRACES naming convention (PEP for peptide, full tryptic peptide amino acid sequence, Protein name, Sampling device (=Pump)). Quality flags follow each peptide column and use the GEOTRACES convention of 1 for good, 6 for below detection limit. These data were published in Saito et al., 2020 as Supplementary Table 1.

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Coverage

Spatial Extent: **N**:17 **E**:-154.4 **S**:-3.5 **W**:140
Temporal Extent: 2011-10-05 - 2016-01-26

Dataset Description

Nitrite Oxidoreductase targeted metaproteomics from R/V Kilo Moana cruise KM1128 and R/V Falkor cruise FK160115 in the Central Pacific Ocean in 2011 and 2016. NxrA and NxrB peptide concentrations in fmol/L. Peptide names are using the GEOTRACES naming convention (PEP for peptide, full tryptic peptide amino acid sequence, Protein name, Sampling device (=Pump)). Quality flags follow each peptide column and use the GEOTRACES convention of 1 for good, 6 for below detection limit. These data were published in Saito et al., 2020 as Supplementary Table 1.

Methods & Sampling

Metaproteomics samples were collected by McLane pump onto 0.2 micron Supor membrane filters, with 51 and 3.0 micron prefilters. Global metaproteomic analyses were conducted using 1-dimensional (1D) and 2-dimensional (2D) chromatographic separation for the Metzyme and ProteOMZ expeditions respectively. Following global metaproteomic analyses, targeted metaproteomic assays were designed and samples were analyzed again by parallel reaction monitoring (PRM) mass spectrometry using mass spectral information from the global proteomic analyses. See methods in Saito et al., 2020 Nature Geosciences for full details.

Samples were analyzed on a Thermo Fusion Orbitrap mass spectrometer. See methods in Saito et al., 2020 Nature Geosciences for full details.

Data quality flags are included following GEOTRACES conventions for results below detection limit (flag= 6).

Parameters were named using the prior GEOTRACES IDP parameter naming convention used for peptides (PEP), although these are new parameters not previously submitted here or elsewhere.

The peptide parameter naming convention was developed in collaboration with the GEOTRACES program. In other to avoid the sustainability challenge of having to maintain a parameter key of codes that represent protein and peptide sequences, the tryptic peptide amino acid sequences are inserted into the parameter name, with prefixes and suffixes for additional metadata. For example, the parameter name "PEP_MTIQWGK_NxrA_PUMP" has the following components. The prefix PEP refers to the peptides datatype, "MTIQWGK" refers to the specific amino acid sequence of the measured peptide, using the IUPAC-IUB 1 letter amino acid naming convention. This sequence can be used to calculate the molecular weight and elemental formula of the molecule that was measured (e.g. see https://web.expasy.org/compute_pi/ and https://web.expasy.org/protparam/). "NxrA" refers to the protein name, in this case nitrite oxidoreductase subunit A. "PUMP" refers to the samping methodology, in order to differentiate when samples may be collected from the same location and depth but by different methods. This approach is useful as tryptic peptides are short enough in sequence to allow their use within parameter names, and each parameter name uniquely describes the molecule being measured, even when, as in this Nxr study, many different peptides are being measured from within a single protein.
Reference: https://febs.onlinelibrary.wiley.com/doi/pdf/10.1111/j.1432-1033.1984.tb07877.x

Data Processing Description

Metaproteome datasets were processed using Skyline (version 4.1) and Metatryp (version 2) software for quantitation and taxonomic least common ancestor results.

BCO-DMO Data Manager Processing Notes:
* First sheet from originally submitted Excel file "031920_nxr_add_metadata_V3.xlsx" extracted to csv
* Column "Long180" (in -180 to 180 degree coordinates) added in addition to "Long" (degrees in 0 to 360) column for import into our data system and to support interoperability.
* DateTime in UTC and ISO 8601 format added to the dataset based on local date and time provided in HST.
* After discussion with submitter, lat and on degrees that were integers given one decimal place of precision. Coordinates were selected to fall on integer degrees.

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Data Files

File
nxr.csv (Comma Separated Values (.csv), 15.73 KB) MD5:84e98d574cf913cc0e7e78450adaac14 Primary data file for dataset ID 806510

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Related Publications

(2018, January 11). Skyline Targeted Mass Spec Environment. Version 4.1. MacCoss Lab Software. https://skyline.ms/announcements/home/software/Skyline/releases/thread.view?rowId=34803 https://skyline.ms/wiki/__r6/page.view?name=new-4-1-features
Software

Gasteiger, E., Hoogland, C., Gattiker, A., Wilkins, M. R., Appel, R. D., & Bairoch, A. (2005). Protein identification and analysis tools on the ExPASy server. In The proteomics protocols handbook (pp. 571-607). Humana press. https://web.expasy.org/compute_pi/pi_tool-doc.html
Methods

METATRYP (2020), METATRYP GitHub repository, Version 2, https://github.com/saitomics/metatryp https://metatryp.whoi.edu/
Software

Nomenclature and Symbolism for Amino Acids and Peptides. Recommendations 1983. (1984). European Journal of Biochemistry, 138(1), 9–37. doi:10.1111/j.1432-1033.1984.tb07877.x
Methods

SIB Swiss Institute of Bioinformatics. (n.d.). Compute pI/Mw tool. SIB Swiss Institute of Bioinformatics. Retrieved March 27, 2020, from https://web.expasy.org/compute_pi/
Methods

SIB Swiss Institute of Bioinformatics. (n.d.). ProtParam tool. SIB Swiss Institute of Bioinformatics. Retrieved March 27, 2020, from https://web.expasy.org/protparam/
Methods

Saito, M. A., McIlvin, M. R., Moran, D. M., Santoro, A. E., Dupont, C. L., Rafter, P. A., … Waterbury, J. B. (2020). Abundant nitrite-oxidizing metalloenzymes in the mesopelagic zone of the tropical Pacific Ocean. Nature Geoscience, 13(5), 355–362. doi:10.1038/s41561-020-0565-6
Results

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Parameters

Parameter	Description	Units
Expedition	Expedition (Cruise) identifier	unitless
Station	Station	unitless
Long	Longitude (0 to 360 degrees)	decimal degrees
Lat	Lattitude	decimal degrees
McLane_cast	McLane pump cast identifier	unitless
McLane_time_local	McLane pump date and time (local time zone HST, UTC-10) in format yyyy-mm-ddTHH:MM	unitless
Depth	Sample depth	meters (m)

PEP_LANQVALLDSIIR_NxrA_PUMP	Peptide amino acid sequence concentration [LANQVALLDSIIR] from protein NxrA from McLane pump samples.	femtomoles per liter (fmol/L)
PEP_LANQVALLDSIIR_NxrA_PUMP_FLAG	Quality flag for column PEP_LANQVALLDSIIR_NxrA_PUMP. Quality flags follow each peptide column and use the GEOTRACES convention of 1 for good, 6 for below detection limit.	unitless
PEP_GGTLVAVAPEYNPPATK_NxrA_PUMP	Peptide amino acid sequence concentration [GGTLVAVAPEYNPPATK] from protein NxrA from McLane pump samples.	femtomoles per liter (fmol/L)
PEP_GGTLVAVAPEYNPPATK_NxrA_PUMP_FLAG	Quality flag for column PEP_GGTLVAVAPEYNPPATK_NxrA_PUMP. Quality flags follow each peptide column and use the GEOTRACES convention of 1 for good, 6 for below detection limit.	unitless
PEP_MTIQWGK_NxrA_PUMP	Peptide amino acid sequence concentration [MTIQWGK] from protein NxrA from McLane pump samples.	femtomoles per liter (fmol/L)
PEP_MTIQWGK_NxrA_PUMP_FLAG	Quality flag for column PEP_MTIQWGK_NxrA_PUMP. Quality flags follow each peptide column and use the GEOTRACES convention of 1 for good, 6 for below detection limit.	unitless
PEP_LHPDDFIPGYK_NxrA_PUMP	Peptide amino acid sequence concentration [LHPDDFIPGYK] from protein NxrA from McLane pump samples.	femtomoles per liter (fmol/L)
PEP_LHPDDFIPGYK_NxrA_PUMP_FLAG	Quality flag for column PEP_LHPDDFIPGYK_NxrA_PUMP. Quality flags follow each peptide column and use the GEOTRACES convention of 1 for good, 6 for below detection limit.	unitless
PEP_ALIVNTPR_NxrA_PUMP	Peptide amino acid sequence concentration [ALIVNTPR] from protein NxrA from McLane pump samples.	femtomoles per liter (fmol/L)
PEP_ALIVNTPR_NxrA_PUMP_FLAG	Quality flag for column PEP_ALIVNTPR_NxrA_PUMP. Quality flags follow each peptide column and use the GEOTRACES convention of 1 for good, 6 for below detection limit.	unitless
PEP_TQFYNDEPEAIEYGENFIVHR_NxrA_PUMP	Peptide amino acid sequence concentration [TQFYNDEPEAIEYGENFIVHR] from protein NxrA from McLane pump samples.	femtomoles per liter (fmol/L)
PEP_TQFYNDEPEAIEYGENFIVHR_NxrA_PUMP_FLAG	Quality flag for column PEP_TQFYNDEPEAIEYGENFIVHR_NxrA_PUMP. Quality flags follow each peptide column and use the GEOTRACES convention of 1 for good, 6 for below detection limit.	unitless
PEP_GLWEPVR_NxrA_PUMP	Peptide amino acid sequence concentration [GLWEPVR] from protein NxrA from McLane pump samples.	femtomoles per liter (fmol/L)

PEP_GLWEPVR_NxrA_PUMP_FLAG	Quality flag for column PEP_GLWEPVR_NxrA_PUMP. Quality flags follow each peptide column and use the GEOTRACES convention of 1 for good, 6 for below detection limit.	unitless
PEP_AIHGVYEGVTIFEAPAK_NxrB_PUMP	Peptide amino acid sequence concentration [AIHGVYEGVTIFEAPAK] from protein NxrB from McLane pump samples.	femtomoles per liter (fmol/L)
PEP_AIHGVYEGVTIFEAPAK_NxrB_PUMP_FLAG	Quality flag for column PEP_AIHGVYEGVTIFEAPAK_NxrB_PUMP. Quality flags follow each peptide column and use the GEOTRACES convention of 1 for good, 6 for below detection limit.	unitless
PEP_IGLNQQAVGYVPTDEEWR_NxrB_PUMP	Peptide amino acid sequence concentration [IGLNQQAVGYVPTDEEWR] from protein NxrB from McLane pump samples.	femtomoles per liter (fmol/L)
PEP_IGLNQQAVGYVPTDEEWR_NxrB_PUMP_FLAG	Quality flag for column PEP_IGLNQQAVGYVPTDEEWR_NxrB_PUMP. Quality flags follow each peptide column and use the GEOTRACES convention of 1 for good, 6 for below detection limit.	unitless
PEP_FPNFGEDTAHGR_NxrB_PUMP	Peptide amino acid sequence concentration [FPNFGEDTAHGR] from protein NxrB from McLane pump samples.	femtomoles per liter (fmol/L)
PEP_FPNFGEDTAHGR_NxrB_PUMP_FLAG	Quality flag for column PEP_FPNFGEDTAHGR_NxrB_PUMP. Quality flags follow each peptide column and use the GEOTRACES convention of 1 for good, 6 for below detection limit.	unitless
PEP_ICNHCTYPGCLAACPR_NxrB_PUMP	Peptide amino acid sequence concentration [ICNHCTYPGCLAACPR] from protein NxrB from McLane pump samples.	femtomoles per liter (fmol/L)
PEP_ICNHCTYPGCLAACPR_NxrB_PUMP_FLAG	Quality flag for column PEP_ICNHCTYPGCLAACPR_NxrB_PUMP. Quality flags follow each peptide column and use the GEOTRACES convention of 1 for good, 6 for below detection limit.	unitless
PEP_DLLGILQLFR_NxrB_PUMP	Peptide amino acid sequence concentration [DLLGILQLFR] from protein NxrB from McLane pump samples.	femtomoles per liter (fmol/L)
PEP_DLLGILQLFR_NxrB_PUMP_FLAG	Quality flag for column PEP_DLLGILQLFR_NxrB_PUMP. Quality flags follow each peptide column and use the GEOTRACES convention of 1 for good, 6 for below detection limit.	unitless
McLane_ISO_DateTime_UTC	McLane pump date and time (time zone UTC) in ISO 8601 format yyyy-mm-ddTHH:MMZ	untiless
Long180	Longitude (-180 to 180)	decimal degrees
NxrA_mean	Average of NxrA peptides	femtomoles per liter (fmol/L)
NxrA_std	Standard Deviation of NxrA peptides	femtomoles per liter (fmol/L)

NxrB_mean	Average of NxrB peptides	femtomoles per liter (fmol/L)
NxrB_std	Standard Deviation of NxrB peptides	femtomoles per liter (fmol/L)
NxrAB_mean	Average of NxrA and NxrB averages	femtomoles per liter (fmol/L)
Fe_as_NxrAB	Concentration of Fe within NxrAB	picomoles per liter (pmol/L)

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Instruments

Dataset-specific Instrument Name	Thermo Fusion Orbitrap mass spectrometer
Generic Instrument Name	Mass Spectrometer
Generic Instrument Description	General term for instruments used to measure the mass-to-charge ratio of ions; generally used to find the composition of a sample by generating a mass spectrum representing the masses of sample components.

Dataset-specific Instrument Name	
Generic Instrument Name	McLane Pump
Generic Instrument Description	McLane pumps sample large volumes of seawater at depth. They are attached to a wire and lowered to different depths in the ocean. As the water is pumped through the filter, particles suspended in the ocean are collected on the filters. The pumps are then retrieved and the contents of the filters are analyzed in a lab.

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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.

FK160115

Website	https://www.bco-dmo.org/deployment/708387
Platform	R/V Falkor
Report	https://service.rvdata.us/data/cruise/FK160115/doc/FK160115_OfficialCruiseReport_Saito_v3.pdf
Start Date	2016-01-16
End Date	2016-02-11
Description	Project: Using Proteomics to Understand Oxygen Minimum Zones (ProteOMZ) More information is available from the ship operator at https://schmidtocean.org/cruise/investigating-life-without-oxygen-in-the... Additional cruise information is available from the Rolling Deck to Repository (R2R): https://www.rvdata.us/search/cruise/FK160115

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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.

The ProteOMZ Expedition: Investigating Life Without Oxygen in the Pacific Ocean (ProteOMZ (Proteomics in an Oxygen Minimum Zone))

Website: https://schmidtocean.org/cruise/investigating-life-without-oxygen-in-the-tropical-pacific/#team

Coverage: Central Pacific Ocean (Hawaii to Tahiti)

From Schmidt Ocean Institute's ProteOMZ Project page:

Rising temperatures, ocean acidification, and overfishing have now gained widespread notoriety as human-caused phenomena that are changing our seas. In recent years, scientists have increasingly recognized that there is yet another ingredient in that deleterious mix: a process called deoxygenation that results in less

oxygen available in our seas.

Large-scale ocean circulation naturally results in low-oxygen areas of the ocean called oxygen deficient zones (ODZs). The cycling of carbon and nutrients – the foundation of marine life, called biogeochemistry – is fundamentally different in ODZs than in oxygen-rich areas. Because researchers think deoxygenation will greatly expand the total area of ODZs over the next 100 years, studying how these areas function now is important in predicting and understanding the oceans of the future. This first expedition of 2016 led by Dr. Mak Saito from the Woods Hole Oceanographic Institution (WHOI) along with scientists from University of Maryland Center for Environmental Science, University of California Santa Cruz, and University of Washington aimed to do just that, investigate ODZs.

During the 28 day voyage named “ProteOMZ,” researchers aboard R/V *Falkor* traveled from Honolulu, Hawaii to Tahiti to describe the biogeochemical processes that occur within this particular swath of the ocean’s ODZs. By doing so, they contributed to our greater understanding of ODZs, gathered a database of baseline measurements to which future measurements can be compared, and established a new methodology that could be used in future research on these expanding ODZs.

Collaborative Research: Iron and phosphorus balanced limitation of nitrogen fixation in the oligotrophic ocean (TriCoLim)

Coverage: Tropical Atlantic

NSF abstract:

Marine cyanobacteria are able to use or "fix" atmospheric nitrogen gas, and so supply much of the essential nutrient nitrogen that supports open ocean food chains. Oceanographers have usually thought that the growth of these nitrogen-fixing cyanobacteria is limited at any particular time and place by the supply of either iron, or of phosphorus. Preliminary experiments have shown, though, that these nitrogen fixers instead grow best when both iron and phosphorus are scarce at the same time. In this project, the researchers will use cellular indicators that are specific for iron and phosphorus limitation to determine how important this type of "balanced limitation" of nitrogen-fixing cyanobacteria is in controlling the productivity of ocean food chains in the tropical Atlantic Ocean. Two graduate students will be trained at the University of Southern California (USC) and Woods Hole Oceanographic Institution, as well as a postdoctoral researcher at USC. Educational outreach efforts will take place at a Los Angeles inner city high school with a student body that is over 98% Hispanic and African-American, and with underrepresented undergraduates in the USC Global Environmental Microbiology course. In addition, two Research Experiences for Undergraduates students will be supervised for summer research projects to help them learn about science career options.

The researchers will investigate the biological and biogeochemical consequences of this unique balanced iron/phosphorus-limited phenotype, using both laboratory and fieldwork approaches. During the first year of this project, the nitrogen-fixing cyanobacteria will be cultured under iron and/or phosphorus limitation, followed by application of proteomics and transcriptomics to identify genes that are potential diagnostic biomarkers for iron/phosphorus balanced limitation. Preliminary work has already identified one promising candidate biomarker in one cyanobacterium, an EzrA protein domain that appears to be associated with the cell size decreases seen specifically under balanced limitation, and the researchers have identified numerous other potential candidates for similar biomarkers. During the second year, these new co-limitation biomarkers and others previously validated for iron limitation (IsiB) and phosphorus limitation (SphX) will be used to investigate balanced limitation during a research cruise transecting from relatively high-iron, low-phosphorus North Atlantic waters, to the relatively high-phosphorus, low-iron South Atlantic. This fieldwork component will survey nitrogen fixing cyanobacteria populations across this natural iron/phosphorus gradient for genetic, proteomic, and physiological indicators of balanced limitation, as well as testing their responses to iron and phosphorus manipulations in shipboard incubation experiments. The third year will be devoted to sample analysis, and publications exploring the responses of oceanic nitrogen fixers to simultaneous limitation by both iron and phosphorus.

US GEOTRACES PMT: Cobalt Biogeochemical Cycling and Connections to Metalloenzymes in the Pacific Ocean (PMT Cobalt and Metalloenzymes)

Coverage: Laboratory Study and Cultures from Northeast Pacific Line P Transect 48.8167 N 128.667 W

NSF abstract:

Cobalt is important for many forms of marine life, yet it is one of the scarcest nutrients in the sea. Cobalt's oceanic abundance and distribution, along with other scarce nutrients, can influence the growth of microscopic plants (phytoplankton). This in turn can influence carbon cycles in the ocean and atmosphere. Therefore, knowledge of the controls on cobalt's abundance and chemical forms in seawater is a valuable component of our ability to understand the ocean's influence on global carbon cycling. Within phytoplankton and other marine microbes, metals such as cobalt, iron, nickel, and copper are used as critical components of enzymes responsible for key cellular reactions. Since these enzymes require metals to work, they are named metalloenzymes. Participating in a Pacific Ocean cruise from Alaska to Tahiti, this project will study the oceanic distributions of dissolved cobalt and the cellular content of a group of metalloenzymes known to influence biogeochemical cycles. The project will provide scientific impact by creating new knowledge about oceanic micronutrients in regions of economic interest with regard to fisheries and deep-sea mining. Measurement of proteins in the North Pacific will provide data of broad biological and chemical interest and will be made available through a new NSF-funded "EarthCube Ocean Protein Portal" data base. Educational impact will stem from participation of a graduate student and two young technicians, as well as the PI's development of a high school chemistry curriculum for use in two local high schools, thus allowing teachers to include real oceanic and environmental data at their first introduction to chemistry.

Cobalt has a complex biogeochemical cycle. Both its inorganic and organic forms are used by biology in the upper ocean and it is removed from solution by being scavenged in the intermediate and deep ocean. This scavenging removal results in cobalt having the smallest oceanic inventory of any biologically utilized element. Recent studies, however, have found that large dissolved cobalt plumes occur in major oxygen minimum zones due to a combination of less scavenging and additions from sedimentary and remineralization fluxes. The GP15 US GEOTRACES Pacific Meridional Transect (PMT) provides an opportunity to examine the influence of oxygen depletion on cobalt chemistry. Moreover, the study of the protein component of microbial communities using new proteomic techniques will provide evidence of how different major microorganisms respond to the chemical environment (e.g. through transporter production for specific nutrients and micronutrients) as well as the biochemical basis for metal requirements related to the use of specific metalloenzymes. Specifically, the PMT provides an opportunity to confirm that the Pacific oxygen minimum zones contain a large amount of cobalt and to test the hypotheses that simultaneous zinc scarcity could induce wide-scale biochemical substitution of cobalt for zinc in the North Pacific Ocean.

Marine Microbial Investigator Award: Investigator Mak Saito (MM Saito)

In support of obtaining deeper knowledge of major biogeochemically relevant proteins to inform a mechanistic understanding of global marine biogeochemical cycles.

Collaborative Research: Evolutionary, biochemical and biogeochemical responses of marine cyanobacteria to warming and iron limitation interactions (Cyanobacteria Warming Responses)

NSF abstract:
The oceans absorb much of the heat generated by human activities, and this warming of the surface ocean has consequences for important groups of marine organisms. Marine cyanobacteria are one such key group of organisms, since they supply much of the essential carbon and nitrogen that supports nearly all the rest of the marine food web. Currently, the growth of cyanobacteria is mostly constrained by scarce supplies of the micronutrient element iron, but they are also very sensitive to the ongoing increases in seawater temperature. Preliminary results suggest that warming could partly mitigate the negative effects of iron limitation on marine cyanobacteria. This project examines in depth how these interactions between warming and iron limitation will affect the future ocean carbon and nitrogen cycles, using laboratory culture experiments showing how cyanobacteria respond to simultaneously changing temperature and iron supplies. Both short-term response

studies and long-term evolutionary experiments testing for adaptation use a comprehensive set of molecular biology tools targeting genes to proteins. The final goal is to apply the results of these experiments to improve quantitative models predicting how the ocean's carbon and nitrogen cycles, biological productivity, and living resources will respond to a warming future climate. Two graduate students, a postdoc and 3-4 underrepresented undergraduate researchers are supported, and the investigators also mentor summer science interns from largely Hispanic local high schools.

The physiology, biochemistry and biogeography of nitrogen-fixing cyanobacteria and unicellular picocyanobacteria are strongly influenced by temperature, subjecting them to intense selective pressure as the modern ocean steadily warms up. These groups have likewise been rigorously selected under chronic iron (Fe) scarcity, and the availability of this crucial micronutrient is also changing with a shifting climate. This project examines short-term acclimation and long-term evolutionary responses of Fe-stressed marine cyanobacteria to a warmer environment. Preliminary data show that Iron Use Efficiencies (IUE, mols N fixed.hr-1 mol cellular Fe-1) of Fe-limited Trichodesmium increase 4 to 5-fold with a 5oC temperature increase, allowing the cells to much more efficiently leverage scarce available Fe supplies to grow and fix nitrogen. This means that warming can to a large degree mitigate the negative effects of Fe limitation on Trichodesmium, resulting in a modelled 22% increase in global nitrogen fixation by 2100 in a warmer climate. This project aims to uncover the cellular biochemical mechanisms involved in this Fe-limitation/thermal IUE effect in a four-year experimental evolution study of the diazotrophs Trichodesmium and Crocosphaera and the picocyanobacteria Synechococcus and Prochlorococcus, under a multi-variate selection matrix of temperature and Fe availability. The objectives are to 1) Assess the long-term adaptive responses of fitness, IUE and physiology to Fe limitation and warming interactions in these four major cyanobacterial groups; 2) Determine the molecular and biochemical mechanisms behind the surprising Fe/warming interactive effect on IUE using genomics, transcriptomics and quantitative proteomics coupled with 'metalloproteomics' determinations of Fe content in critical proteins; 3) Compare and contrast acclimation and adaptation responses to Fe limitation and warming in key cyanobacteria taxa, and 4) Integrate results using a published biogeochemical modeling approach to assess global consequences for marine productivity and nitrogen fixation. This project offers a mechanistic and predictive understanding of adaptation to Fe and warming co-stressors in a rapidly changing future ocean environment for some of the most important photoautotrophic functional groups in the ocean.

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

Collaborative Research: Underexplored Connections between Nitrogen and Trace Metal Cycling in Oxygen Minimum Zones Mediated by Metalloenzyme Inventories (CliOMZ)

Coverage: Eastern Tropical Pacific

NSF abstract:
Though scarce and largely insoluble, trace metals are key components of sophisticated enzymes (protein molecules that speed up biochemical reactions) involved in biogeochemical cycles in the dark ocean (below 1000m). For example, metalloenzymes are involved in nearly every reaction in the nitrogen cycle. Yet, despite direct connections between trace metal and nitrogen cycles, the relationship between trace metal distributions and biological nitrogen cycling processes in the dark ocean have rarely been explored, likely due to the technical challenges associated with their study. Availability of the autonomous underwater vehicle (AUV) Clio, a sampling platform capable of collecting high-resolution vertical profile samples for biochemical and microbial measurements by large volume filtration of microbial particulate material, has overcome this challenge. Thus, this research project plans an interdisciplinary chemistry, biology, and engineering effort to test the hypothesis that certain chemical reactions, such as nitrite oxidation, could become limited by metal availability within the upper mesopelagic and that trace metal demands for nitrite-oxidizing bacteria may be increased under low oxygen conditions. Broader impacts of this study include the continued development and application of the Clio Biogeochemical AUV as a community resource by developing and testing its high-resolution and adaptive sampling capabilities. In addition, metaproteomic data will be deposited into the recently launched Ocean Protein Portal to allow oceanographers and the metals in biology community to examine the distribution of proteins and metalloenzymes in the ocean. Undergraduate students will be supported by this project at all three institutions, with an effort to recruit minority students. The proposed research will also be synergistic with the goals of early community-building efforts for a potential global scale microbial biogeochemistry program modeled after the success of the GEOTRACES program, provisionally called "Biogeoscapes: Ocean metabolism and nutrient cycles on a changing planet".

The proposed research project will test the following three hypotheses: (1) the microbial metalloenzyme distribution of the mesopelagic is spatially dynamic in response to environmental gradients in oxygen and trace metals, (2) nitrite oxidation in the Eastern Tropical Pacific Ocean can be limited by iron availability in the upper mesopelagic through an inability to complete biosynthesis of the microbial protein nitrite oxidoreductase, and (3) nitrite-oxidizing bacteria increase their metalloenzyme requirements at low oxygen, impacting the distribution of both dissolved and particulate metals within oxygen minimum zones. One of the challenges to characterizing the biogeochemistry of the mesopelagic ocean is an inability to effectively sample it. As a sampling platform, we will use the novel biogeochemical AUV Clio that enables high-resolution vertical profile samples for biochemical and microbial measurements by large volume filtration of microbial particulate material on a research expedition in the Eastern Tropical Pacific Ocean. Specific research activities will be orchestrated to test the hypotheses. Hypothesis 1 will be explored by comparison of hydrographic, microbial distributions, dissolved and particulate metal data, and metaproteomic results with profile samples collected by Clio. Hypothesis 2 will be tested by incubation experiments using 15NO2- oxidation rates on Clio-collected incubation samples. Hypothesis 3 will be tested by dividing targeted nitrite oxidoreductase protein copies by qPCR (quantitative polymerase chain reaction)-based nitrite oxidizing bacteria abundance (NOB) to determine if cellular copy number varies with oxygen distributions, and by metalloproteomic analyses of NOB cultures. The demonstration of trace metal limitation of remineralization processes, not just primary production, would transform our understanding of the role of metals in biogeochemical cycling and provide new ways with which to interpret sectional data of dissolved and particulate trace metal distributions in the ocean. The idea that oxygen may play a previously underappreciated role in controlling trace metals due not just to metals' physical chemistry, but also from changing biological demand, will improve our ability to predict trace metal distributions in the face of decreasing ocean oxygen content.

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

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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.

Marine Microbiology Initiative (MMI)

Website: https://www.moore.org/initiative-strategy-detail?initiativeId=marine-microbiology-initiative

A Gordon and Betty Moore Foundation Program.

Forging a new paradigm in marine microbial ecology:

Microbes in the ocean produce half of the oxygen on the planet and remove vast amounts of carbon dioxide, a greenhouse gas, from the atmosphere. Yet, we have known surprisingly little about these microscopic organisms. As we discover answers to some long-standing puzzles about the roles that marine microorganisms play in supporting the ocean's food webs and driving global elemental cycles, we realized that we still need to learn much more about what these organisms do and how they do it—including how they evolved and contribute to our ocean's health and productivity.

The Marine Microbiology Initiative seeks to gain a comprehensive understanding of marine microbial communities, including their diversity, functions and behaviors; their ecological roles; and their origins and evolution. Our focus has been to enable researchers to uncover the principles that govern the interactions among microbes and that govern microbially mediated nutrient flow in the sea. To address these opportunities, we support leaders in the field through investigator awards, multidisciplinary team research projects, and efforts to create resources of broad use to the research community. We also support development of new instrumentation, tools, technologies and genetic approaches.

Through the efforts of many scientists from around the world, the initiative has been catalyzing new science through advances in methods and technology, and to reduce interdisciplinary barriers slowing progress. With our support, researchers are quantifying nutrient pools in the ocean, deciphering the genetic and biochemical bases of microbial metabolism, and understanding how microbes interact with one another. The initiative has five grant portfolios:

Individual investigator awards for current and emerging leaders in the field.
Multidisciplinary projects that support collaboration across disciplines.
New instrumentation, tools and technology that enable scientists to ask new questions in ways previously not possible.
Community resource efforts that fund the creation and sharing of data and the development of tools, methods and infrastructure of widespread utility.
Projects that advance genetic tools to enable development of experimental model systems in marine microbial ecology.
We also bring together scientists to discuss timely subjects and to facilitate scientific exchange.

Our path to marine microbial ecology was a confluence of new technology that could accelerate science and an opportunity to support a field that was not well funded relative to potential impact. Around the time we began this work in 2004, the life sciences were entering a new era of DNA sequencing and genomics, expanding possibilities for scientific research – including the nascent field of marine microbial ecology. Through conversations with pioneers inside and outside the field, an opportunity was identified: to apply these new sequencing tools to advance knowledge of marine microbial communities and reveal how they support and influence ocean systems.

After many years of success, we will wind down this effort and close the initiative in 2021. We will have invested more than $250 million over 17 years to deepen understanding of the diversity, ecological activities and evolution of marine microbial communities. Thanks to the work of hundreds of scientists and others involved with the initiative, the goals have been achieved and the field has been profoundly enriched; it is now positioned to address new scientific questions using innovative technologies and methods.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1031271
Gordon and Betty Moore Foundation: Marine Microbiology Initiative (MMI)	GBMF3782
NSF Division of Ocean Sciences (NSF OCE)	OCE-1657766
NSF Division of Ocean Sciences (NSF OCE)	OCE-1736599
NSF Division of Ocean Sciences (NSF OCE)	OCE-1850719
NSF Division of Ocean Sciences (NSF OCE)	OCE-1924554

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