# Net tow metaproteome of Trichodesmium species mapped to a Trichodesmium metagenome plus cyanoGEBA species genomes in units of normalized protein spectral counts from samples collected in the Atlantic and Pacific Ocean between 2000 and 2018

Website: https://www.bco-dmo.org/dataset/787147

Data Type: Cruise Results

Version: 1

Version Date: 2020-04-30

#### **Project**

- » <u>Collaborative Research: Iron and phosphorus balanced limitation of nitrogen fixation in the oligotrophic ocean</u> (TriCoLim)
- » New technology for high resolution analysis of proteins and other organic materials produced by marine microorganisms (MM Proteins and Organics Tech)
- » Marine Microbial Investigator Award: Investigator Mak Saito (MM Saito)
- » <u>Collaborative Research: Evolutionary, biochemical and biogeochemical responses of marine cyanobacteria to warming and iron limitation interactions</u> (Cyanobacteria Warming Responses)

# **Program**

» Marine Microbiology Initiative (MMI)

Contributors	Affiliation	Role
Saito, Mak A.	Woods Hole Oceanographic Institution (WHOI)	Principal Investigator
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#### **Abstract**

Net tow metaproteome of Trichodesmium species mapped to a Trichodesmium metagenome plus cyanoGEBA species genomes, analyzed by 2D LC-MS/MS in units of normalized protein spectral counts. Samples were collected in North Atlantic surface waters, at station BATS (Bermuda Atlantic Time-series Study), and station ALOHA (A Long-Term Oligotrophic Habitat Assessment) between 2000 and 2018.

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#### Coverage

**Spatial Extent**: N:23.22 E:-21.59 S:0.17 W:-158 **Temporal Extent**: 2000-07-27 - 2018-03-18

# **Dataset Description**

Net tow metaproteome of Trichodesmium species mapped to a Trichodesmium metagenome plus cyanoGEBA species genomes, analyzed by 2D LC-MS/MS in units of normalized protein spectral counts. Samples were collected in North Atlantic surface waters, at station BATS (Bermuda Atlantic Time-series Study), and station ALOHA (A Long-Term Oligotrophic Habitat Assessment) between 2000 and 2018. An alternative form of these data are available as dataset: "Trichodesmium field metaproteomes - protein spectral counts in alternative format" <a href="https://www.bco-dmo.org/dataset/787078">https://www.bco-dmo.org/dataset/787078</a> Related datasets: Trichodesmium field metaproteomes - sequence fasta: <a href="https://www.bco-dmo.org/dataset/787181">https://www.bco-dmo.org/dataset/787181</a> Trichodesmium field metaproteomes - peptide spectral counts: <a href="https://www.bco-dmo.org/dataset/787168">https://www.bco-dmo.org/dataset/787168</a> Trichodesmium sample provenance: <a href="https://www.bco-dmo.org/dataset/787093">https://www.bco-dmo.org/dataset/787093</a> - Sample provenance file, which includes sample locations, filter sizes

## Methods & Sampling

A 200um plankton net net was deployed to  $\sim$ 20m depth, then recovered to just below surface, repeating five times. Trichodesmium colonies were hand picked into 0.2um filtered surface seawater, rinsed twice in 0.2um filtered surface seawater, and decanted onto a 0.2-4um Supor filter (indicated in sample provenance table, see dataset <a href="https://www.bco-dmo.org/dataset/787093">https://www.bco-dmo.org/dataset/787093</a>).

Proteins were extracted and tryps in digested in-gel following Saito et al., 2014 (Science). Peptides were analyzed by LC-MS/MS in data discovery mode on a Thermo Orbitrap Fusion.

#### **Data Processing Description**

Thermo proteome discoverer 2.2 was used to search the data with the SEQUEST algorithm. Statistical validation was performed in Scaffold (Proteome Software) at the 1% protein and peptide false discovery rate (FDR) levels.

A publicly available Trichodesium metagenome was used in this search (IMG ID 2821474806) as well as the contents of the CyanoGEBA project (Shih et al, 2013).

**BCO-DMO Data Manager Processing Notes:** 

- \* Time values "00009" changed to 09:00 after checking the sample log.
- \* Removed column of row numbers called "Unnamed:0"
- \* Column names were switched kegg\_pathway and pfams\_id so those column names were switched to correct that. kegg id and ncbi name were also switched.

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#### **Data Files**

# File

## Trichodesmium field metaproteomes - protein spectral counts

filename: proteins.csv

(Comma Separated Values (.csv), 27.15 MB) MD5:7e32e3f238062d90ccdf711acd189373

Net tow metaproteome of Trichodesmium species mapped to a Trichodesmium metagenome plus cyanoGEBA species genomes in units of normalized protein spectral counts from samples collected in the Atlantic and Pacific Ocean between 2000 and 2018.

Parameters in this comma delimited file are included in the Parameter section of the Dataset Landing Page "Trichodesmium field metaproteomes - protein spectral counts" https://www.bco-dmo.org/dataset/787147.

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# **Related Publications**

Saito, M. A., McIlvin, M. R., Moran, D. M., Goepfert, T. J., DiTullio, G. R., Post, A. F., & Lamborg, C. H. (2014). Multiple nutrient stresses at intersecting Pacific Ocean biomes detected by protein biomarkers. Science, 345(6201), 1173–1177. https://doi.org/10.1126/science.1256450

Methods

Shih, P. M., Wu, D., Latifi, A., Axen, S. D., Fewer, D. P., Talla, E., ... Kerfeld, C. A. (2012). Improving the coverage of the cyanobacterial phylum using diversity-driven genome sequencing. Proceedings of the National Academy of Sciences, 110(3), 1053–1058. doi:10.1073/pnas.1217107110

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#### **Related Datasets**

### **Different Version**

Held, N., & Saito, M. (2020). *Metaproteomes of Trichodesmium from samples collected in North Atlantic surface waters, station BATS, and station ALOHA between 2000 and 2018* [Data set]. Biological and Chemical Oceanography Data Management Office (BCO-DMO). https://doi.org/10.26008/1912/BCO-DMO.787078.1 https://doi.org/10.26008/1912/bco-dmo.787078.1

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#### **Parameters**

Parameter	Description	Units
sample_id	Unique sample location ID	unitless
cruise_id	Cruise name	unitless
station_id	Station name	unitless
latitude_dd	Latitude (South is negative)	decimal degrees
longitude_dd	Longitude (West is negative)	decimal degrees
depth_m	Sample depth	meters (m)
date_y-m-d	Date (Local ship time) in format yyyy-mm-dd (various time zones, see sample provenance dataset http://lod.bco-dmo.org/id/dataset/787093)	unitless
time_h-m-d	Time (Local ship time) of sampling in format HH:MM (various time zones, see sample provenance dataset http://lod.bco-dmo.org/id/dataset/787093)	unitless
minimum_filter_size_microns	Maxiumum phytoplankton net size	microns (uM)
maximum_filter_size_microns	Minimum phytoplankton net size	microns (uM)
protein_id	Unique protein accession number. Uniquely identifies a protein within the dataset and FASTA file	unitless
protein_name	Protein descriptive name	unitless
top3_precursor	Normalized precursor ion intensity of the top 3 most abundant peptide	unitless
molecular_weight_kDa	Molecular weight of the sample (no data, column included to comply with Ocean Protein Portal template)	kilodaltons (kDa)
ncbi_id	NCBI protein ID. NCBI taxon identifier (txid)	unitless
ncbi_name	NCBI protein name. NCBI taxon name associated with the NCBI taxon identifier (ncbi_id)	unitless
kegg_id	KO ontology identifier	unitless
kegg_description	Kegg description as it correlates to the Kegg identifier (kegg_id).(no data, column included to comply with Ocean Protein Portal template)	unitless
kegg_pathway	Kegg pathway as it correlates to the Kegg identifier (kegg_id). (no data, column included to comply with Ocean Protein Portal template)	unitless
pfams_id	PFams identifier	unitless
pfams_name	pfams name (no data, column included to comply with Ocean Protein Portal template)	unitless
uniprot_id	Uniprot database identifier (no data, column included to comply with Ocean Protein Portal template)	unitless
enzyme_comm_id	Enzyme Commission identifer (no data, column included to comply with Ocean Protein Portal template)	unitless
other_identified_proteins	Other protein identifiers in the FASTA file (no data, column included to comply with Ocean Protein Portal )	unitless
sample_type	Sample type "Trichodesmium net tow"	unitless

# Instruments

Dataset- specific Instrument Name	Thermo Orbitrap Fusion 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.

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# **Deployments**

#### AT39-05

Website	https://www.bco-dmo.org/deployment/765978
Platform	R/V Atlantis
Start Date	2018-02-11
End Date	2018-03-14
Description	For study of iron and phosphorus balanced limitation of nitrogen fixation in the oligotrophic ocean.

# JC150

Website	/ebsite https://www.bco-dmo.org/deployment/78668	
Platform	RRS James Cook	
Start Date	2017-06-26	
End Date	2017-08-12	

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# **Project Information**

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.

New technology for high resolution analysis of proteins and other organic materials produced by marine microorganisms (MM Proteins and Organics Tech)

Website: <a href="https://www.moore.org/grant-detail?grantId=GBMF3934">https://www.moore.org/grant-detail?grantId=GBMF3934</a>

In support of acquiring a high resolution mass spectrometer that incorporates the latest technologies for analyzing proteins and other organic materials.

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.

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# **Program Information**

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
Gordon and Betty Moore Foundation: Marine Microbiology Initiative (MMI)	<u>GBMF3934</u>
Gordon and Betty Moore Foundation: Marine Microbiology Initiative (MMI)	<u>GBMF3782</u>
NSF Division of Ocean Sciences (NSF OCE)	OCE-1657766
NSF Division of Ocean Sciences (NSF OCE)	OCE-1850719

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