

Semi-quantitative cell proteome of marine *Synechococcus* WH8102 using DIA-MS, interactive nutrient-temperature responses in stable chemostat bioreactors from laboratory experiments conducted in 2019

Website: <https://www.bco-dmo.org/dataset/923159>

Data Type: experimental

Version: 1

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Project

» [Convergence: RAISE: Linking the adaptive dynamics of plankton with emergent global ocean biogeochemistry](#) (Ocean Stoichiometry)

» [Collaborative Research: The stoichiometric trait distribution of the marine microbiome](#) (StoichTraitD)

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Abstract

This proteomic data set was prepared to determine how the distribution of protein-based traits contribute to cellular elemental stoichiometry of a globally-abundant, surface ocean phytoplankton in response to nutrient stress under variable temperature conditions. Cellular proteins are from laboratory chemostat cultures of marine *Synechococcus* isolate WH8102 (clade III). Proteins were extracted from cells grown under stable, steady-state conditions in a low-phosphorus medium (N:P=80) and a low-nitrate medium (N:P=1.7) across 3 temperatures of 20, 24, and 28°C (for a total of 6 chemostat cultures) at a white light density of 125 $\mu\text{mol quanta}^{-1} \text{m}^{-2} \text{s}^{-1}$ on a 12-hour-light:12-hour-dark diel cycle, at a continuous dilution rate of 0.18 d^{-1} , in fall of 2019. Cells and proteins were collected at 5 mid-light-period time points on non-consecutive days to yield a total of 30 samples. Culture work was performed at the University of California, Irvine by Adam Martiny's group. Proteins were analyzed with data-independent acquisition mass spectrometry proteomics methods by Mak Saito's group at the Woods Hole Oceanographic Institution. Data are exclusive peak area intensities of proteins with 2 or more representative peptides, normalized with Scaffold (2.2.1) DIA proteome software. Peptides were analyzed using a Michrom Advance HPLC system coupled to a Q-Exactive mass spectrometer (Thermo Scientific instrument version 2.8) with a Michrom Advance CaptiveSpray source, using the constant injection concentration of 1 $\mu\text{g}/\mu\text{L}$ to allow uniformity across the dataset. Data with more details are published in Garcia et al. (2024) Proteome trait regulation of marine *Synechococcus* elemental stoichiometry under global change (DOI:10.1093/ismej/wrae046).

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Coverage

Location: Laboratory in the Department Earth System Science, University of California, Irvine

Temporal Extent: 2019 - 2019

Methods & Sampling

Experimental design

Synechococcus cultures (WH8102) were grown in polycarbonate bottles with a continuous chemostat method used previously (Garcia et al. 2016) in artificial seawater. We used two concentration ratios of macronutrients (NO₃:PO₄³⁻ = 1.7 and 80) and 3 levels of temperature (20, 24 and 28°C) with a slow dilution rate (0.18 d⁻¹) to ensure treatment-wise culture stability. White light was supplied at 125 μmol quanta m⁻² s⁻¹ on a 12h:12h light:dark cycle. Chemostat equilibria were monitored by measuring culture cell density and forward scatter (FSC_H) with a Novocyte flow cytometer 1000 (Acea Biosciences, Inc, San Diego, CA). Cells for proteome analysis were collected after an acclimation period on days 38, 43, 47, 50 and 57 with a 47 mm polycarbonate filter (0.2 mm pore size) 7-8 hours into the light period, pelleted by centrifugation (21,130 g for 3 minutes), flash frozen in liquid nitrogen, and stored at -80°C.

Protein extraction and peptide preparation

Proteins were extracted by heating pelleted cells at 95°C for 10 min and gently shaking at room temperature for 30 min in a buffer solution (400 μL - 1760 μL; 50 mM HEPES pH 8.5 (Boston BioProducts #BB-2082), 1% SDS in HPLC grade water) before centrifuging at 14100 g for 20 min at room temperature and removing the supernatant. Sodium dodecyl sulfate (1%) is a strong detergent for diverse matrices including cell membranes (Hughes et al. 2014). Benzoylase nuclease (50 units; Novagen #70746-3) was added to 400 μL extracted protein sample and incubated at 37°C for 30 min. Samples were reduced by adding 20 μL of 200 mM DTT (Fisher #BP172-5) in 50 mM HEPES pH 8.5 at 45°C for 30 min and alkylated with 40 μL of 400 mM iodoacetamide (Acros #122270050) in HEPES pH 8.5 for 30 min at 24°C. The reaction was quenched by adding 40 μL of 200 mM DTT in 50 mM HEPES pH 8.5. SpeedBead Magnetic Carboxylate Modified Particles (GE Healthcare #65152105050250 and #45152105050250) were prepared according to [3] and added (20 μg/μL) to 400 μL of extracted protein sample. Samples were incubated with formic acid (pH of 2-3) and washed with ethanol and acetonitrile using a magnetic rack. Protein was measured with the BCA method (Thermo Scientific Micro BCA Protein Assay Kit #23235) and digested overnight at 37°C with 1 part trypsin (Promega #V5280; dissolved in HEPES pH 8.0, 0.5 μg/μL) and 25 parts protein. Peptides were washed with acetonitrile and ethanol using a magnetic rack and diluted to a target concentration of 0.1% trifluoroacetic acid or 1% formic acid and a final concentration of 1 μg/μL.

Mass spectrometry of peptides

Similar to other analyses (Searle et al. 2018), peptides were analyzed using a Michrom Advance HPLC system coupled to a Q-Exactive mass spectrometer (Thermo Scientific instrument version 2.8) with a Michrom Advance CaptiveSpray source, using the constant injection concentration of 1 mg/mL to allow uniformity across the dataset. Samples were concentrated onto a C18 column (Reprosil-Gold, Dr. Maisch GmbH) and eluted in a non-linear, 200-min gradient of formic acid and acetonitrile buffers. Full MS1 scans were performed (35,000 resolution, 3e6 AGC target, 60 ms maximum IT, 385 to 1015 m/z) with overlapping DIA scans (17,500 resolution, 1e6 AGC target, 60 ms maximum IT, 24.0 m/z isolation windows, normalized collision energy of 27, loop count 25).

Proteomic data analysis

Data-independent acquisition mass spectrometry sample data were analyzed using Scaffold DIA (2.2.1), converted to mzML format (ProteoWizard 3.0.11748), and individually searched against Syn8102_uniprot-proteome_UP000001422.fasta with a peptide and fragment mass tolerance of 10.0 ppm. Percolator (3.01) filtered peptides for a maximum false discovery rate of 0.01. Charged peptides (2-3) with length (6-30) were considered. EncyclopeDIA (0.9.6) selected the 5 highest quality fragment ions for quantitation (Searle et al. 2018).

Data Processing Description

Data-independent acquisition mass spectrometry sample data were analyzed using Scaffold DIA (2.2.1), converted to mzML format (ProteoWizard 3.0.11748), and individually searched against Syn8102_uniprot-proteome_UP000001422.fasta with a peptide and fragment mass tolerance of 10.0 ppm. Percolator (3.01) filtered peptides for a maximum false discovery rate of 0.01. Charged peptides (2-3) with length (6-30) were considered. EncyclopeDIA (0.9.6) selected the 5 highest quality fragment ions for quantitation (Searle et al. 2018).

BCO-DMO Processing Description

* Sheet 1 of submitted file "Normalized peak areas (exclusive intensity for BCO-DMO).xlsx" (submitted Apr 2nd, 2024) was modified to add a row of sample ID numbers (1-30) before import into the bco-dmo data system, discussed with the file submitter. This modified excel file was added as a supplemental file (alternate format, wide-form data table).

* The table was then imported into the BCO-DMO data system. Values "NaN" imported as missing data values.

** Missing data values are displayed differently based on the file format you download. They are blank in csv files, "NaN" in MatLab files, etc.

* Column names adjusted to conform to BCO-DMO naming conventions designed to support broad re-use by a variety of research tools and scripting languages. [Only numbers, letters, and underscores. Can not start with a number]

* Data table was transformed from wide format (normalized peak area columns per sample) to one column of normalized peak area values with additional columns with the sample ID, and treatment measurements (temperature, NO₃_to_PO₄, etc).

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Parameters

Parameters for this dataset have not yet been identified

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Project Information

Convergence: RAISE: Linking the adaptive dynamics of plankton with emergent global ocean biogeochemistry (Ocean Stoichiometry)

NSF Award Abstract:

Due to their sheer abundance and high activity, microorganisms have the potential to greatly influence how ecosystems are affected by changes in their environment. However, descriptions of microbial physiology and diversity are local and highly complex and thus rarely considered in Earth System Models. Thus, the researchers focus on a convergence research framework that can qualitatively and quantitatively integrate eco-evolutionary changes in microorganisms with global biogeochemistry. Here, the investigators will develop an approach that integrates the knowledge and tools of biologists, mathematicians, engineers, and geoscientists to understand the link between the ocean nutrient and carbon cycles. The integration of data and knowledge from diverse fields will provide a robust, biologically rich, and computationally efficient prediction for the variation in plankton resource requirements and the biogeochemical implications, addressing a fundamental challenge in ocean science. In addition, the project can serve as a road map for many other research groups facing a similar lack of convergence between biology and geoscience.

Traditionally, the cellular elemental ratios of Carbon, Nitrogen, and Phosphorus (C:N:P) of marine communities have been considered static at Redfield proportions but recent studies have demonstrated strong latitudinal variation. Such regional variation may have large - but poorly constrained - implications for marine biodiversity, biogeochemical functioning, and atmospheric carbon dioxide levels. As such, variations in ocean community C:N:P may represent an important biological feedback. Here, the investigators propose a convergence

research framework integrating cellular and ecological processes controlling microbial resource allocations with an Earth System model. The approach combines culture experiments and omics measurements to provide a molecular understanding of cellular resource allocations. Using a mathematical framework of increasing complexity describing communicating, moving demes, the team will quantify the extent to which local mixing, environmental heterogeneity and evolution lead to systematic deviations in plankton resource allocations and C:N:P. Optimization tools from engineering science will be used to facilitate the quantitative integration of models and observations across a range of scales and complexity levels. Finally, global ocean modeling will enable understanding of how plankton resource use impacts Earth System processes. By integrating data and knowledge across fields, scales and complexity, the investigators will develop a robust link between variation in plankton C:N:P and global biogeochemical cycles.

Collaborative Research: The stoichiometric trait distribution of the marine microbiome (StoichTraitD)

Coverage: Indian Ocean; Laboratory Ecophysiology

NSF abstract:

The elemental ratios of carbon, nitrogen, and phosphorus (C:N:P) have been considered fixed proportions in marine environments but recent work has demonstrated changes across latitudes. Such variation can have large implications for marine biogeochemistry and atmospheric CO₂ levels. As such, future variations in ocean community C:N:P could be a key feedback to global change. The elemental composition of particulate organic matter (POM) represents the aggregate value of diverse microorganisms as well as non-living particles. However, we currently do not understand how individual cells and particles contribute to observed variation in the C:N:P of POM. This project is determining the biomass C:N:P of individual microbial cells grown under a range of conditions and sampled from diverse ocean biomes. Because different individuals are likely to have different fates (e.g., loss to sinking, lysis, predation), understanding how the trait distribution of microbial biomass C:N:P relates to cell size and trophic mode and how environmental conditions affect each trait's distribution offers new perspectives and insight into marine C, N, and P cycles. This project supports two PhD students and multiple undergraduate students. The PhD students are integrated into existing networks on microbiome science at each institution with opportunities to collaborate across diverse disciplines. Undergraduate students at both institutions are being recruited through existing training programs that target underrepresented groups. In addition, PI Hall is part of a collaborative of Ecologists and Poets at CSU, that look at ways to translate ecological relationships to non-traditional media to make it more accessible and impactful to the general public. This group is exploring the nature of individuality within the marine microbiome by creating trait distributions of written text and removing different modes of individuals (e.g., words) to better understand and communicate how individuals from the smallest organisms on the planet (marine microorganisms) can have large effects on the surrounding ecosystem (i.e., the planet). Results from this project are being incorporated into future projects of the working group at Colorado State University including public presentations, art installations, and published materials.

The aim of this project is to quantify the relationship between environmental conditions and marine microbial C:N:P by assessing the individuality in microbial elemental stoichiometry. To achieve this the project uses energy dispersive spectroscopy (EDS) to assess the stoichiometric trait distribution of populations and communities under different resource and temperature treatments and oceanographic field work across a broad latitudinal gradient. The researchers hypothesize that the relationship between the stoichiometry of particulate organic matter (POM) and environmental conditions are masked by distinct responses of individual constituents of the marine microbiome. They hypothesize that these distinct responses result in a multi-modal distribution of particulate carbon (C), nitrogen (N), and phosphorus (P) of the marine microbiome. The investigators are characterizing the distribution of three stoichiometric traits (biomass C:N, C:P, and N:P) for 50 marine isolates (both autotrophs and heterotrophs) under different resource and temperature environments. They are characterizing the same trait distribution for marine communities sampled from different regions of the ocean for the same resource and temperature environments as the population experiments. They are participating on an ocean going cruise to characterize stoichiometric trait distribution of the marine microbiome across natural gradients of resources and temperature. Understanding how constituent members of microbial communities alter their biomass in response to environmental change is providing a missing link between the variation in the ocean's environment and particulate C:N:P ratios for diverse marine environments.

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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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1848576
NSF Division of Ocean Sciences (NSF OCE)	OCE-2135035
NSF Division of Integrative Organismal Systems (NSF IOS)	IOS-2137339

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