Single-cell elemental quotas and stoichiometry of Synechococcus sp. WH8102 measured by X-ray microanalysis (XRMA) under varying phosphorus conditions

Website: https://www.bco-dmo.org/dataset/1005186
Data Type: experimental
Version: 1
Version Date: 2026-08-18

Project
» Collaborative Research: Assessing the role of polyphosphate production and cycling in marine ecosystem functioning (Polyphosphate production and cycling)

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Abstract
This dataset contains single-cell elemental stoichiometry measurements of Synechococcus sp. WH8102, obtained using XRMA (X-ray microanalysis) combined with microscopy-based cell size measurements. Cells were analyzed under different experimental phosphorus (P) conditions and timepoints to quantify cellular carbon (C), nitrogen (N), and phosphorus (P) content at the single-cell level. Cell size was determined using two independent approaches: 1)XRMA-associated SEM measurements (A_um2_xray) 2)ImageJ-based image analysis (A_um2_imageJ). Cellular volume was estimated using a rod-shaped cell geometry model, allowing conversion from projected cell area to volume (µm³). Elemental quotas (C_fg_cell, N_fg_cell, P_fg_cell) were measured in femtograms per cell, and normalized to volume where applicable. The dataset supports analysis of cellular stoichiometry (C:N:P ratios), elemental plasticity, and physiological responses to phosphorus availability across time and experimental replicates.

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Coverage

Location: Laboratory experiment conducted in the Duhamel Lab at the University of Arizona; XRMA analysis conducted at the Microscopy Platform at ICM-CSIC, Spain

Methods & Sampling

For XRMA analysis, ~ 0.4 mL of *Synechococcus* culture cells were transferred to a 1.5 mL microtube and washed with 0.8 mL of cold buffered ultrapure water (pH 8) to remove salts. After centrifugation (1 min at 7000 rpm at 15 ºC), 1.1 mL supernatant was replaced by cold buffered ultrapure water and centrifuged under the same conditions. This last step was repeated twice, and 1.1 mL was carefully discarded. The concentrated cells were homogenized before transferring 6 µL onto a 3 mm TEM grid coated with formvar and evaporated carbon (Ted Pella, Redding, CA, USA). The grid was air-dried and stored in a vacuum cabinet until analysis. Cells were analyzed for elemental composition on a Hitachi SU8600 Field-Emission Scanning Electron Microscope (FE-SEM) equipped with two high-resolution (123 eV) Bruker X-Flash 7|30 Energy Dispersive Spectrometers. To avoid interference with the holder, the grids were attached to the edge of the SEM pins. XRMA spectra were obtained at 20 kV of incident energy during 90 seconds of live time. The absolute beam current was measured using a Faraday cup before each analysis session, which allowed for correcting for beam current drift during the session, and ensured an accurate reference to the calibration. Polystyrene latex spheres with mean particle size of 0.6 and 1.1 µm (Sigma LB6 and LB11) were used to calibrate carbon, and ATP (Sigma, A2383) was used to obtain the calibration constants for N and P, following Norland et al. (1995).

Cell dimensions were measured from SEM micrographs using ImageJ (version 1.54g, Abràmoff et al. 2004). *Synechococcus* cells exhibited rod-shaped to elongated-oval morphologies. As a result, biovolume was calculated assuming a cylindrical shape with two hemispherical ends (Sun & Liu, 2003), using the width (cross section) and length (transapical section) measurements obtained for each cell in ImageJ.

Data Processing Description

Latex spheres with a 0.6 µm mean particle size were used as internal standards and were analyzed at the start and end of each sample analysis session. Detection limits were calculated as 3 times the standard deviation of the blank (formvar film) measured in the energy region of each target element's spectral peak and were 26.3, 0.54, and 0.45 fg $cell^{-1}$ for C, N, and P, respectively. In the present study, only elemental quotas above the detection limit, which account for more than 98% of the quotas measured, are shown and used to calculate ratios.

BCO-DMO Curation Notes

This section documents curation actions performed prior to publication review with the submitter, and additional information relevant to understanding and reusing this dataset. It distinguishes changes made to the submitted (meta)data from unresolved issues and/or enhancements that improve future reuse and interoperability.

CURATION ACTIONS PERFORMED ON DATA

- Loaded Sheet1 from xray_syn_wh8102_dataset_bco.xlsx into table named xrma_single_cell_stoichiometry; configured empty string and "nd" as missing values; applied floating point error adjustment and preserved formatting during load
- Renamed column "Exp" to "exp" to match the lowercase column name used in the README, since the actual data file used "Exp" as the treatment column header
- Output as 1005186_v1_xrma_single_cell_stoichiometry.csv

CURATION ACTIONS PERFORMED ON METADATA

- BCO-DMO's standard metadata entry and text formatting steps were performed. See: https://www.bco-dmo.org/how-to/standard-curation-edits
- Added a citation for ImageJ

ISSUES POTENTIALLY IMPACTING REUSE

- N/A

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Data Files

File
1005186_v1_xrma_single_cell_stoichiometry.csv (Comma Separated Values (.csv), 38.48 KB) MD5:4cf2e50dffa11619b4d785c183b3f25f Primary data file for dataset ID 1005186, version 1

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Supplemental Files

File
README XRMA filename: XRMA_BCO.txt (Plain Text, 2.54 KB) MD5:23a358824349baef37a040c948271a1b this file contains information to undertand the dataset

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Related Publications

Abràmoff, M.D, Magalhães, P.J., Ram, S.J. 2004. Image processing with ImageJ. Biophotonics International 11(7): 36−42
Software

Norland, S., Fagerbakke, K. M., & Heldal, M. (1995). Light element analysis of individual bacteria by x-ray microanalysis. Applied and Environmental Microbiology, 61(4), 1357–1362. https://doi.org/10.1128/aem.61.4.1357-1362.1995
Methods

Sun, J., Liu, D. (2003). Geometric models for calculating cell biovolume and surface area for phytoplankton. Journal of Plankton Research, 25(11), 1331–1346. doi:10.1093/plankt/fbg096
Methods

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Related Datasets

IsRelatedTo

Filella, A. (2026) **RNA-seq gene expression data for Synechococcus sp. WH8102 under continuous high, mid, and low phosphorus treatments in laboratory EFB experiments in 2025.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2026-08-14 doi:10.26008/1912/bco-dmo.998248.1 [view at BCO-DMO]
Relationship Description: Datasets were part of same experiment.

Filella, A., Duhamel, S., Diaz, J. (2026) **Cell abundance, nutrient concentrations, and intracellular phosphorus pools in Synechococcus sp. WH8102 continuous (EFB) culture experiments.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2026-08-13 doi:10.26008/1912/bco-dmo.1005084.1 [view at BCO-DMO]
Relationship Description: Datasets were part of same experiment.

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Parameters

Parameter	Description	Units
SampleID	Unique identifier for each analyzed single-cell sample	unitless
Syn_Cell	Cell index within sample or field of view	unitless
exp	Experimental treatment condition (e.g., Low P, Mid P, High P)	unitless
time	Sampling timepoint in days	days
rep	Biological replicate identifier	unitless
A_um2_xray	Cell projected area measured directly from SEM/XRMA system	µm2
A_um2_imageJ	Cell projected area recalculated using ImageJ image analysis	µm2
Volume_um3	Estimated cell volume calculated assuming rod-shaped geometry	µm3
C_fg_cell	Carbon content per cell	femtograms (fg C / cell)
N_fg_cell	Nitrogen content per cell	femtograms (fg N / cell)
P_fg_cell	Phosphorus content per cell	femtograms (fg P / cell)
C_fg_um3	Carbon concentration normalized to cell volume	fg C / µm3
N_fg_um3	Nitrogen concentration normalized to cell volume	fg N / µm3
P_fg_um3	Phosphorus concentration normalized to cell volume	fg P / µm3
C_P	Carbon-to-phosphorus molar ratio	ratio (unitless)
C_N	Carbon-to-nitrogen molar ratio	ratio (unitless)
N_P	Nitrogen-to-phosphorus molar ratio	ratio (unitless)

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Instruments

Dataset-specific Instrument Name	centrifuge
Generic Instrument Name	Centrifuge
Dataset-specific Description	After centrifugation (1 min at 7000 rpm at 15 ºC), 1.1 mL supernatant was replaced by cold buffered ultrapure water and centrifuged under the same conditions.
Generic Instrument Description	A machine with a rapidly rotating container that applies centrifugal force to its contents, typically to separate fluids of different densities (e.g., cream from milk) or liquids from solids.

Dataset-specific Instrument Name	homogenizer
Generic Instrument Name	Homogenizer
Dataset-specific Description	The concentrated cells were homogenized before transferring 6 µL onto a 3 mm TEM grid coated with formvar and evaporated carbon (Ted Pella, Redding, CA, USA).
Generic Instrument Description	A homogenizer is a piece of laboratory equipment used for the homogenization of various types of material, such as tissue, plant, food, soil, and many others.

Dataset-specific Instrument Name	Hitachi SU8600 Field-Emission Scanning Electron Microscope (FE-SEM)
Generic Instrument Name	Scanning Electron Microscope
Dataset-specific Description	Cells were analyzed for elemental composition on a Hitachi SU8600 Field-Emission Scanning Electron Microscope (FE-SEM) equipped with two high-resolution (123 eV) Bruker X-Flash 7|30 Energy Dispersive Spectrometers.
Generic Instrument Description	A scanning electron microscope (SEM) scans a focused electron beam over a surface to create an image. The electrons in the beam interact with the sample, producing various signals that can be used to obtain information about the surface topography and composition.

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Project Information

Collaborative Research: Assessing the role of polyphosphate production and cycling in marine ecosystem functioning (Polyphosphate production and cycling)

Coverage: Mediterranean Sea and California Current Ecosystem

NSF Award Abstract:
Phosphorus (P) is a vital nutrient required by all forms of life. In the ocean, which sustains half of global photosynthesis and oxygen supply, P can be scarce enough to constrain biological productivity, carbon dioxide uptake, and therefore climate. Human activity is accelerating the delivery of nutrients like P to the ocean, but rates of nitrogen inputs are even greater. This imbalance may increase levels of P stress in marine ecosystems, placing ocean productivity more and more under the control of P supply. Given the critical role of P in sustaining ocean health and ecosystem services now and into the future, a comprehensive understanding of its utilization and fate in the marine environment is necessary. In this project, the research team investigates marine polyphosphate (polyP), a ubiquitous yet poorly understood form of P made by all living organisms. To

close major knowledge gaps on marine polyP, the investigators are overcoming major technical barriers to produce the first quantitative measurements of polyP in marine microorganisms. These measurements are being conducted on laboratory microbial cultures, as well as field samples from environments with high P supply, such as the California Current Ecosystem, or very low P supply, such as the Mediterranean Sea. In addition, the research team is resolving the cellular function of marine polyP across these different organisms and environments in order to clarify its role in biological P nutrition. This work helps advance polyP research across disciplines, including terrestrial science and even cancer research, and has broad application to P bioremediation. This project supports a postdoctoral researcher, a graduate student, and several undergraduate students in the labs of two female scientists. New educational tools to teach the public about marine polyP are being produced and disseminated through this project. A K12 teacher is participating in the work and communicating the findings to their classrooms and to broad audiences online.

PolyP is ubiquitous in marine systems, where it plays critical roles in microbial P nutrition and P mineral formation and sequestration. In these ways, polyP has the potential to shape long- and short-term marine P cycling, primary productivity, microbial ecology, and global climate. However, major knowledge gaps still exist. Due to technical limitations, the scientific community currently lacks a quantitative understanding of marine polyP pools, their chain lengths, and biological origins. Furthermore, given the view of polyP as a P storage molecule, recent observations pointing to the preferential retention of particulate polyP in low phosphate (Pi) environments raise new questions about its ecophysiological functions. To close these knowledge gaps, two research questions are addressed: Q1: What is the total content and chain length distribution of polyP across different microbial groups and environmental conditions? Q2: How do the production of particulate polyP contribute to microbial P demand and stoichiometry across a broad range in Pi availability? The following hypotheses are tested: H1 (Q1): Functionally and environmentally diverse plankton produce a broad range of polyP chain lengths and concentrations. H2 (Q2): The preferential retention of polyP in low Pi environments can be reconciled with its role as a P storage molecule by a combination of taxonomic and physiological factors. These hypotheses are tested in the laboratory using representative cultures of marine plankton and in the field using observational approaches along natural Pi gradients in the Pacific Ocean and Mediterranean Sea. Applying a new P-targeted method using mass spectrometry, the team is resolving an unprecedented level of detail in marine polyP content and speciation. By combining cell sorting with elemental, biochemical, and radiotracer analyses, the team is gaining a mechanistic understanding of polyP physiology and its cycling in the ocean.

This project is supported by the Biological Oceanography and Chemical Oceanography Programs. 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-2245249

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