Cell abundance, nutrient concentrations, and intracellular phosphorus pools in Synechococcus sp. WH8102 continuous (EFB) culture experiments

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

Contributors	Affiliation	Role
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Abstract
This dataset contains measurements of fluorescence, cell abundance, phosphate concentrations, and intracellular phosphorus (P) pools from continuous culture experiments of Synechococcus sp. WH8102 conducted under controlled laboratory P treatments (High-P, Mid-P, and Low-P). Experiments were performed under steady-state conditions in exponentially fed batch (EFB) cultures (Fischer et al., 2014), with three biological replicates per treatment for four to five consecutive days. Intracellular P partitioning across major macromolecular pools, including ATP, DNA, RNA, free phosphate (Pi), phospholipids, and polyphosphate (polyP). Intracellular P pools were quantified using established biochemical and analytical methods appropriate for each fraction, enabling assessment of cellular P allocation under contrasting nutrient regimes. These data were generated to investigate how P availability constrains microbial physiology, cellular resource allocation, and population dynamics in marine cyanobacteria. The dataset provides a comprehensive view of physiological responses across organizational scales, linking P conditions and growth rates to intracellular P allocation and cell physiology in Synechococcus. Together, these measurements provide a resource for examining P-driven changes in cellular composition and nutrient use strategies in marine microbial systems.

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Coverage

Location: Duhamel Lab, Department of Molecular and Cellular Biology, The University of Arizona (lab experiments)

Methods & Sampling

Synechococcus sp. WH8102 was grown in exponentially fed batch (EFB) continuous cultures under controlled laboratory conditions to maintain near steady-state physiology. Cultures were maintained at 26 °C on a 12:12 h light:dark cycle (250 µE m^{-2} s^{-1}) in SN medium with three phosphorus treatments: High-P (36 µM), Mid-P (5 µM), and Low-P (1 µM). Continuous dilution rates were adjusted (0.35, 0.25, and 0.12 d^{-1}, respectively) to match growth rates and maintain stable biomass.

Samples were collected daily and processed immediately. Cell abundance was measured using a Coulter Counter, and growth rates were calculated from changes in in vivo fluorescence (measured by fluorometry), corrected for dilution. Subsamples were filtered onto 0.2 µm polycarbonate or GF/F filters for intracellular phosphorus analyses and stored at −80 °C or extracted immediately, depending on the assay.

Intracellular phosphorus pools were quantified across major macromolecular fractions, including ATP, DNA, RNA, phospholipids, and polyphosphate, using established biochemical and fluorometric methods. ATP was measured via bioluminescence, nucleic acids via fluorometric assays, polyphosphate via dye-based fluorometry, and phospholipid-associated P following extraction and colorimetric phosphate determination. Dissolved and particulate phosphorus fractions, including soluble reactive phosphorus (SRP), total particulate phosphorus (TPP), and intracellular inorganic phosphate (Pi), were measured using standard colorimetric methods. Intracellular phosphorus pools, including ATP, DNA, RNA, phospholipid-associated phosphorus (P_lipid), free inorganic phosphate (free_Pi), and polyphosphate (PolyP), as well as TPP, are reported as bulk concentrations in the culture (µM), phosphorus quotas per cell (fmol $cell^{-1}$), and phosphorus normalized to cell biovolume (fmol $µm^{-3}$).

PolyP was extracted by enzymatic lysis and quantified fluorometrically using the JC-D7 dye following Yang et al. (Yang et al., 2024), calibrated against a 45-chain-length polyP standard

DNA and RNA were extracted by bead-beating and quantified fluorometrically with PicoGreen and RiboGreen assays following Mouginot et al. (Mouginot et al., 2015)

Intracellular ATP was extracted by boiling in Tris buffer and quantified by firefly bioluminescence following Holm-Hansen and Booth (Holm-Hansen & Booth, 1966), with baseline drift corrected using a custom Python pipeline (available at https://github.com/DuhamelLab/ATP, DuhamelLab. (n.d.)).

Phospholipids were extracted using a biphasic isopropanol/ethyl acetate protocol adapted from Bielawski et al. (Bielawski et al., 2009), and phospholipid-bound P was quantified colorimetrically following Bartlett (Bartlett, 1959).

SRP and TPP were determined by standard phosphomolybdenum blue colorimetry (Grasshoff et al., 1983; Strickland & Parsons, 1972), and free intracellular phosphate was recovered by osmotic shock extraction following Raimbault and Mingazzini (Raimbault & Mingazzini, 1987).

Together, these approaches enabled quantification of cellular phosphorus allocation and nutrient dynamics under controlled phosphorus supply conditions.

Data Processing Description

All statistical analyses were conducted in R (v4.3.3; R Core Team, Vienna, Austria). Data were tested for normality using the Shapiro–Wilk test and for homogeneity of variances using Levene's test. When assumptions of normality and homoscedasticity were met, differences among treatments were assessed using one-way ANOVA followed by Tukey's post hoc tests for pairwise comparisons. When assumptions were violated, non-parametric Kruskal–Wallis tests were applied, followed by pairwise Wilcoxon rank-sum tests with multiple-testing correction. Effect sizes for ANOVA were quantified using eta squared (η^2). Within-treatment variability was assessed using coefficients of variation (CV). A significance threshold of $\alpha = 0.05$ was applied throughout.

Because not all intracellular P pools were quantified at the same sampling events, fractional P allocation was evaluated at the treatment level rather than per replicate. Mean concentrations of each P-containing pool were calculated from all available steady-state replicates within each treatment and summed to construct a treatment-level intracellular P budget. The relative contributions of individual pools were then expressed as fractions of this calculated budget. Statistical analyses were performed on absolute pool sizes, whereas proportional allocations are reported descriptively. Relative treatment responses were quantified by calculating

fold changes for each pool or metric relative to the high-P condition. Fold change was computed as the ratio of the mean value in the low-P or mid-P treatment to the mean value in the high-P treatment.

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 data from P_pools_cell_srp_fluo.xlsx (Sheet1), using row 1 as header, table named p_pools_cell_srp_fluo; applied Excel floating point error adjustment and preserved cell display formatting on load; set "" and "nd" as missing value indicators
- Overwrote biovolume_µm3 column for every row based on treatment group (value was identified for each treatment group, but were not filled out in all cells of the table), filling in a fixed per-treatment culture property: 0.844 for Low-P, 0.845 for Mid-P, and 0.436 for High-P
- Renamed columns in compliance with BCO-DMO parameter naming guidance: SRP_µM to SRP, FLUO_RFU to FLUO, cell_mL to cell, biovolume_µm3 to biovolume
- Exported file as 1005084_v1_p_pools_cell_srp_fluo.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
- Used information from README and methods to expand on parameter definitions

ISSUES POTENTIALLY IMPACTING REUSE

- N/A

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Data Files

File
1005084_v1_p_pools_cell_srp_fluo.csv (Comma Separated Values (.csv), 7.01 KB) MD5:2024fa6b2242eaf035bf00e69d5c71ba Primary data file for dataset ID 1005084, version 1

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Supplemental Files

File
README- Synechococcus cellular P allocation filename: README- Synechococcus cellular P allocation .txt (Plain Text, 2.39 KB) MD5:cc5a8460a98ad0d44b542becaf4e413e README file for Synechococcus cellular P allocation; includes the original file formats that were converted. See BCO-DMO Processing section for all changes to data files.

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Related Publications

Bartlett, G. R. (1959). Phosphorus Assay in Column Chromatography. Journal of Biological Chemistry, 234(3), 466–468. https://doi.org/10.1016/s0021-9258(18)70226-3 https://doi.org/10.1016/S0021-9258(18)70226-3
Methods

Bielawski, J., Pierce, J. S., Snider, J., Rembiesa, B., Szulc, Z. M., & Bielawska, A. (2009). Comprehensive Quantitative Analysis of Bioactive Sphingolipids by High-Performance Liquid Chromatography–Tandem Mass Spectrometry. Lipidomics, 443–467. https://doi.org/10.1007/978-1-60761-322-0_22
Methods

DuhamelLab. (n.d.). ATP [Computer software]. GitHub. https://github.com/DuhamelLab/ATP
Methods

Fischer, R., Andersen, T., Hillebrand, H., & Ptacnik, R. (2014). The exponentially fed batch culture as a reliable alternative to conventional chemostats. Limnology and Oceanography: Methods, 12(7), 432–440. Portico. https://doi.org/10.4319/lom.2014.12.432
Methods

Grasshoff, K., Kremling, K., and Ehrhardt, M. (1983). Methods of Seawater Analysis. Verlag Chemia, Florida
Methods

Holm-Hansen, O., & Booth, C. R. (1966). THE MEASUREMENT OF ADENOSINE TRIPHOSPHATE IN THE OCEAN AND ITS ECOLOGICAL SIGNIFICANCE1. Limnology and Oceanography, 11(4), 510–519. Portico. https://doi.org/10.4319/lo.1966.11.4.0510
Methods

Mouginot, C., Zimmerman, A. E., Bonachela, J. A., Fredricks, H., Allison, S. D., Van Mooy, B. A. S., & Martiny, A. C. (2015). Resource allocation by the marine cyanobacterium *Siynechococcus* WH8102 in response to different nutrient supply ratios. Limnology and Oceanography, 60(5), 1634–1641. Portico. https://doi.org/10.1002/lno.10123
Methods

Raimbault, P., & Mingazzini, M. (1987). Diurnal variations of intracellular nitrate storage by marine diatoms: effects of nutritional state. Journal of Experimental Marine Biology and Ecology, 112(3), 217–232. https://doi.org/10.1016/0022-0981(87)90070-0
Methods

Strickland, J. D. H. and Parsons, T. R. (1972). A Practical Hand Book of Seawater Analysis. Fisheries Research Board of Canada Bulletin 157, 2nd Edition, 310 p.
Methods

Yang, X., Gao, R., Zhang, Q., Yung, C. C. M., Yin, H., & Li, J. (2024). Quantification of Polyphosphate in Environmental Planktonic Samples Using a Novel Fluorescence Dye JC-D7. Environmental Science &Amp; Technology, 58(32), 14249–14259. https://doi.org/10.1021/acs.est.4c04545
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., Segura-Noguera, M., Diaz, J. (2026) **Single-cell elemental quotas and stoichiometry of Synechococcus sp. WH8102 measured by X-ray microanalysis (XRMA) under varying phosphorus conditions.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2026-08-18 doi:10.26008/1912/bco-dmo.1005186.1 [view at BCO-DMO]
Relationship Description: Datasets were part of same experiment.

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Parameters

Parameter	Description	Units
treatment	Experimental Phosphorus supply treatment: Low-P, Mid-P, or High-P.	categorical (none)
day	Sampling day (time-point) of the experiment	days
bottle	Biological replicate/sample container ID (a, b, c)	categorical (none)
FLUO	Fluorescence signal (proxy for pigment or cell activity depending on assay)	RFU (relative fluorescence units)
cell	Cell abundance concentration in the culture	cells per mL (cells / mL)
SRP	Soluble reactive phosphorus (SRP) concentration in the culture	µM (micromoles per liter)
biovolume	Average cellular biovolume	µm3 per cell
growth_rates_1_day	Specific growth rate over a one-day interval, calculated from changes in in vivo fluorescence and corrected for culture dilution	per day
ATP_value_uM	ATP-associated phosphorus concentration in the culture	µM (micromoles per liter)
ATP_value_fmol_um3	ATP-associated phosphorus normalized to cell biovolume	fmol μm^{-3}
ATP_value_fmol_cell	ATP-associated phosphorus quotas per cell	fmol $cell^{-1}$
DNA_value_uM	DNA-associated phosphorus concentration in the culture	µM (micromoles per liter)
DNA_value_fmol_um3	DNA-associated phosphorus quota per cell	fmol P μm^{-3}
DNA_value_fmol_cell	DNA-associated phosphorus normalized to cell biovolume	fmol P $cell^{-1}$
RNA_value_uM	RNA-associated phosphorus concentration in the culture	µM (micromoles per liter)
RNA_value_fmol_um3	RNA-associated phosphorus quota per cell	fmol P μm^{-3}
RNA_value_fmol_cell	RNA-associated phosphorus normalized to cell biovolume	fmol P $cell^{-1}$

P_lipid_value_uM	Phospholipid-associated phosphorus concentration in the culture	µM (micromoles per liter)
P_lipid_value_fmol_um3	Phospholipid-associated phosphorus quota per cell	fmol P μm^{-3}
P_lipid_value_fmol_cell	Phospholipid-associated phosphorus normalized to cell biovolume	fmol P $cell^{-1}$
free_Pi_value_uM	Free inorganic phosphate (Pi) concentration in the intracellular phosphorus pool, expressed as a bulk culture concentration	µM (micromoles per liter)
free_Pi_value_fmol_um3	Free inorganic phosphate (Pi) quota per cell	fmol P μm^{-3}
free_Pi_value_fmol_cell	Free inorganic phosphate (Pi) normalized to cell biovolume	fmol P $cell^{-1}$
PolyP_value_uM	Polyphosphate-associated phosphorus concentration in the culture	µM (micromoles per liter)
PolyP_value_fmol_um3	Polyphosphate-associated phosphorus quota per cell	fmol P μm^{-3}
PolyP_value_fmol_cell	Polyphosphate-associated phosphorus normalized to cell biovolume	fmol P $cell^{-1}$
TPP_value_uM	Total particulate phosphorus (TPP) concentration in the culture	µM (micromoles per liter)
TPP_value_fmol_um3	Total particulate phosphorus (TPP) quota per cell	fmol P μm^{-3}
TPP_value_fmol_cell	Total particulate phosphorus (TPP) normalized to cell biovolume	fmol P $cell^{-1}$

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Instruments

Dataset-specific Instrument Name	Coulter Counter Multisizer 4e (Beckman Coulter, Indianapolis, USA)
Generic Instrument Name	Coulter Counter
Dataset-specific Description	Cell abundance was measured using a Coulter Counter, and growth rates were calculated from changes in in vivo fluorescence (measured by fluorometry), corrected for dilution.
Generic Instrument Description	An apparatus for counting and sizing particles suspended in electrolytes. It is used for cells, bacteria, prokaryotic cells and virus particles. A typical Coulter counter has one or more microchannels that separate two chambers containing electrolyte solutions. from https://en.wikipedia.org/wiki/Coulter_counter

Dataset-specific Instrument Name	Fluorometer (AquaFluor, Turner Designs)
Generic Instrument Name	Fluorometer
Dataset-specific Description	Cell abundance was measured using a Coulter Counter, and growth rates were calculated from changes in in vivo fluorescence (measured by fluorometry), corrected for dilution.
Generic Instrument Description	A fluorometer or fluorimeter is a device used to measure parameters of fluorescence: its intensity and wavelength distribution of emission spectrum after excitation by a certain spectrum of light. The instrument is designed to measure the amount of stimulated electromagnetic radiation produced by pulses of electromagnetic radiation emitted into a water sample or in situ.

Dataset-specific Instrument Name	Luminometer (Berthold LUMAT LB 9510 l)
Generic Instrument Name	Luminometer
Dataset-specific Description	ATP was measured via bioluminescence, nucleic acids via fluorometric assays, polyphosphate via dye-based fluorometry, and phospholipid-associated P following extraction and colorimetric phosphate determination.
Generic Instrument Description	A luminometer is an instrument that measures light and other optical properties of specimens in chemiluminescent and bioluminescent applications.

Dataset-specific Instrument Name	Spectrophotometer (SpectraMax iD5, Molecular Devices, San Jose, USA)
Generic Instrument Name	Spectrophotometer
Dataset-specific Description	Dissolved and particulate phosphorus fractions, including soluble reactive phosphorus (SRP), total particulate phosphorus (TPP), and intracellular inorganic phosphate (Pi), were measured using standard colorimetric methods.
Generic Instrument Description	An instrument used to measure the relative absorption of electromagnetic radiation of different wavelengths in the near infra-red, visible and ultraviolet wavebands by samples.

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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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