Synechococcus batch culture data (cell quotas and ratios (C,N,P), size, and diameter) from laboratory experiments in 2021 to 2022 with related isolates cultured across a range of temperatures (16-25C)

Website: https://www.bco-dmo.org/dataset/926311
Data Type: experimental
Version: 1
Version Date: 2024-04-30

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

Contributors	Affiliation	Role
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Abstract
Diverse phytoplankton modulate the coupling between the ocean carbon and nutrient cycles through life-history traits such as cell size, elemental quotas, and ratios. Biodiversity is mostly considered at broad functional levels, but major phytoplankton lineages are themselves highly diverse. As an example, Synechococcus is found in nearly all ocean regions, and we demonstrate contains extensive intraspecific variation. Here, we grew four closely related Synechococcus isolates in serially transferred cultures across a range of temperatures (16-25°C) to quantify for the relative role of intraspecific trait variation vs. environmental change. We collected data at the time of sampling, after cultures grew for seven doublings or one month. Experiments were conducted from September of 2021 to early 2022. This dataset includes cell quotas (fmol) for carbon (QC), nitrogen (QN), and phosphorus (QP). It also includes N:P, C:N, and C:P stoichiometry, cell size, and cell diameter (µm) for each Synechococcus strain and clade under each thermal condition.

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Coverage

Spatial Extent: **N**:48.72 **E**:3.55 **S**:31.91 **W**:-124.17
Temporal Extent: 2021 - 2022

Dataset Description

These data were published in the results publication Harcourt R, Garcia NS, Martiny AC (2024). The data table for this dataset was derived from table S8 in Harcourt R, Garcia NS, Martiny AC (2024), with additional culture information (sample date, lat, lon, site location) from the Roscoff Culture Collection (RCC), Station Biologique de Roscoff. See Harcourt R, Garcia NS, Martiny AC (2024) for additional related supplemental figures and tables.

Methods & Sampling

We incubated serially transferred cultures of *Synechococcus* (CC9311, BL107, ROS8604, CC9902, representing two clades) in triplicate 1 L flasks at 16°C, 18°C, 20°C, 22°C, 25°C, and 27°C. We supplied ambient light (60 µmol quanta m^{-2} s^{-1}) using white fluorescent lamps on a 12:12 light-dark cycle. We grew cultures in modified artificial seawater media. To ascertain that we did not impose growth rates upon cultures and the observed growth rates are a product of strain and environmental conditions, we used nutrient replete media and sampled prior to cultures encountering nutrient limitation; we supplied nitrate (NO_3^-) and phosphate (PO_4^{3-}) in concentrations of 125 µM and 10 µM, respectively. Cells utilized a mean of 17% of the supplied nitrogen, and 22% of the supplied phosphorus, indicating cultures did not reach nutrient limitation. We transferred media and diluted cultures using an open flame in a hood in order to avoid contamination. We diluted weekly by approximately an order of magnitude to avoid nutrient limitation and maintain a stable growth rate**.**

We measured particulate organic carbon (POC), particulate organic nitrogen (PON), and particulate organic phosphorus (POP), as well as cell enumeration by flow cytometry, following the methods outlined in Garcia et al. (2016). We sampled after seven doublings or one month to acclimate cells to the temperature conditions. We vacuum filtered POC, PON (150 mL), and POP (50 mL) samples onto pre-combusted GF/F Whatman glass filters (450°C) at 10 psi. We dried particulate organic carbon and particulate organic nitrogen samples at 50-80°C for a minimum of 48 hours and pelletized prior to analysis using a Flash EA 1112 NC Soil Analyzer (Thermo-Scientific, MA). We rinsed filtered particulate organic phosphorus samples with 0.17 M $NaSO_4$, immersed the filter in 2 mL of $MgSO_4$, dried at 80°C overnight, and combusted at 450°C for 2 hours. We then added 5 mL of 0.2 M HCl and baked the samples at 80-90°C. We measured particulate organic phosphorus samples via colorimetric assay following the Bermuda Atlantic Time-series methodology using a Genesys 10S UV-vis spectrophotometer (Thermo-Scientific) at 885 nm.

We measured culture cell density every two-three days and immediately prior to sampling using a NovoCyte 1000 flow cytometer (excitation laser 488 nm, emission peak 575 nm) and forward scatter. We assessed growth rate using flow cytometry based on increases in biomass measured across time points using the following equation: $\mu = \ln(CD2)-\ln(CD1))/(T2-T1)$, in which *CD2* and *CD1* are cell counts in cells/mL^{-1} on the most recent count and the previous count, respectively, and *T2* and *T1* represent time points. We accounted for recent dilutions in accounting for growth rate; we counted cells prior to and after dilutions to ensure growth rate was accurately assessed. We counted the cells at a flow rate of 35 µL/min. To assess heterotrophic populations, we stained the cultures with SYBR Green (Thermo-Fisher) for 15 minutes at room temperature, vortexed them, and counted using the (excitation laser 488 nm, emission peak 520 nm) channel. We recorded duplicate cell counts at each sampling.

We measured cell diameter by microscopy under oil immersion at 1000x magnification using the Axioplan2 and AxioView 1.4.5 sizing software (Carl Zeiss, Goettingen, Germany) with reference to a staged micrometer (Ted Pella Inc., Redding, CA). To estimate cell diameter, we created a conversion factor determined by plotting the mean observed cell diameters and mean forward scatter values for several strains of *Synechococcus*.

Locations: Laboratory batch cultures in incubators from 16-25°C. The original isolation locations for the Synechococcus strains used were:
31.91, -124.17, Pacific Ocean, California Current
32.87, -117.26, Pacific Ocean, California Current
41.72, 3.55, Mediterranean Sea, Balearic Sea
48.72, -3.98, Atlantic Ocean, Britanny coast

Organism Identifier (LifeSciences Identifier, LSID):
Synechococcus, urn:lsid:marinespecies.org:taxname:160572

Data Processing Description

We used the software NovoExpress 1.5.6 to count cells. We used NovoExpress 1.5.6 and AxioView 1.4.5 to determine cell size.

BCO-DMO Processing Description

* File "S8_TableRevised.csv" was imported into the BCO-DMO data system for this dataset. Values "N/A" 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]

* Links to strain information in the Roscoff Culture Collection (RCC) that were included in a "Reference" column in the data table were corrected by obtaining the correct links in the corresponding results journal publication Harcourt, R., Garcia, N. S., & Martiny, A. C. (2024).

* Additional isolation sample collection information was added to the data table from the RCC collection metadata (sample date, lat, lon, additional site location descriptions, and identifier for the strain in the RCC collection).

* Date converted to ISO 8601 format

* "m" removed from depth values and instead included in column description metadata, depth column was then typed as numeric type.

* LSID added for Synecococcus in metadata text (from World Register of Marine Species)

Problem Description

Some replicates were lost as a consequence of a malfunction of the EA Analyazer.

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Data Files

File	
926311_v1_syn-batch-cultures.csv	(Comma Separated Values (.csv), 15.62 KB) MD5:0dea9ff74bffe353e442495b23312ac3
Primary data file for dataset ID 926311, version 1	

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Related Publications

Agilent Technologies, Inc. (n.d.) Flow Cytometry Software: NovoExpress version 1.5.6. Available from https://www.agilent.com/en/product/research-flow-cytometry/flow-cytometry-software/novocyte-novoexpress-software-1320805
Software

Carl Zeiss (n.d.) AxioView 1.4.5 Software. https://www.micro-shop.zeiss.com/en/us/softwarefinder/
Software

Garcia, N. S., Sexton, J., Riggins, T., Brown, J., Lomas, M. W., & Martiny, A. C. (2018). High Variability in Cellular Stoichiometry of Carbon, Nitrogen, and Phosphorus Within Classes of Marine Eukaryotic Phytoplankton Under

Sufficient Nutrient Conditions. Frontiers in Microbiology, 9. https://doi.org/10.3389/fmicb.2018.00543
Methods

Harcourt, R., Garcia, N. S., & Martiny, A. C. (2024). Intraspecific trait variation modulates the temperature effect on elemental quotas and stoichiometry in marine Synechococcus. PLOS ONE, 19(3), e0292337. https://doi.org/10.1371/journal.pone.0292337
Results

Knap, A.H., Michaels, A.F., Steinberg, D.K., Bahr, F., Bates, N.R., Bell, S., Countway, P., Close, A.R., Doyle, A.P., Dow, R.L., Howse, F.A., Gundersen, K., Johnson, R.J., Kelly, R., Little, R., Orcutt, K., Parsons, R., Rathburn, C., Sanderson, M. and Stone, S. (1997) BATS Methods Manual, Version 4 Woods Hole, MA, US. U.S. JGOFS Planning Office 136pp. http://eprints.soton.ac.uk/id/eprint/361194
Methods

Station Biologique de Roscoff (1998). RCC32 (Strain ROS8604) Synechococcus_sp. Roscoff Culture Collection: Marine Microalgae, Macroalgae, Protists, Bacteria and Viruses. Accessed 7 Apr 2023. https://roscoff-culture-collection.org/rcc-strain-details/32
IsSupplementedBy

Station Biologique de Roscoff (2002). RCC515 (Strain BL107) Synechococcus_sp. Roscoff Culture Collection: Marine Microalgae, Macroalgae, Protists, Bacteria and Viruses. Accessed 7 Apr 2023. https://roscoff-culture-collection.org/rcc-strain-details/515
IsSupplementedBy

Station Biologique de Roscoff (2007). RCC1086 (Strain CC9311) Synechococcus_sp. Roscoff Culture Collection: Marine Microalgae, Macroalgae, Protists, Bacteria and Viruses. Accessed 7 Apr 2023. https://roscoff-culture-collection.org/rcc-strain-details/1086
IsSupplementedBy

Station Biologique de Roscoff (2012). RCC2673 (Strain CC9902) Synechococcus_sp. Roscoff Culture Collection: Marine Microalgae, Macroalgae, Protists, Bacteria and Viruses. Accessed 7 Apr 2023. https://roscoff-culture-collection.org/rcc-strain-details/2673
IsSupplementedBy

Strickland, Parsons, Michaels, A., Dow, R., Elardo, K., and Bates, N. (1997) BATS Methods Manual. Version 4. Chapter 21. The Determination of Phosphorus in Sea Water. Chapter available on April 30th, 2024 from https://bats.bios.asu.edu/wp-content/uploads/2017/07/chapter11.pdf
https://eprints.soton.ac.uk/361194/#chapter11
Methods

Strickland, Parsons, Michaels, A., Dow, R., Howse, F., and Bates, N. (1997) BATS Methods Manual. Version 4. Chapter 10. The Determination of Nitrite in Sea Water. Chapter available on April 30th, 2024 from https://bats.bios.asu.edu/wp-content/uploads/2017/07/chapter10.pdf
https://eprints.soton.ac.uk/361194/#chapter10
Methods

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Related Datasets

IsSupplementedBy

Harcourt, R., Garcia, N. S., & Martiny, A. C. (2024). Table 1. Synechococcus strain information. https://doi.org/10.1371/journal.pone.0292337.t001

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Parameters

Parameter	Description	Units
Rep	biological replicate.	unitless
Temp	Temperature.	degrees Celsius (degC)
Strain	Strain of Synechococcus.	unitless
Clade	Clade of Synechococcus	unitless
GR	Growth rate (μ) per day	Growth rate per day (d-1)
QN	Cell nitrogen quota	femtomoles (fmol)
QP	Cell phosphorus quota	femtomoles (fmol)
QC	Cell carbon quota	femtomoles (fmol)
FSCH	Forward scatter height (cell size proxy).	unitless
CN	Carbon:nitrogen ratio.	unitless
NP	Nitrogen:phosphorus ratio	unitless
CP	Carbon:phosphorus ratio	unitless
Cell_Diameter_um	Cell diameter	micrometers (um)
Grams_N	Grams of nitrogen	grams (g)
Grams_P	Grams of phosphorus	grams (g)
Grams_C	Grams of carbon	grams (g)
isolation_sample_date	Sample date of strain isolation source.	unitless
isolation_lat	Latitude of strain isolation source.	decimal degrees
isolation_lon	Longitude of strain isolation source.	decimal degrees
isolation_depth	Depth of strain isolation source.	meters (m)
isolation_location	Location description (Sea or Ocean) for the strain isolation source.	unitless
isolation_site	Site description for stain isolation source.	unitless
isolation_ecosystem	Ecosystem (coastal, pelagic) of strain isolation source.	unitless
RCC_Number	Identifier in the Roscoff Culture Collection (RCC) for the strain.	unitless
RCC_Link	Link to the Roscoff Culture Collection (RCC) page for the strain.	unitless

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Instruments

Dataset-specific Instrument Name	Flash EA 1112 NC Soil Analyzer (Thermo-Scientific, MA)
Generic Instrument Name	Elemental Analyzer
Generic Instrument Description	Instruments that quantify carbon, nitrogen and sometimes other elements by combusting the sample at very high temperature and assaying the resulting gaseous oxides. Usually used for samples including organic material.

Dataset-specific Instrument Name	NovoCyte 1000 (Agilent, Santa Clara, CA)
Generic Instrument Name	Flow Cytometer
Generic Instrument Description	Flow cytometers (FC or FCM) are automated instruments that quantitate properties of single cells, one cell at a time. They can measure cell size, cell granularity, the amounts of cell components such as total DNA, newly synthesized DNA, gene expression as the amount messenger RNA for a particular gene, amounts of specific surface receptors, amounts of intracellular proteins, or transient signalling events in living cells. (from: http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm)

Dataset-specific Instrument Name	Axioplan2 microscope (Carl Zeiss, Goettingen, Germany)
Generic Instrument Name	Microscope - Optical
Dataset-specific Description	Axioplan2 microscope (Carl Zeiss, Goettingen, Germany) and Stage micrometer (Ted Pella Inc., Redding, CA)
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of reflection and absorption of visible light. Includes conventional and inverted instruments. Also called a "light microscope".

Dataset-specific Instrument Name	Genesys 10S UV-vis spectrophotometer (Thermo-Scientific)
Generic Instrument Name	Spectrophotometer
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: 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 CO_2 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-2134950
NSF Division of Ocean Sciences (NSF OCE)	OCE-2135035

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