Physiology data from Crocosphaera iron and phosphorus colimitation culture experiment

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Project
» Collaborative Research: Iron and phosphorus balanced limitation of nitrogen fixation in the oligotrophic ocean (TriCoLim)

Contributors	Affiliation	Role
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Abstract
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Methods & Sampling

Culturing methods: Triplicate cultures of Crocosphaera watsonii strain WH0005 were grown under low iron (Fe, 5 nM) and low phosphorus (P, 2 uM) conditions for 3 months at 27C to acclimate to simultaneously low concentrations of both nutrients. Fe and P were then “added back” at different concentrations to create nutrient treatments to assess the physiological effects of Fe and P co-limitation. The treatments were: Fe/P Replete (Replete, 250 nM Fe and 10 uM P), Fe-limited (Felim, 3 nM and 10 uM P), P-limited (Plim, 250 nM Fe and 0.15 uM P), Fe/P Co-limited (Colim, 3 nM Fe and 0.15 uM P). Phosphate was passed through an activated Chelex 100 resin column (BioRad Laboratories, Hercules, CA, USA) to remove contaminating iron. Aquil concentrations of vitamins and a modified trace metals mix were also added (1.21 x 10-7 M Mn, 7.97 x 10-8 M Zn, 1.00 x 10-7 M Mo, and 5.03 x 10-8 M Co).

Cultures were maintained under semi-continuous culturing conditions on a 12:12 light:dark cycle in temperature-controlled incubators at 150 µmol photons m^-2 s^-1 and diluted every three days to maintain steady-state exponential growth. Cultures were grown in microwave-sterilized media made with 0.2 micron-filtered Aquil-base artificial seawater (Sunda et al., 2005) for ~1 month and then grown for ~2 weeks (3-8 generations) in media made with Aquil artificial seawater passed through an activated Chelex 100 resin column to remove contaminating iron. All media was buffered with 25 uM EDTA. The limiting nutrient was directly added to the culture bottles during periodic dilutions.

Dilutions were conducted based on in-vivo fluorescence measured in real-time on a 10AU Fluorometer (Turner Designs, San Jose, CA), and growth rates were later validated with 1%, 0.2-µm filtered glutaraldehyde preserved cell samples.

Growth Rates & Cell size: The specific growth rate (µ) was then calculated using the equation µ = (ln N1 – ln N0) / t, where N refers to cell densities and t is time in days. The cell size was determined by measuring the

cell diameters of at least 20 cells per sample using the CaptaVision Imaging Software (Commack, NY, USA).

Chlorophyll A: Chlorophyll concentrations were measured by filtering 30 mL of culture GFF filters (Whatman, Grade GF/F), extracted overnight at -20°C in 90% acetone (Welschmeyer 1994), and measured on a Trilogy fluorometer (Turner Designs).

Nitrogen fixation rates: Nitrogen-fixation rates were measured using the acetylene reduction assay during the night-time following previously described methods (Garcia et al., 2013). Briefly, 40 mL culture sub-samples were collected from the triplicate experimental cultures and 6 mL of acetylene was injected into 35 mL of headspace at the start of the dark period. All-night (~12 hours) accumulation of acetylene was measured at the end of the incubation period on a gas chromatograph GC-8a (Shimadzu Scientific Instruments, Columbia, Maryland), and the measured ethylene was converted to fixed nitrogen using a ratio of 3:1 and a Bunsen coefficient of 0.086. Converted nitrogen-fixation rates were then normalized to particulate organic nitrogen (N-specific rates) and cell counts (cell-specific rates).

Carbon fixation rates: To approximate net primary productivity, 10 ml sub-cultures from each experimental replicate were incubated for 3-5 hours with H14CO3 under the same experimental growth conditions (e.g. light, temperature, etc.). Samples were then filtered onto glass microfiber filters (GF/F) and stored in the dark overnight before analysis using a Wallac System 1400 liquid scintillation counter (Jiang et al., 2018). Carbon fixation rates were then normalized to particulate organic C (C-specific rates) and cell counts (cell-specific rates).

Elemental stoichiometry analysis: Particulate organic carbon and nitrogen (POC and PON) samples were filtered on pre-combusted glass microfiber filters (Whatman, Grade GF/F), dried in an oven at ~60C, and then pelleted and analyzed on a 4010 Costech Elemental Analyzer calibrated with methionine and acetanilide (Jiang et al., 2018). Particulate organic phosphorus (POP) samples were filtered on pre-combusted GF/F, dried, and analyzed following Fu et al., (2005). Briefly, the dried POP samples were combusted for 2 hours at 500C to convert organic phosphorus to inorganic orthophosphate, which was then measured spectrophotometrically.

Phosphorus Use Efficiency: Nitrogen-specific Phosphorus Use Efficiencies (NPUEs) were calculated by normalizing measured nitrogen-fixation rates to POP content (mol N fixed hr^-1 mol cellular P^-1). Similarly, Carbon-specific Phosphorus Use Efficiencies (CPUEs) were calculated by normalizing measured carbon fixation (C-fixation) rates to intracellular phosphorus (Jiang et al., 2018).

Data Processing Description

BCO-DMO Processing Notes:

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Related Publications

Fu, F.-X., Zhang, Y., Bell, P. R. F., & Hutchins, D. A. (2005). PHOSPHATE UPTAKE AND GROWTH KINETICS OFTRICHODESMIUM(CYANOBACTERIA) ISOLATES FROM THE NORTH ATLANTIC OCEAN AND THE GREAT BARRIER REEF, AUSTRALIA. Journal of Phycology, 41(1), 62–73. doi:10.1111/j.1529-8817.2005.04063.x
Results

Garcia, N. S., Fu, F.-X., Breene, C. L., Yu, E. K., Bernhardt, P. W., Mulholland, M. R., & Hutchins, D. A. (2013). Combined effects of CO2 and light on large and small isolates of the unicellular N2-fixing cyanobacterium Crocosphaera watsonii from the western tropical Atlantic Ocean. European Journal of Phycology, 48(1), 128–139. doi:10.1080/09670262.2013.773383
Methods

Jiang, H.-B., Fu, F.-X., Rivero-Calle, S., Levine, N. M., Sañudo-Wilhelmy, S. A., Qu, P.-P., … Hutchins, D. A. (2018). Ocean warming alleviates iron limitation of marine nitrogen fixation. Nature Climate Change, 8(8), 709–712. doi:10.1038/s41558-018-0216-8
Methods

Sunda, W. , N. Price, and François MM Morel. 2005. “Trace Metal Ion Buffers And Their Use In Culture Studies”. In Algal Culturing Techniques, 35-63. Algal Culturing Techniques. Burlington, MA: Academic Press.

Methods

Welschmeyer, N. A. (1994). Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and pheopigments. Limnology and Oceanography, 39(8), 1985–1992. doi:10.4319/lo.1994.39.8.1985
Methods

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Related Datasets

IsRelatedTo

Hutchins, D. A., Fu, F. (2021) **Particulate organic phosphorus data from TriCoLim incubation experiments done aboard R/V Atlantis AT39-05 in the Tropical Atlantic.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2021-07-13 http://lod.bco-dmo.org/id/dataset/855661 [view at BCO-DMO]
Relationship Description: Part of same experiment.

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Parameters

Parameters for this dataset have not yet been identified

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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.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1657757

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