Physiological measurements during Cyanobacteria Crocosphaera iron/warming experiments

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Project
» Collaborative Research: Evolutionary, biochemical and biogeochemical responses of marine cyanobacteria to warming and iron limitation interactions (Cyanobacteria Warming Responses)

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Abstract
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Dataset Description

The data describes Figures 1 and 2 and Supplmentary Figures of the results paper (Yang et al., 2021).

Methods & Sampling

Culturing Methods: Triplicate cultures of *Crocosphaera watsonii* strain WH0005 were grown at five ecologically-relevant temperatures spanning the thermal range of *Crocosphaera*: 20C, 22C, 27C, 32C, and 36C. Cultures were maintained under two Fe conditions, Fe-replete and Fe-limited, in microwave-sterilized medium made with 0.2 micron-filtered surface seawater collected from the Sargasso Sea using a trace metal clean towfish system. The medium was amended with Aquil concentrations of phosphate (10 µM) passed through an activated Chelex 100 resin column (BioRad Laboratories, Hercules, CA, USA) to remove contaminating Fe, and with vitamins and a modified Aquil trace metals stock (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) (Sunda et al. 2005). Fe-replete medium was amended with 250 nM Fe, while 5 nM was directly added to Fe-limited cultures during periodic dilutions. The media was buffered with 25 µM EDTA, and the resulting average concentration of dissolved free inorganic iron, which is the form most bioavailable to phytoplankton, was calculated for the different experimental conditions following Jabre and Bertrand (2020) (see Supplementary Methods, Table S1, Figure S1). The total iron concentrations include the added Fe-EDTA, and a measured background Fe concentration in the Sargasso seawater of 0.54 nM.

Cultures were maintained semi-continuously in 2.5 L polycarbonate bottles on a 12:12 light:dark cycle in temperature-controlled incubators at 150 µmol photons m-2 s-1, and diluted every three days with media adjusted to the experimental temperature to maintain steady-state exponential growth for at least 2 months. All bottles used in the study were soaked in a 1% Citranox detergent for 24 hours, rinsed in Milli-Q (18.2 Ω)

water, and then soaked in 10% HCl for a week, rinsed in Milli-Q and microwave-sterilized before use. 0.2 micron filter-sterilized nutrients, trace metals, and vitamins were amended to the natural seawater base using sterile pipette tips rinsed three times with 1% HCl and three times with microwave sterilized Milli-Q water immediately prior to use.

Dilutions were conducted based on *in-vivo* fluorescence measured in real-time on a 10AU Fluorometer (Turner Designs, San Jose, CA). Cell samples were preserved in 0.5% 0.2-µm filtered glutaraldehyde to validate *in-vivo* growth rates using on an Olympus BX51 epifluorescence microscope. 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).

Nitrogen Fixation Rates: N2-fixation rates were measured using the acetylene reduction assay following previously described methods (Garcia et al. 2013). Briefly, duplicate 40 mL culture 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 in 75 mL sealed-top bottles. 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 N2 using a ratio of 3:1 and a Bunsen coefficient of 0.086. Converted N2-fixation rates were then normalized to particulate organic nitrogen (N-specific N2-fixation rates).

Carbon Fixation Rates: To approximate net primary productivity (C-fixation), 10 mL sub-cultures from each experimental replicate were incubated for 5 hours with H14CO3 beginning 2 hours after the start of the light period 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). Calculated rates were then normalized to particulate organic carbon (C-specific C-fixation rates).

Elemental Stoichiometry: To measure particulate organic carbon and nitrogen (POC and PON), 30-40 mL of culture from each experimental treatment was filtered onto 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).

Intracellular Iron Content: Intracellular Fe samples were obtained by filtering cultures onto acid-washed 0.2µm Supor polyethersulfone filters (Pall Laboratory) and rinsed with oxalate reagent to remove extracellular trace metals (Tovar-Sanchez et al. 2003). All filtration and sample processing steps were conducted in a class 100 trace metal clean environment (Cunningham and John 2017). Filters were then digested with 5 mL of 50% nitric acid (HNO3) amended with 10 ppb Indium as an internal standard at 95C for 5 days in individual, 30 mL perfluoroalkoxy vials (Savillex). Following acid-digestion, the filters were removed with plastic tweezers, and samples were dried overnight at 100ºC. Samples were resolubilized in 200 µL of 1:1 concentrated HNO3 and hydrochloric acid (HCl), sealed, and heated for ~2-3 hours and then allowed to cool. The sample was dried and resuspended in 5 mL of 0.1M distilled HNO3 and then analyzed by inductively coupled plasma mass spectrometry (ICP-MS, Element 2, Thermo). Intensities of 56Fe were calibrated with a 0.1-300 ppb metal reference standard curve. 115In was monitored as an internal standard to correct for matrix suppression and any sample loss during digestion. Two procedural blank filters for each treatment were also analyzed and subtracted from the measured sample values.

Fe Quotas and Resource Use Efficiencies: Fe quotas (mol Fe / µmol POC) were calculated using Fe concentrations measured via ICP-MS and POC (see above). Nitrogen-specific Iron Use Efficiencies (N-IUEs) were calculated by normalizing measured N2-fixation rates to intracellular Fe content (mol N fixed hr-1 mol cellular Fe-1) (Kustka et al. 2003). Similarly, Carbon-specific Iron Use Efficiencies (C-IUEs, mol C fixed hr-1 mol cellular Fe-1) were calculated by normalizing measured C-fixation rates to intracellular iron.

Data Processing Description

Data processing has been done using Excel.

BCO-DMO processing notes:

- Renamed parameter names to comply with database requirements

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Related Publications

Yang, N., Merkel, C. A., Lin, Y.-A., Levine, N. M., Hawco, N. J., Jiang, H.-B., ... Hutchins, D. A. (2021). Warming Iron-Limited Oceans Enhance Nitrogen Fixation and Drive Biogeographic Specialization of the Globally Important Cyanobacterium Crocosphaera. Frontiers in Marine Science, 8. doi:10.3389/fmars.2021.628363
Results

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Parameters

Parameter	Description	Units
Fe_Treatment	Experimental Iron concentrations for Replete and Limited cultures	nanomolar
Temperature	Experimental temperatures for culture experiments	Degrees Celsius (°C)
Growth_Rate	Average specific growth rate calculated from cell counts.	d^-1
Particulate_Organic_Carbon	Average particulate organic carbon.	micromolar (uM)
Particulate_Organic_Nitrogen	Average particulate organic nitrogen.	micromolar (uM)
Particulate_Organic_Phosphorus	Average particulate organic phosphorus.	micromolar (uM)
Intracellular_Fe	Average of intracellular iron.	micromolar (uM)
Nspecific_N2_fixation	Average PON-specific nitrogen fixation rates, nd = no data for that replicate.	hr^-1
Cspecific_CO2_fixation	Average POC-specific carbon fixation rates.	hr^-1
Carbon_Iron_Use_Efficiency	CIUE, average carbon-specific iron use efficiency.	mol C hr^-1 per mol Fe^-1
Nitrogen_Iron_Use_Efficiency	NIUE, average nitrogen-specific iron use efficiency, nd = no data for that replicate.	mol N hr^-1 per mol Fe^-1

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Instruments

Dataset-specific Instrument Name	4010 Costech Elemental Analyzer
Generic Instrument Name	Elemental Analyzer
Dataset-specific Description	4010 Costech 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	Olympus BX51 epifluorescence microscope
Generic Instrument Name	Fluorescence Microscope
Dataset-specific Description	Olympus BX51 epifluorescence microscope
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of fluorescence and phosphorescence instead of, or in addition to, reflection and absorption of visible light. Includes conventional and inverted instruments.

Dataset-specific Instrument Name	Gas chromatograph GC-8a (Shimadzu Scientific Instruments, Columbia, Maryland),
Generic Instrument Name	Gas Chromatograph
Dataset-specific Description	Gas chromatograph GC-8a (Shimadzu Scientific Instruments, Columbia, Maryland),
Generic Instrument Description	Instrument separating gases, volatile substances, or substances dissolved in a volatile solvent by transporting an inert gas through a column packed with a sorbent to a detector for assay. (from SeaDataNet, BODC)

Dataset-specific Instrument Name	Inductively coupled plasma mass spectrometry (ICP-MS, Element 2, Thermo)
Generic Instrument Name	Inductively Coupled Plasma Mass Spectrometer
Dataset-specific Description	Inductively coupled plasma mass spectrometry (ICP-MS, Element 2, Thermo)
Generic Instrument Description	An ICP Mass Spec is an instrument that passes nebulized samples into an inductively-coupled gas plasma (8-10000 K) where they are atomized and ionized. Ions of specific mass-to-charge ratios are quantified in a quadrupole mass spectrometer.

Dataset-specific Instrument Name	Wallac System 1400 liquid scintillation counter
Generic Instrument Name	Liquid Scintillation Counter
Dataset-specific Description	Wallac System 1400 liquid scintillation counter
Generic Instrument Description	Liquid scintillation counting is an analytical technique which is defined by the incorporation of the radiolabeled analyte into uniform distribution with a liquid chemical medium capable of converting the kinetic energy of nuclear emissions into light energy. Although the liquid scintillation counter is a sophisticated laboratory counting system used the quantify the activity of particulate emitting (ß and a) radioactive samples, it can also detect the auger electrons emitted from 51Cr and 125I samples.

Dataset-specific Instrument Name	10AU Fluorometer (Turner Designs, San Jose, CA)
Generic Instrument Name	Turner Designs Fluorometer 10-AU
Dataset-specific Description	10AU Fluorometer (Turner Designs, San Jose, CA)
Generic Instrument Description	The Turner Designs 10-AU Field Fluorometer is used to measure Chlorophyll fluorescence. The 10AU Fluorometer can be set up for continuous-flow monitoring or discrete sample analyses. A variety of compounds can be measured using application-specific optical filters available from the manufacturer. (read more from Turner Designs, turnerdesigns.com, Sunnyvale, CA, USA)

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Project Information

Collaborative Research: Evolutionary, biochemical and biogeochemical responses of marine cyanobacteria to warming and iron limitation interactions (Cyanobacteria Warming Responses)

NSF abstract:
The oceans absorb much of the heat generated by human activities, and this warming of the surface ocean has consequences for important groups of marine organisms. Marine cyanobacteria are one such key group of organisms, since they supply much of the essential carbon and nitrogen that supports nearly all the rest of the marine food web. Currently, the growth of cyanobacteria is mostly constrained by scarce supplies of the micronutrient element iron, but they are also very sensitive to the ongoing increases in seawater temperature. Preliminary results suggest that warming could partly mitigate the negative effects of iron limitation on marine cyanobacteria. This project examines in depth how these interactions between warming and iron limitation will affect the future ocean carbon and nitrogen cycles, using laboratory culture experiments showing how cyanobacteria respond to simultaneously changing temperature and iron supplies. Both short-term response studies and long-term evolutionary experiments testing for adaptation use a comprehensive set of molecular biology tools targeting genes to proteins. The final goal is to apply the results of these experiments to improve quantitative models predicting how the ocean's carbon and nitrogen cycles, biological productivity, and living resources will respond to a warming future climate. Two graduate students, a postdoc and 3-4 underrepresented undergraduate researchers are supported, and the investigators also mentor summer science interns from largely Hispanic local high schools.

The physiology, biochemistry and biogeography of nitrogen-fixing cyanobacteria and unicellular picocyanobacteria are strongly influenced by temperature, subjecting them to intense selective pressure as the modern ocean steadily warms up. These groups have likewise been rigorously selected under chronic iron (Fe) scarcity, and the availability of this crucial micronutrient is also changing with a shifting climate. This project examines short-term acclimation and long-term evolutionary responses of Fe-stressed marine cyanobacteria to a warmer environment. Preliminary data show that Iron Use Efficiencies (IUE, mols N fixed.hr-1 mol cellular Fe-1) of Fe-limited Trichodesmium increase 4 to 5-fold with a 5oC temperature increase, allowing the cells to much more efficiently leverage scarce available Fe supplies to grow and fix nitrogen. This means that warming can to a large degree mitigate the negative effects of Fe limitation on Trichodesmium, resulting in a modelled 22% increase in global nitrogen fixation by 2100 in a warmer climate. This project aims to uncover the cellular biochemical mechanisms involved in this Fe-limitation/thermal IUE effect in a four-year experimental evolution study of the diazotrophs Trichodesmium and Crocosphaera and the picocyanobacteria Synechococcus and Prochlorococcus, under a multi-variate selection matrix of temperature and Fe availability. The objectives are to 1) Assess the long-term adaptive responses of fitness, IUE and physiology to Fe limitation and warming interactions in these four major cyanobacterial groups; 2) Determine the molecular and biochemical mechanisms behind the surprising Fe/warming interactive effect on IUE using genomics, transcriptomics and quantitative proteomics coupled with 'metalloproteomics' determinations of Fe content in critical proteins; 3) Compare and contrast acclimation and adaptation responses to Fe limitation and warming in key cyanobacteria taxa, and 4) Integrate results using a published biogeochemical modeling approach to assess global consequences for marine productivity and nitrogen fixation. This project offers a mechanistic and predictive understanding of adaptation to Fe and warming co-stressors in a rapidly changing future ocean environment for some of the most important photoautotrophic functional groups in the ocean.

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-1851222

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