Physiology responses to experimental iron warming interactions of coastal and oceanic Synechococcus collected from the South China Sea in April 2014

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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
The unicellular cyanobacterium Synechococcus is one of the most important primary producers in the ocean, and its growth and distribution are regionally limited by iron (Fe) concentration and temperature. However, the potential interactions between Fe availability and ocean warming in Synechococcus remain largely unexplored. We cultivated coastal (XM24) and oceanic (YX04-1) Synechococcus isolates from South China Sea under a matrix of two Fe concentrations (2 nM, 250 nM) and temperatures (24°C, 27°C) to investigate their physiological and transcriptomic responses.

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Coverage

Location: South China Sea
Spatial Extent: **N**:24 **E**:118 **S**:17 **W**:112
Temporal Extent: 2014-04 - 2020-12

Methods & Sampling

Culturing Conditions and experimental design

Synechococcus strains XM24 and YX 04-1 were isolated from the coastal region and offshore water of the South China Sea, respectively (Schiksnis et al., 2024; Zheng et al., 2018). Phylogenetic analysis classified them into subclade II clade CB5 and subclade I clade II, respectively.

The cultures were grown in Aquil medium using trace metal clean artificial seawater (Sunda et al., 2005). The experiments were conducted using a matrix of two Fe concentrations (2 nM and 250 nM) and two temperatures (24℃ and 27℃) under a 12:12 dark/light cool-white fluorescent light with an intensity of ~30 µmol quanta m-2 s-1. The cultures were isolated at ~25C, and hence 24℃ and 27℃ were used in the experiments to bracket this ambient temperature. A semi-continuous approach (Yang et al., 2021) was employed to grow the cultures under each treatment condition for at least two months (12 generations or more) prior to measuring physiological responses and collecting RNA samples. The cultures were diluted every other day based on in vivo fluorescence readings. Physiological parameters determined included growth rates, chlorophyll a, Fe quotas and carbon fixation rates. RNA samples were flash-frozen and stored in liquid nitrogen until extraction and sequencing.

To compare and contrast the responses of both isolates to warming and Fe limitation individually and in combination, six-treatment comparisons were conducted: 1) -Fe@27oC: Fe-limited vs Fe-replete at 27oC; 2) -Fe@24oC: Fe-limited vs Fe-replete at 24oC; 3) -Fe/warming interaction (-Fe+warming): 27oC Fe-limited vs 24oC Fe-replete; 4) 27oC@+Fe: 27oC Fe-replete vs 24oC Fe-replete; 5) 27C@-Fe: 27oC Fe-limited vs 24oC Fe-limited; 6) +Fe/warming interaction (+Fe+warming): Fe-replete at 27oC vs Fe-limited at 24oC.

Growth rates, Carbon fixation rates and elemental stoichiometry

Cell growth rates were determined by measuring in vivo fluorescence every other day, using the equation $\mu=\ln(N/N0)/(t-t0)$, where N represented the final cellular in vivo fluorescence at time t, and N0 represented the initial in vivo fluorescence at time t0. Carbon fixation rates were assessed using 14C-labeled bicarbonate. Specifically, 50 mL cultures were extracted from each bottle, incubated with 14C for 3 hours, and then filtered onto GF/F membranes. Subsequently, 14C radioactivity of the filters was measured using a Beckman System 6500 liquid scintillation counter, converted to carbon fixation rates and normalized to particulate organic carbon concentration (Fu et al., 2008). To determine the particulate organic carbon and nitrogen (POC and PON), the cultures were filtered onto pre-combusted glass microfiber filters. The filters were then dried in an oven and analyzed using a Costech Elemental Analyzer that was calibrated with methionine and acetanilide (Fu et al., 2008).

Fe quota measurements

To obtain iron quota results, cell samples were filtered, digested, and analyzed by mass spectrometry following published methods (Hawco et al., 2021; Yang et al., 2021). Briefly, cultures were filtered using acid-washed 0.2 µm Supor polyethersulfone filters and rinsed with an oxalate reagent to eliminate extracellular trace metals (Kustka et al., 2004). The filters were digested with 5 ml of 50% nitric acid (HNO3) at 95°C for five days in 30 mL perfluoroalkoxy vials (Savillex). After removing the filters and drying the samples at 100°C, they were resolubilized in 200 µL of 1:1 concentrated HNO3 and hydrochloric acid (HCl), sealed and heated for approximately 2-3 hours. The samples were dried down again and then resuspended in 5 mL of 0.1 M distilled HNO3 for Fe and P concentration analysis using a Thermo Scientific Element2 inductively coupled plasma mass spectrometry (ICP-MS). The cellular Fe quota was represented by the Fe concentration normalized to phosphate concentration and POC (Kustka et al., 2004).

Data Processing Description

The significance of the physiological parameters between Fe and temperature changes was assessed via two-way ANOVA and a Tukey multiple comparison test at p-value<0.05 in Graphpad prism v9.5.1, including growth rates, Fe quota, and carbon fixation rates.

After RNA sequencing, adapter sequences and low-quality bases were trimmed from raw reads in fastq format using Atropos. The quality of the trimmed reads was confirmed using FastQC v0.11.2. Ribosomal RNAs were removed using SortMeRNA v2.0 with default parameters and the remaining clean non-rRNA reads were aligned to *Synechococcus* reference genomes using BWA MEM v0.7.12 with default parameters. The number of reads aligned to each gene feature was counted using featureCounts v1.6.0 and differentially expressed genes were identified using DESeq2 v1.24.0 with specified cutoffs for log2 fold change > 1 and adjusted p-value < 0.05. TMM-normalized read counts in counts per million (CPM) were also calculated using edgeR v3.26.8 to compare gene expression across treatments. KEGG functional enrichment analysis was performed using clusterProfiler v3.12. Heatmaps were generated using the pheatmap v1.0.12 and all other figures were generated using ggplot2 v3.3.6 in R studio.

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Related Publications

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https://doi.org/10.18129/B9.BIOC.EDGER https://doi.org/10.18129/B9.bioc.edgeR
Software

Didion, J. P., Martin, M., & Collins, F. S. (2017). Atropos: specific, sensitive, and speedy trimming of sequencing reads. PeerJ, 5, e3720. Portico. https://doi.org/10.7717/peerj.3720
Software

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Methods

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Software

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Software

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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.
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Software

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
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Among Gene Clusters. OMICS: A Journal of Integrative Biology, 16(5), 284–287. https://doi.org/10.1089/omi.2011.0118
Software

Zheng, Q., Wang, Y., Xie, R., Lang, A. S., Liu, Y., Lu, J., Zhang, X., Sun, J., Suttle, C. A., & Jiao, N. (2018). Dynamics of Heterotrophic Bacterial Assemblages within Synechococcus Cultures. Applied and Environmental Microbiology, 84(3). https://doi.org/10.1128/aem.01517-17 https://doi.org/10.1128/AEM.01517-17
Methods

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Parameters

Parameters for this dataset have not yet been identified

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Instruments

Dataset-specific Instrument Name	Costech Elemental Analyzer ECS 4010
Generic Instrument Name	Costech International Elemental Combustion System (ECS) 4010
Generic Instrument Description	The ECS 4010 Nitrogen / Protein Analyzer is an elemental combustion analyser for CHNSO elemental analysis and Nitrogen / Protein determination. The GC oven and separation column have a temperature range of 30-110 degC, with control of +/- 0.1 degC.

Dataset-specific Instrument Name	Thermo Scientific Element2 inductively coupled plasma mass spectrometry (ICP-MS)
Generic Instrument Name	Inductively Coupled Plasma Mass Spectrometer
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	Beckman System 6500 liquid scintillation counter
Generic Instrument Name	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	10-AU Fluorometer (Turner Designs)
Generic Instrument Name	Turner Designs Fluorometer 10-AU
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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