In vivo chlorophyll and photosynthetic efficiencies for G. huxleyi RCC874 and RCC914 N amendment laboratory-based culture experiment

Website: https://www.bco-dmo.org/dataset/999184
Data Type: experimental
Version: 1
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Project
» Collaborative Research: Defining the role of the pan genome in Emiliania huxleyi ecology and biogeography (ECO-PAN-EHUX)

Contributors	Affiliation	Role
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Abstract
Environmental factors associated with climate change alter the interactions between bacteria and their algal hosts, known as the phycosphere, with broader implications for marine microbial community ecology. We examined the interactive effects of warming and nitrogen limitation on two strains of Gephyrocapsa huxleyi (RCC874 and RCC914), a widely abundant and physiologically flexible coccolithophore, and their phycospheres in response to N-replete and low N conditions. We measured in vivo chlorophyll a fluorescence and photosynthetic efficiency (Fv/Fm) to assess the impact of co-stress on G. huxleyi photophysiology.

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Coverage

Location: Laboratory-based experiment
Temporal Extent: 2024-01-18 - 2024-12-14

Methods & Sampling

G. huxleyi strains RCC874 and RCC914 were obtained from the Roscoff Culture Collection (RCC). The cultures were transferred from the maintenance stock into artificial seawater (40 g Instant Ocean® Sea Salt per L Milli-Q® water) amended with nutrients, trace metals, and vitamins according to the L1 recipe (https://ncma.bigelow.org/algae-media-recipes) without silica (herein reported as L1-Si). The temperatures were selected to simulate no heat stress (18°C), moderate heat stress (22°C), and high heat stress conditions (25°C and 28°C for RCC874 and RCC914, respectively). The cultures were acclimated to the temperature conditions under a 14:10 light/dark cycle for approximately 10 days prior to the start of the experiment. Following acclimation, cultures were transferred in triplicate into 2.8 L Fernbach flasks at an initial concentration of 1.0×10^5 cells mL^{-1}. The cultures were divided into two conditions: nitrogen-replete (N replete; L1-Si with standard 8.82×10^{-4} M $NaNO_3$) and nitrogen-limited (N limited; L1-Si with 8.82×10^{-5} M $NaNO_3$).

Growth was monitored daily at the same time of day by in vivo chlorophyll fluorescence (relative fluorescence units, RFUs) on a Turner Designs Aquafluor handheld fluorometer (in vivo chlorophyll *a* channel). Briefly, each culture was mixed by swirling 3 times clockwise and then 3 times counterclockwise before aseptically removing a 2 mL subsample with a serological pipette. The subsample was transferred to a 10 mm × 10 mm methacrylate cuvette and placed in the Aquafluor for RFU measurement. The efficiency of photosystem II (PSII), Fv/Fm, was determined daily for each replicate using a Turner Designs AquaFlash handheld fluorometer (factory calibrated). Again, each culture was mixed (as described above) before aseptically removing a 3 mL subsample with a serological pipette. The subsample was transferred to a 10 mm × 10 mm quartz cuvette and placed in the AquaFlash for Fv/Fm measurement. If in vivo chlorophyll *a* fluorescence exceeded the maximum linear range, samples were diluted with artificial seawater L1-Si media containing the corresponding nitrogen concentration to bring the reading within range.

Data Processing Description

Data was processed in R version 4.4.0. To arrive at a final in vivo chlorophyll a RFU, readings were multiplied by dilution factors, if applied.

BCO-DMO Processing Description

- Loaded data from "Chla_FvFm_GhuxleyiCultures_WHOI_Oct2023-Jan2024.xlsx" (sheet 1), treating empty strings and "nd" as missing values
- Converted the `date` column from format `%m-%d-%y` to ISO date format `%Y-%m-%d`, output as date type
- Renamed column `Fv/Fm` to `Fv_Fm`
- Set column types: `F0` and `Fm` as string; `Fv_Fm`, `chla_read`, `chla_total`, and `dilution_factor` as number; `date` as date with format `%Y-%m-%d`; `nitrogen` and `strain` as integer; `replicate` as string; `temperature_c` as integer
- Output written to "999184_v1_G_huxleyi_chlorophyll_and_photosynthetic_efficiencies.csv"

Problem Description

No problems or issues have been reported by the dataset authors.

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Parameters

Parameters for this dataset have not yet been identified

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Instruments

Dataset-specific Instrument Name	Aquafluor Handheld Fluorometer (Turner Designs, USA)
Generic Instrument Name	Fluorometer
Dataset-specific Description	A Aquafluor Handheld Fluorometer (Turner Designs, USA) was used to measure in vivo chlorophyll a fluorescence.
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	AquaFlash Handheld Fluorometer (Turner Designs, USA)
Generic Instrument Name	Fluorometer
Dataset-specific Description	A AquaFlash Handheld Fluorometer (Turner Designs, USA) was used to measure photosynthetic efficiency (Fv/Fm). The AquaFlash uses two measuring LEDs to estimate photosynthetic efficiency. The first LED (monitoring) excites the sample with very low light intensity so as not to induce a change in chlorophyll reaction centers. While continuously monitoring the sample using the monitoring LED, the second LED (saturating) exposes the sample to a high intensity of light to effectively close chlorophyll reaction centers and bring the sample to a maximum fluorescence state (Fm). The difference between the maximum (Fm) and minimum (Fo) fluorescence states is called variable fluorescence (Fv).
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.

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Project Information

Collaborative Research: Defining the role of the pan genome in Emiliania huxleyi ecology and biogeography (ECO-PAN-EHUX)

NSF Award Abstract:
Emiliania huxleyi is a numerically and ecologically important phytoplankton species in the ocean known for its cosmopolitan distribution and ability to form large blooms in coastal and open ocean regions. Studies of E. huxleyi variants in culture have found differences in growth, function and activity potential among them. The E. huxleyi variants also differ in some of the genes they carry. It has been hypothesized that this genomic variability may underlie the global success of this phytoplankton species by allowing adaptation of variants to diverse environments. Yet, the direct connection between genomic content and ecological success remains unclear. This project investigates how the conserved and variable portions of E. huxleyi genome may be connected to its success and its dynamics under varied environmental conditions. This work is critical to our understanding of how this important phytoplankton species may shift and respond to future changes. This project also supports the development of a series of hands-on activities designed to teach middle school students advance computational data analysis in ocean science. These activities are in collaboration with the

Girls Who Code Club at the Our Sisters School, a tuition-free, non-sectarian, independent school for girls from low-income families, located in New Bedford, MA.

Understanding how phytoplankton diversity and phenotype are driven by changes in the environment is crucial for better predicting carbon cycle dynamics in the future ocean. While much work has investigated competition among phytoplankton species, intraspecific diversity and dynamics remain largely unknown for many eukaryotic phytoplankton. The overarching goal of this project is to define the role of the pan genome (set of variable genes) in E. huxleyi ecology and biogeography through a series of genomic analyses, computational field surveys, and laboratory-based experiments. This project is sequencing the genomes of several E. huxleyi isolates from across the global ocean and combining them with existing genome sequences to constrain the core and variable portions of the pan genome. Using this new pan genome reference database and leveraging global scale metagenomics and metatranscriptomic surveys, this project is estimating ecotype diversity of E. huxleyi across ocean regions to identify patterns of environmental selection. This project additionally focuses on identifying the physiological and transcriptional responses of a selection of sequenced strains and their responses to shifts in their nutrient environments in controlled laboratory studies. As E. huxleyi plays such a significant role in marine ecosystems and the global carbon cycle it is important that its pan genome and its impact on the biogeography and ecology of E. huxleyi is taken into consideration. It is likely that these dynamics are acutely important to predicting how this genus, and perhaps others, respond to changing environmental conditions in the future 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-1948025
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