Reactive oxygen species for G. huxleyi RCC874 and RCC914 N amendment laboratory-based culture experiment

Website: https://www.bco-dmo.org/dataset/999330
Data Type: experimental
Version: 1
Version Date: 2026-05-22

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 its phycosphere under nitrogen-replete and low-nitrogen conditions. Reactive oxygen species (ROS) are a by-product of metabolic processes and are produced in response to environmental stress. We measured the fluorescence of CM-H2DCFDA as an indicator of ROS in G. huxleyi to assess the impact of combined stressors on G. huxleyi physiology.

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Coverage

Location: Laboratory-based experiment
Temporal Extent: 2023-10-13 - 2023-12-11

Dataset Description

This dataset relates to both the growth (relative fluorescence) and growth rates datasets as these were the strains and conditions used to generate the growth data. Supplemental file 998934_v1_G_huxleyi_strain_growth_conditions.csv identifies the strain ID, the media type the strain was grown in, and the incubator temperature. The primary data file, 998934_v1_G_huxleyi_relative_fluorescence_units.csv, identifies the daily relative fluorescence units (in vivo chlorophyll a) as a proxy for biomass for the individual strains. Finally, the growth rates data file, 998934_v1_G_huxleyi_strain_exponential_daily_growth_rates.csv, identifies the exponential growth rate for all the strains over the linear portion of the growth curve.

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 $NaNO_3$).

For reactive oxygen species (ROS) analysis, samples were stained with 5 µM CM-H2DCFDA (Invitrogen). Samples were incubated at room temperature in the dark for 1 hour before analysis, as per Sheyn et al. (2016). The fluorescence of stained samples was measured using a SpectraMax M3 Multi-Mode Microplate Reader (ex: 485 nm and em: 525 nm) (Molecular Devices, USA).

Data Processing Description

Data was processed in R version 4.4.0.

BCO-DMO Processing Description

- Loaded sheet 1 from Excel file "ROS_GhuxleyiCultures_WHOI_Oct2023-Jan2024.xlsx" with headers from row 1; treated "", "nd", "N/A", and "spike" as missing values
- Converted the `date` column from format "%m-%d-%y" to ISO format "%Y-%m-%d" as a date type
- Set column types: `date` as date (%Y-%m-%d), `fluorescence` and `nitrogen` as number, `replicate` as string, `strain` as integer, `temperature_c` as integer
- Output written to "999330_v1_G_huxleyi_reactive_oxygen.csv"

Problem Description

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

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Related Publications

Sheyn, U., Rosenwasser, S., Ben-Dor, S., Porat, Z., & Vardi, A. (2016). Modulation of host ROS metabolism is essential for viral infection of a bloom-forming coccolithophore in the ocean. The ISME Journal, 10(7), 1742–1754. https://doi.org/10.1038/ismej.2015.228
Methods

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Parameters

Parameters for this dataset have not yet been identified

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Instruments

Dataset-specific Instrument Name	SpectraMax M3 Multi-Mode Microplate Reader (Molecular Devices, USA)
Generic Instrument Name	plate reader
Dataset-specific Description	The fluorescence of stained samples was measured using a SpectraMax M3 Multi-Mode Microplate Reader (ex: 485 nm and em: 525 nm) (Molecular Devices, USA).
Generic Instrument Description	Plate readers (also known as microplate readers) are laboratory instruments designed to detect biological, chemical or physical events of samples in microtiter plates. They are widely used in research, drug discovery, bioassay validation, quality control and manufacturing processes in the pharmaceutical and biotechnological industry and academic organizations. Sample reactions can be assayed in 6-1536 well format microtiter plates. The most common microplate format used in academic research laboratories or clinical diagnostic laboratories is 96-well (8 by 12 matrix) with a typical reaction volume between 100 and 200 uL per well. Higher density microplates (384- or 1536-well microplates) are typically used for screening applications, when throughput (number of samples per day processed) and assay cost per sample become critical parameters, with a typical assay volume between 5 and 50 µL per well. Common detection modes for microplate assays are absorbance, fluorescence intensity, luminescence, time-resolved fluorescence, and fluorescence polarization. From: http://en.wikipedia.org/wiki/Plate_reader, 2014-09-0-23.

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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
NSF Division of Ocean Sciences (NSF OCE)	OCE-1948409

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