Alkaline phosphatase activity for G. huxleyi CCMP371 grown under low N and low P N:P ratios in laboratory-based culture experiments

Website: https://www.bco-dmo.org/dataset/998945
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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
Gephyrocapsa huxleyi, a ubiquitous marine coccolithophore, plays a significant role in marine ecosystems and the global carbon cycle. Broadly, this project seeks to define the role of the pan-genome in G. huxleyi ecology and biogeography and provide insight into how this taxon's genotypic variability influences its biogeography and ecology, and ultimately how this genus may adapt to changing environmental conditions in the future ocean. This project leveraged the G. huxleyi pan-genome database to inform a series of additional laboratory-based culture experiments to relate genotype to phenotype in different strains under shifting response drivers, specifically nitrogen (N) and phosphorus (P). Here, we grew G. huxleyi CCMP371, a highly calcifying, axenic strain, under low N and low P conditions by modulating the dissolved inorganic nitrogen (DIN) to dissolved inorganic phosphorus (DIP) ratios. The CCMP371 experiment began as batch cultures until a maximum in vivo chlorophyll fluorescence was reached (based on a previous growth experiment; data contributed to BCO-DMO in this same project). At this point, an equal volume of culture was removed from triplicate flasks and replaced with an equal volume of modified L1/5-ASW, with an N:P of 0.5:1 (0.5 µM N and 1 µM P) for low N conditions. Likewise, an equal volume of culture was removed from triplicate flasks and replaced with an equal volume of L1/5-ASW, with an N:P of 125:1 (25 µM N and 0.2 µM P) for low P conditions. Dilutions continued until steady-state conditions, determined by microscopic cell counts, were reached. Once the low N and low P triplicate cultures reached steady state, samples were collected for a suite of physicochemical parameters, including alkaline phosphatase activity reported in this dataset. Alkaline phosphatase activity (APA) is a common physiological marker of P stress, where increased APA indicates a phenotypic shift toward the use of organic P sources. In this experiment, APA was used to confirm P-deficiency in the low P cultures relative to the low N cultures. These data were used to inform additional nutrient amendment experiments with G. huxleyi strains (reported elsewhere), and they can be used to better constrain existing tools, such as the Global Alkaline Phosphatase Activity Dataset (GAPAD).

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Coverage

Location: Laboratory-based culture experiments with G. huxleyi CCMP371

Methods & Sampling

For this alkaline phosphatase activity dataset, *G. huxleyi* CCMP371 was grown under low N and low P conditions by modulating dissolved inorganic nitrogen (DIN) to dissolved inorganic phosphorus (DIP) ratios. This experiment began as a single batch culture until a maximum in vivo chlorophyll fluorescence was reached (based on a previous growth experiment; data contributed to BCO-DMO in this same project), which initiated a semi-continuous phase of the experiment. Briefly, CCMP371 (in L1/5-Sargasso seawater base media) was used to inoculate L1/5-artificial seawater base media (ASW) in each of six 2 L Erlenmeyer flasks. The cultures were kept at 18ºC (the optimum in terms of its thermal range; see Krinos et al. 2025) on a 14 h:10 h light-dark cycle with a light intensity of 100 µE m-2 s-1. Growth was monitored daily by in vivo fluorescence and by microscopic cell counts (reported elsewhere). A total of 3 flasks were randomly selected as low N treatment flasks, and the remaining 3 were selected as low P treatment flasks for the semi-continuous phase of the experiment, with daily removal of 0.4 L of culture and replacement with 0.4 L modified L1/5-ASW, with an N:P of 0.5:1 (0.5 µM N and 1 µM P) for low N and an N:P of 125:1 (25 µM N and 0.2 µM P) for low P until steady-state growth, as determined by in vivo fluorescence and microscopic cell counts (reported elsewhere), was maintained. Dilutions were made at the same time each day, roughly 5 h into the light cycle, to avoid diel effects. Daily dilutions continued until the cultures reached a steady state that was maintained for at least 4 days after the daily removal/dilution began. Once the low N and low P triplicate cultures reached steady state, samples were collected for a suite of physicochemical parameters, including alkaline phosphatase activity (reported here).

For APA analysis, samples (5 mL) from each experimental treatment (low N and low P) and replicate were filtered onto 47-mm polycarbonate membranes (0.2 µm) and stored at −20°C until analysis. APA was assayed after Dyhrman and Ruttenberg (2006) on a Biotek Synergy H1 hybrid microplate reader in fluorescence mode (with an excitation wavelength of 360 nm and an emission wavelength of 460 nm) using the fluorogenic phosphatase substrate 6,8-difluoro-4-methylumbelliferyl phosphate. Subsamples (0.2 mL) from each sample were transferred to a black, flat-bottom, untreated 96-well microplate and assayed immediately and then at intervals adjusted to the activity of the sample, such that all readings fell within the linear range of the assay (at least four measurements within 1 h or less). A standard curve from 0 to 600 nM DiFMU in artificial seawater was generated and used to calculate the rate of DiFMUP hydrolysis. Values were normalized per *G. huxleyi* cell (cell counts reported elsewhere).

Details for the reagents used are as follows:

6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP; Invitrogen/Thermo Scientific D6567); Abs/Em = 358/455

6,8-difluoro-7-hydroxy-4-methylcoumarin (DiFMU; Invitrogen/Thermo Scientific D-6566); reference standard

Data Processing Description

A standard curve from 0 to 600 nM DiFMU in artificial seawater was generated and used to calculate the rate of DiFMUP hydrolysis. Rates were divided by the number of G. huxleyi cells collected on the filter to obtain a rate per cell for each treatment and replicate.

BCO-DMO Processing Description

- Loaded data from "APA_CCMP371_BCO_DMO.xlsx" (sheet 1), treating empty strings and "nd" as missing values
- Renamed column "G. huxleyi_ID" to "G_huxleyi_ID"
- Set column types: "APA_nmolP_cell_hour" as number with scientific notation output format, "Experimental_Treatment" as string, "G_huxleyi_ID" as string, and "Replicate" as integer
- Output written to "998945_v1_G_huxleyi_CCMP371_alkaline_phosphatase_activity.csv"

Problem Description

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

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Related Publications

Dyhrman, S. T., & Ruttenberg, K. C. (2006). Presence and regulation of alkaline phosphatase activity in eukaryotic phytoplankton from the coastal ocean: Implications for dissolved organic phosphorus remineralization. Limnology and Oceanography, 51(3), 1381–1390. doi:10.4319/lo.2006.51.3.1381
Methods

Krinos, A. I., Shapiro, S. K., Li, W., Haley, S. T., Dyhrman, S. T., Dutkiewicz, S., Follows, M. J., & Alexander, H. (2025). Intraspecific Diversity in Thermal Performance Determines Phytoplankton Ecological Niche. Ecology Letters, 28(1). Portico. https://doi.org/10.1111/ele.70055
Methods

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Parameters

Parameters for this dataset have not yet been identified

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Instruments

Dataset-specific Instrument Name	Biotek Synergy H1 hybrid microplate reader
Generic Instrument Name	plate reader
Dataset-specific Description	APA was assayed on a Biotek Synergy H1 hybrid microplate reader in fluorescence mode. Subsamples (0.2 mL) from each sample were transferred to a black, flat-bottom, untreated 96-well microplate and assayed immediately, with subsequent measurements taken at intervals adjusted to the activity of the sample such that all readings fell within the linear range of the assay (at least four measurements within 1 h or less).
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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