Laboratory-based growth studies with multiple G. huxleyi strains for genome sequencing

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

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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. To construct a pan-genomic G. huxleyi database, 16 different G. huxleyi strains (CCMP1280, CCMP375, CCMP377, CCMP371, RCC3492, RCC4567, RCC1222, RCC847, RCC1256, RCC914, RCC1215, RCC1239, RCC3963, RCC1212, RCC6856, and RCC6071) obtained from national (NCMA) and international (Roscoff) culture collections were grown in the laboratory in 1 L nutrient-replete L1 media with a natural seawater base. Growth of each strain was monitored daily using in vivo chlorophyll a fluorescence, expressed as relative fluorescence units (RFUs), as a proxy for biomass. Exponential growth rates were also calculated for each strain from the linear portion of the growth curves. At the conclusion of each growth experiment, biomass was collected by filtration for DNA extraction and downstream genome sequencing (reported elsewhere). The growth data generated here supported the extraction of high-quality DNA for a pan-genome database (reported elsewhere) and additional culture experiments to relate genotype to phenotype in G. huxleyi strains under varying environmental conditions, including different nitrogen:phosphorus ratios, low nitrogen, and low phosphorus conditions (reported elsewhere). More broadly, the G. huxleyi growth data provided here can be used to inform primary productivity models to better parameterize inputs such as nutrient uptake, temperature sensitivity, and bloom duration.

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Coverage

Location: Laboratory-based experiment

Methods & Sampling

All *G. huxleyi* isolates were obtained from either the National Center for Marine Algae and Microbiota (East

Boothbay, ME) or the Roscoff Culture Collection (Roscoff, France) and maintained in incubators with ~100 µE m-2 s-1 light on a 14:10 light:dark cycle in autoclaved L1 media with a 0.2-µm filtered Vineyard Sound (MA) seawater base. Isolates were grown at different temperatures, as determined by their collection location (reported in strain metadata from NCMA and/or Roscoff; e.g., CCMP371 was collected in June 1987 in the Sargasso Sea at 32°N, 62°W with a culture collection maintenance temperature of 20 °C). To generate enough biomass needed for downstream genome sequencing (details reported elsewhere), large (1 L) batch cultures of each isolate were grown. For each separate isolate, a 25 mL nutrient-replete (L1) culture was used to inoculate 1 L nutrient-replete (L1) media in a Fernbach flask. No replicate cultures were grown for this experiment, as replicates were not necessary given the goal of obtaining DNA for genome sequencing.

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. A blank measurement was also taken daily using deionized water in place of a culture sample in the same type of cuvette. The RFU value obtained from the blank was subtracted from the RFU value of the culture for a “blank-corrected” RFU. Negative values for the blank were a result of instrument drift and were subtracted as described above. No RFUs in the dataset were missing or anomalous.

The exponential population growth rate for each *G. huxleyi* strain was calculated by measuring changes in blank-corrected RFUs (in vivo chlorophyll *a*) as a proxy for biomass over time during the exponential growth phase (minimally 4 days), using the formula µ = (ln(N2) - ln(N1))/t2-t1, where N1 is the in vivo chlorophyll *a* (RFU) at the start, N2 is the in vivo chlorophyll *a* (RFU) at the end, t1 is the first timepoint (day), and t2 is the final timepoint (day). RFUs were ln-transformed and fitted with a linear regression of ln(RFU) versus time (days). The regression slope over the most linear portion (where R2 > 98%) of the growth curve was taken as the best estimate of mean daily population growth rate.

The resulting datasets from applying this method included *G. huxleyi* strain growth conditions, *G. huxleyi* growth (biomass proxied by relative fluorescence units from in vivo chlorophyll *a*), and *G. huxleyi* exponential growth rates.

Data Processing Description

Relative fluorescence unit (RFU) values for each day and for each *G. huxleyi* strain were calculated by subtracting the blank RFU for that day from the RFU of the strain for that day. This yielded a “blank-corrected” RFU. Negative values for the blank were a result of instrument drift. The exponential population growth rate for each *G. huxleyi* strain was calculated from RFUs from the linear portion of the growth curve (minimally 4 days). RFUs were ln-transformed in Prism 11 (GraphPad) and fitted with a linear regression of ln(RFU) versus time (days). The regression slope over the most linear portion (where R2 > 98%) of the growth curve was taken as the best estimate of mean daily population growth rate.

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	Turner Designs Aquafluor
Generic Instrument Name	Turner Designs Aquafluor 8000
Dataset-specific Description	Turner Designs Aquafluor (Turner Designs 8000-406), in vivo chlorophyll a channel (Ex: 395/130 nm; Em: ≥ 660 nm). 10 mm x 10 mm methacrylate cuvette.
Generic Instrument Description	The Turner Designs Aquafluor 8000 is a lightweight, handheld fluorometer/turbidimeter ideal for field use. It can be configured with one or two channels to measure turbidity, chlorophyll, algae, dyes, ammonium, CDOM, and more. Detailed description at https://www.turnerdesigns.com/aquafluor-handheld-fluorometer

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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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