Cell abundance, forward scatter, chlorophyll, growth rate, and FvFm.0 measurements from reciprocal transplant assays

Website: https://www.bco-dmo.org/dataset/854010
Data Type: experimental
Version: 1
Version Date: 2021-06-17

Project
» BEE: Testing the evolutionary responses of mixotrophs to future ocean conditions (MixoEvo)

Contributors	Affiliation	Role
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Abstract
This dataset includes cell abundance, forward scatter, chlorophyll, growth rate and FvFm.0 measurements from reciprocal transplant assays.

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Methods & Sampling

Six lineages of each of two strains of the genus *Ochromonas* (CCMP1393, and CCMP2951) have been evolving at three temperatures, the ancestral temperature of 24C, and cooler and warmer temperatures of 18C and 30C. A third strain, CCMP 1391 does not survive at 30C and is only evolving at 18C and 24C. The experiment is run at two light levels, 100uE and 50uE. Cultures are maintained in batch culture in K medium made by adding pre-mixed nutrients (ordered from the NCMA) to 0.2 µm filtered Santa Barbara coastal seawater in three light- and temperature-controlled incubators.

Evolving lineages are regularly tested for several characteristics including growth rate, cell size, chlorophyll content, and photosynthetic efficiency. To show that characteristic changes are indeed from an evolutionary response as opposed to phenotypic plasticity, reciprocal transplant assays are conducted every three months. This involved placing subsamples of each evolving lineage into all three temperatures, and comparing their performance in characteristic tests (growth rate, photosynthetic efficiency etc.).

Sampling and analytical procedures:

Guava count:
Cell density and relative size are measured every day for a 4-day growth period using a Guava easyCyte Flow Cytometer (Luminex Corporation, Austin, TX).

Chlorophyll Extractions:
Cellular chlorophyll-*a* (chl-*a*) content is measured by filtering cells onto a 25mm-diameter GF/F filter (Whatman) and extracting for 24 hours in a 90% acetone solution. Chl-*a* concentration in the acetone solution is read using a Trilogy fluorometer (Turner Designs, San Jose, CA).

FIRe Dark-acclimated Fv/Fm measurement:
Photosynthetic efficiency is measured using a mini Fluorescence Induction and Relaxation (FIRe) system

(custom built by M. Gorbunov, Rutgers University).

Known Issues/Problems:
There was no chlorophyll collected during the first three backtransfers. Some FvFm data is missing because the FIRe was unable to process the data.

Data Processing Description

Data processing:
Guava Suite Software 3.4 (Luminex Corporation, Austin, TX), fpro.exe

FIRe Analysis: To run the analysis program on a separate PC, copy the program (fpro.exe), together with the file EGAVGA.bgi (a graphic driver). The program fprope.exe must be stored in the same directory as the raw FIRe data files. Use DOSBox emulator to run fpro.exe.

BCO-DMO Processing:
- concatenated separate .csv files into one dataset;
- re-named fields to comply with BCO-DMO naming conventions (replaced special characters with underscores).

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Data Files

File
cell_abund.csv (Comma Separated Values (.csv), 300.30 KB) MD5:6b11b1a999f7902bebd3f1e7080ea812 Primary data file for dataset ID 854010

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Parameters

Parameter	Description	Units
Strain	The strain of Ochromonas	unitless
Light	Light intensity	micromoles quanta per square meter per second (umol quanta m-2 s-1)
Ev	Temperature that the strain was evolved at	degrees Celsius (°C)
Acc	Temperature that the strain was briefly acclimated to (for 5 days) prior to measurements	degrees Celsius (°C)
Rep	Replicate; Which of 6 replicates the data were collected from	unitless
Week	Weeks since start of experiment	unitless

D1Pop	Day one population size	cells per milliliter (Cells/mL)
D2Pop	Day two population size	cells per milliliter (Cells/mL)
D3Pop	Day three population size	cells per milliliter (Cells/mL)
D4Pop	Day four population size	cells per milliliter (Cells/mL)
D1FSC	Day one forward scatter	relative fluorescence units
D2FSC	Day two forward scatter	relative fluorescence units
D3FSC	Day three forward scatter	relative fluorescence units
D4FSC	Day four forward scatter	relative fluorescence units
Growth_Rate	Rate of growth of cell cultures	per day (d^(-1))
PE_Count	Cell density at the time of the PE measurement	cells per milliliter (Cells/mL)
Chl_a_read	Amount of chlorophyll in the sample	nanograms chl-a per milliliter (ng chl-a mL-1)
Chl_a_per_cell	Amount of chlorophyll a per cell	picograms chl-a per cell (pg chl-a cell-1)
FvFm_0	Day 0 Maximum quantum yield of photochemistry in PSII, measured in a dark-adapted state (dimensionless). This parameter characterizes the efficiency of primary photosynthetic reactions.	dimensionless
file_name	Name of the original csv file	unitless

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Instruments

Dataset-specific Instrument Name	Guava easyCyte flow cytometer
Generic Instrument Name	Flow Cytometer
Dataset-specific Description	Guava easyCyte flow cytometer (Luminex Corporation, Austin, TX)
Generic Instrument Description	Flow cytometers (FC or FCM) are automated instruments that quantitate properties of single cells, one cell at a time. They can measure cell size, cell granularity, the amounts of cell components such as total DNA, newly synthesized DNA, gene expression as the amount messenger RNA for a particular gene, amounts of specific surface receptors, amounts of intracellular proteins, or transient signalling events in living cells. (from: http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm)

Dataset-specific Instrument Name	mini-FIRe
Generic Instrument Name	Fluorometer
Dataset-specific Description	mini-FIRe (Fluorescence Induction and Relaxation System) custom built by M. Gorbunov, Rutgers University.
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	Trilogy fluorometer
Generic Instrument Name	Fluorometer
Dataset-specific Description	Trilogy fluorometer (Turner Designs, San Jose, CA)
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

BEE: Testing the evolutionary responses of mixotrophs to future ocean conditions (MixoEvo)

NSF Award Abstract:
Aquatic ecosystems host a wide variety of single-celled, microscopic organisms. Many of these species live near the surface of the water, where they grow and reproduce using different metabolic strategies that shape their place in the marine food web. For example, biologists have traditionally grouped planktonic microbes into either primary producers (which use photosynthesis to create new organic matter) or heterotrophs (which eat organic matter - such as the bodies - produced by other organisms). However, a large number of species are actually mixotrophic: they "mix" these two forms of metabolism by simultaneously conducting photosynthesis and eating smaller cells, including bacteria. Furthermore, many mixotrophs are metabolically flexible: they may rely more or less on each source of metabolism depending on environmental conditions. Because photosynthesis (which takes carbon out of the atmosphere and locks it into organic matter) and heterotrophy (which respires organic matter back into carbon dioxide) control whether or not oceanic food webs act as carbon sinks (having a net removal of carbon dioxide from the atmosphere), understanding mixotroph metabolism is critical to predicting the effects of marine plankton on atmospheric carbon. This project advances understanding of mixotroph metabolism by quantifying the extent to which mixotrophs can alter their reliance on photosynthesis over short and long timescales. The project tests how quickly mixotrophs can adapt to both warmer and colder water conditions, and how these adaptations alter their role in the carbon cycle. Researchers - including graduate students, a postdoctoral researcher, and undergraduate trainees - will measure the physiological responses of experimentally evolved mixotrophs and use mathematical models to connect these changes to global oceanic carbon cycling. As data are collected, they are shared with the public through outreach seminars, annual open house events, and weekly scientific presentations at the local Santa Barbara Museum of Natural History.

In order to predict biologically mediated feedbacks in the climate system, we must understand how marine plankton will respond to future ocean conditions. While a number of studies have sought to quantify the potential evolutionary response of phytoplankton, much less is known about the impacts of shifting conditions (e.g., increased temperature) on mixotrophs. What data are available suggest that mixotrophs may modulate a positive climate feedback loop: when warmed, mixotrophs become more heterotrophic, thus reducing their contribution to the biological pump and enhancing local respiration of organic carbon. Warming may also result in reductions in cell size, reducing sinking fluxes and carbon export from the upper ocean. Furthermore, because the predicted increase in oceanic stratification is expected to favor mixotrophs, their metabolic responses may be increasingly significant to understanding the global carbon cycle. The PI of this project is experimentally evolving mixotrophs under a range of temperature conditions in a fully factorial design that also manipulates the availability of light (photosynthesis) and prey (heterotrophy). She quantifies the carbon budget, grazing activity, nutrient content, and grazer palatability of evolved lineages in order to estimate the impact of any observed adaptations on carbon cycling. Specifically, the investigator asks how evolved lineages compare to ancestral lineages in their ability to tolerate altered thermal conditions, and connects differences in fitness to shifts in reliance on photosynthesis versus heterotrophy. Simultaneously, she incorporates a mixotrophy module into a global ocean biogeochemistry model, allowing the quantification of the impact of mixotrophs with either contemporary or evolved physiological traits. This work will provide some of the first known data on mixotroph plastic and evolutionary responses, and allow the scaling of these responses to their potential impacts on upper ocean biogeochemistry.

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-1851194

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