Quantification of photosynthetic capacity of cells using a laser induction of chlorophyll autofluorescence

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Data Type: experimental
Version: 1
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Project
» BEE: Testing the evolutionary responses of mixotrophs to future ocean conditions (MixoEvo)

Contributors	Affiliation	Role
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Abstract
This dataset includes a quantification of photosynthetic capacity of cells using a laser induction of chlorophyll autofluorescence.

Table of Contents

Coverage

Temporal Extent: 2018-05-18 - 2020-05-17

Methods & Sampling

Six independent lineages of each Ochromonas strain were grown in batch cultures in three light- and temperature-controlled incubators (18°C, 24°C, and 30°C), and 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 were conducted every three months for two years. This involved placing subsamples of each evolving lineage into all three temperatures, and comparing their performance in characteristic tests (growth rate, photosynthetic efficiency etc.). Evolved lineages performing equally at all "acclimation temperatures" is evidence of plasticity, while differences in performance indicates adaptation. Using growth rate and photosynthetic rate to compute the relative contributions of autotrophy versus heterotrophy for each evolved lineage.

Procedures for Photosynthesis-Irradiance curves:
Dark-acclimate cells to be sampled for at least 15 minutes. This operation requires the actinic light source (ALS). In the DOS prompt Type 'fview'. Press enter. The data acquisition program will open. Adjust the number of samples and PAR steps to 20. Adjust the max PAR to 1001. MTF and MTRP stay set to 0. Adjust the acclimation time between each sample to 15 seconds. Homogenize sample before reading. Insert your test tube with dark-acclimated sample. Press 's' (for 'start/stop'). Adjust the 'Gain:' so that the fluorescence trace is as close to, but not greater than, 100%.

Missing Data:
Occasionally, at high light levels and especially high temperatures, the signal to noise ratio of the fluorescence

signal is very low. This causes noisy instrument output, which fails at the analysis (fprope.exe) stage. These data are missing from the dataset, and can be identified when a run (unique combination of week, light level, food level, strain, evolutionary temperature, acclimation temperature, and replicate) has fewer than twenty rows of data.

Data Processing Description

Data processing procedures:
fprope.exe, EGAVGA.bgi (graphic driver), DOSBox emulator

FIRe Analysis: To run the analysis program on a separate PC, copy the program (fprope.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 fprope.exe.

Calculating ETR data from .RES file: Copy the .RES file into the ETR template excel sheet. This sheet has prepopulated equations for base FvFm, base Sigma, and ETR. The base FvFm equation is =IF(Par=0, Fv/Fm, base FvFm) The equation for base Sigma is =IF(par=0, Sigma, base sigma) The equation for ETR is =(+par * sigma * 2.04 * (FvFm/(FvFm)) * .00602). Export this completed excel sheet as a CSV file to be used in the R script.

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);
- converted date format to YYYY-MM-DD.

[table of contents | back to top]

Data Files

File
PI_curves.csv (Comma Separated Values (.csv), 6.35 MB) MD5:f836a09b5de6e824b5dd08a31325efed Primary data file for dataset ID 853945

[table of contents | back to top]

Parameters

Parameter	Description	Units
Week	Week of sampling	unitless
Light	Light intensity	microEinsteins (uE)
Food	Xenic vs Axenic	unitless
Strain	The strain of Ochromonoas	unitless
Evol	Temperature that the strain was evolved at	degrees Celsius (°C)

Acc	Temperature that the strain was acclimated to	degrees Celsius (°C)
Rep	Replicate; Which of 6 replicates the data were collected from	unitless
File	The output file number from the FIRe	unitless
DATE	Date of sampling; format: YYYY-MM-DD	unitless
PAR	Photosynthetically active radiation	microEinsteins per square meter per second (uE/m2/s)
Fo_or_F	Minimum fluorescence yield	a.u.
Fm	Maximum fluorescence yield	a.u.
FmDec	Minimum fluorescence yield retrieved from the relaxation phase	a.u.
Fv	Variable fluorescence yield (=Fm-Fo)	a.u.
FvDEC	Variable fluorescence yield (=FmDec-Fo) retrieved from the relaxation phase	a.u.
Fv_Fm	Fv/Fm. Maximum quantum yield of photochemistry in PSII(dimensionless). This parameter characterizes the efficiency of primary photosynthetic reactions.	dimensionless
FvD_FmD	FvD/FmD. Quantum yield of photochemistry in PSII retrieved from the relaxation phase	dimensionless
Sigma	The functional absorption cross-section of PSII. Reported in relative units. Must be multiplied by a calibration coefficient to be converted into absolute units (Angstrom^2). This calibration coefficient is determined for each instrument.	Relative units (see description)
p	"Connectivity parameter" that determines the probability of excitation energy transfer between individual photosynthetic units	dimensionless
Fm_PAM	Fm(PAM). Maximum fluorescence yield recorded at the end of MTF flash when the plastoquinone (PQ) pool is fully reduced	a.u.
Fv_Fm_PAM	Fv/Fm(PAM). Maximum quantum yield of photochemistry in PSII estimated from the MTF protocol	unitless

GAIN	Gain of the detector	unitless
Fo_prime	Minimum fluorescence yield	a.u.
qp_coeff	Coefficient of photochemical quenching, defined as a fraction of open PSII RCs	dimensionless
baseFvFm	Maximum quantum yield of photochemistry in PSII, measured in a dark-adapted state (PAR=0).	dimensionless
baseSigma	The functional absorption cross-section of PSII, measured in a dark-adapted state (PAR=0). Reported in relative units. Must be multiplied by a calibration coefficient to be converted into absolute units (Angstrom^2). This calibration coefficient is determined for each instrument.	Relative units (see description)
ETR	Electron Transport Rate	Electrons per second per reaction center
file_name	Name of the original csv file	unitless

[table of contents | back to top]

Instruments

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.

[table of contents | back to top]

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.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1851194

[table of contents | back to top]