Series 1B-3: Multiple stressor experiments on T. pseudonana (CCMP1335) – computed data from the LC3 protocol for samples at 4 temperatures, 15-26C

Website: https://www.bco-dmo.org/dataset/829009
Data Type: experimental
Version: 1
Version Date: 2020-11-12

Project
» Collaborative Research: Effects of multiple stressors on Marine Phytoplankton (Stressors on Marine Phytoplankton)

Contributors	Affiliation	Role
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Abstract
Four follow-up experiments on the combined effect of light and temperature changes on the growth rate (mu) and photophysiology of Thalassiosira pseudonana CCMP 1335 were conducted to supplement / repeat series 1A experiments. This was necessary because doubt existed regarding the growth during 1A experiments. 1A experiments were conducted in artificial seawater. 1B experiments were conducted in artificial seawater supplemented with 5% sterilized seawater. The experiments were designed to test the combined effects of four temperatures, and eight light intensities on the growth and photophysiology of the diatom T. pseudonana CCMP1335 in a multifactorial design. This dataset contains measurements of photophysiology using the Light curve (LC3) protocol of the Aquapen-C AP-C 100 fluorometer.

Table of Contents

Coverage

Temporal Extent: 2018-11-17 - 2019-04-16

Dataset Description

The raw fluorescence data can be found under the Data Files section as an Excel file and as individual .csv files.

Methods & Sampling

Experimental setup:

The experiments were designed to test the combined effects of four temperatures, and eight light intensities on growth and photophysiology of the diatom T. pseudonana CCMP1335 in a multifactorial design. Four temperatures were tested: 15°C, 18°C, 22°C, and 26°C. Within each temperature, eight light levels were tested: 30, 40, 70,90,105,125,140 and 265 µmol photons · m-2 · s-1. All lights were set at a 12 h day: 12 h dark cycle. For logistical reasons, experiments were partially conducted in series.

Experiments were conducted in Multicultivator MC-1000 OD units (Photon Systems Instruments, Drasov, Czech Republic). Each unit consists of eight 85 ml test-tubes immersed in a thermostated water bath, each independently illuminated by an array of cool white LEDs set at specific intensity and timing. A 0.2µm filtered ambient air was bubbled through sterile artificial seawater, and the humidified air was supplied to each tube Each experiment was split into two phases: An acclimation phase spanning 3 days, was used to acclimate cultures to their new environment. Pre-acclimated, exponentially-growing cultures were then inoculated into fresh media and incubated through a 4-day experimental phase during which assessments of growth, photophysiology, and nutrient cycling were carried out daily. All sampling started 6 hours into the daily light cycle to minimize the effects of diurnal cycles.

Experiments were conducted with artificial seawater (ASW) prepared using previously described methods (Kester et. al 1967), and enriched with 50mL per liter of UV sterilized natural seawater and nitrate (NO3), phosphate (PO4), silicic acid (Si[OH]4), at levels ensuring that the cultures would remain nutrient-replete over the course of the experiment. Trace metals and vitamins were added as in f/2 (Guillard 1975). The pH of the growth media was measured spectrophotometrically using the m-cresol purple method (Dickson 1993), and adjusted using 0.1N HCl or 0.1M NaOH.

Photophysiology

Photophysiology was assessed daily using a handheld Pulse Amplitude Modulated (PAM) fluorometer (AquaPen-C AP-C 100, Photon System Instruments, Czech Republic). A sample was collected from each light treatment for each, 5 hours after the start of the daily light cycle, and placed in the dark for a minimum of 30 minutes prior to measurements. The dark-adapted sample was used to generate light curves that provide measurements of in-vivo chlorophyll autofluorescence (F0), the maximum quantum yield (QYmax or Fv/Fm), and relative photosynthesis rates based on PSII quantum yields at varying light intensities - using the instrument's LC3 protocol. The LC3 protocol involves measurements of baseline and maximal fluorescence over seven 60-second phases, with each phase representing a light intensity from 10 to 1000 µmol photons m-2 · s-1. Blue light (455 nm) was used as actinic light in these experiments, and measurements were made at measuring illumination (f-pulse) intensity of 0.03 µmol photons m-2 · s-1, and saturating (F-pulse) illumination of 2100 µmol photons m-2 · s-1, and actinic illumination (A-pulse) controlled by the instrument's protocol were set at 10, 20, 50, 100, 300, 500, and 1000 µmol photons m-2 · s-1 (for each 60-second phase).

Data Processing Description

BCO-DMO Processing Notes:
- data submitted in Excel file "BCODMO_Series 1B - 3_photophysiology_4Aug2020.xlsx" sheets "15C COMPUTED", "18C COMPUTED", "22C COMPUTED", and "26C COMPUTED".
- the four tables were concatenated and transformed so rows became columns.
- extracted the table to csv
- added conventional header with dataset name, PI name, version date

[table of contents | back to top]

Data Files

File	
1B_photophys.csv	(Comma Separated Values (.csv), 26.57 KB) MD5:895cb8ce9e1c7b75d275fd3175c41f78
Primary data file for dataset ID 829009	
Raw Fluorescence Data - parameter descriptions	
filename: Passow_Series_1B_3_photophys_param_descriptions_RawData.csv	(Comma Separated Values (.csv), 2.66 KB) MD5:946696ceb6f1b4414f02692e4172afa4
Descriptions of rows and columns for fluorescence Raw Data.	
Raw Fluorescence Data 15C	
filename: Passow_Series_1B_3_photophys_15C_RawData.csv	(Comma Separated Values (.csv), 17.43 KB) MD5:4a42412df5258c87e0539cffa2db9442
Raw data: fluorescence measurements from the LC3 protocol for samples at 15C at eight light levels.	
Raw Fluorescence Data 18C	
filename: Passow_Series_1B_3_photophys_18C_RawData.csv	(Comma Separated Values (.csv), 13.75 KB) MD5:6d96efeea568281191e7d108217135bd
Raw data: fluorescence measurements from the LC3 protocol for samples at 18C and 8 light levels.	
Raw Fluorescence Data 22C	
filename: Passow_Series_1B_3_photophys_22C_RawData.csv	(Comma Separated Values (.csv), 18.05 KB) MD5:fae88908e6a0fa681078ed8ff31646a9
Raw data: fluorescence measurements from the LC3 protocol for samples at 22C and 8 light levels.	
Raw Fluorescence Data 26C	
filename: Passow_Series_1B_3_photophys_26C_RawData.csv	(Comma Separated Values (.csv), 20.31 KB) MD5:b1bf46482ceeebf2c68dc3b790dcf25e
Raw data: fluorescence measurements from the LC3 protocol for samples at 26C and 8 light levels.	
Raw Fluorescence Data	
filename: Passow_Series_1B_3_photophys_RawData.xlsx	(Microsoft Excel, 77.53 KB) MD5:1ab7778f5afc7bfa7edda6db116ea1e2
Raw data: fluorescence measurements from the LC3 protocol for samples at 15C, 18C, 22C, and 26C and 8 light levels.	

[table of contents | back to top]

Related Publications

Dickson, A. G. (1993). The measurement of sea water pH. Marine Chemistry, 44(2-4), 131–142. doi:10.1016/0304-4203(93)90198-w https://doi.org/10.1016/0304-4203(93)90198-W
Methods

Dickson, A. G., & Millero, F. J. (1987). A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep Sea Research Part A. Oceanographic Research Papers, 34(10), 1733–1743. doi:10.1016/0198-0149(87)90021-5
Methods

Guillard, R. R. L. (1975). Culture of Phytoplankton for Feeding Marine Invertebrates. Culture of Marine Invertebrate Animals, 29–60. doi:10.1007/978-1-4615-8714-9_3
Methods

Kester, D. R., Duedall, I. W., Connors, D. N., & Pytkowicz, R. M. (1967). Preparation of Artificial Seawater 1. Limnology and Oceanography, 12(1), 176–179. doi:10.4319/lo.1967.12.1.0176
Methods

Mehrbach, C., Culberson, C. H., Hawley, J. E., & Pytkowicx, R. M. (1973). Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnology and Oceanography, 18(6), 897–907. doi:10.4319/lo.1973.18.6.0897
Methods

Pierrot, D. E. Lewis,and D. W. R. Wallace. 2006. MS Excel Program Developed for CO2 System Calculations. ORNL/CDIAC-105a. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee. doi: 10.3334/CDIAC/otg.CO2SYS_XLS_CDIAC105a.
Methods

[table of contents | back to top]

Parameters

Parameter	Description	Units
Phase	Indicates whether the sample was collected during the acclimation phase or the experiment phase of the experiment.	unitless
Temperature	Indicates the temperature at which the samples were incubated.	degrees Celsius
Day	Indicates the timepoint (day) of sampling. D0 = day 0; D1 = day 1; etc.	unitless
Replicate	Indicates replication within a treatment. "NA" indicates "not applicable"	unitless
Irradiance	Irradiance level: SOL = sub-optimum light; OL = optimum light; EL = extreme light	unitless
Fo	minimum fluorescence in dark-adapted state.	RFU (Relative Fluorscence Units)
Fm	the maximum fluorescence in dark-adapted state; measured during the first saturation flash after dark adaptation	RFU (Relative Fluorscence Units)
QY_max	The maximum Quantum yield. A measure of the Photosystem II efficiency. In a dark-adapted sample this is equivalent to Fv/Fm. In a light-adapted sample it is equivalent to Fv'/Fm'.	unitless
Fm_L1	The first measurement of the maximum fluorescence following exposure to actinic light at 10 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	RFU (Relative Fluorscence Units)
Fm_L2	The second measurement of the maximum fluorescence following exposure to actinic light at 20 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	RFU (Relative Fluorscence Units)
Fm_L3	The third measurement of the maximum fluorescence following exposure to actinic light at 50 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	RFU (Relative Fluorscence Units)
Fm_L4	The fourth measurement of the maximum fluorescence following exposure to actinic light at 100 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	RFU (Relative Fluorscence Units)

Fm_L5	The fifth measurement of the maximum fluorescence following exposure to actinic light at 300 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	RFU (Relative Fluorscence Units)
Fm_L6	The sixth measurement of the maximum fluorescence following exposure to actinic light at 500 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	RFU (Relative Fluorscence Units)
Fm_L7	The seventh measurement of the maximum fluorescence following exposure to actinic light at 1000 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	RFU (Relative Fluorscence Units)
Ft_L1	The first measurement of the maximum fluorescence following exposure to actinic light at 10 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	RFU (Relative Fluorscence Units)
Ft_L2	The second measurement of the maximum fluorescence following exposure to actinic light at 20 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	RFU (Relative Fluorscence Units)
Ft_L3	The third measurement of the maximum fluorescence following exposure to actinic light at 50 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	RFU (Relative Fluorscence Units)
Ft_L4	The fourth measurement of the maximum fluorescence following exposure to actinic light at 100 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	RFU (Relative Fluorscence Units)
Ft_L5	The fifth measurement of the maximum fluorescence following exposure to actinic light at 300 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	RFU (Relative Fluorscence Units)
Ft_L6	The sixth measurement of the maximum fluorescence following exposure to actinic light at 500 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	RFU (Relative Fluorscence Units)
Ft_L7	The seventh measurement of the maximum fluorescence following exposure to actinic light at 1000 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	RFU (Relative Fluorscence Units)
QY_L1	The first measurement of the instantenous photosystem II quantum yield following exposure to actinic light at 10 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	unitless
QY_L2	The second measurement of the instantenous photosystem II quantum yield following exposure to actinic light at 20 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	unitless

QY_L3	The third measurement of the instantenous photosystem II quantum yield following exposure to actinic light at 50 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	unitless
QY_L4	The fourth measurement of the instantenous photosystem II quantum yield following exposure to actinic light at 100 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	unitless
QY_L5	The fifth measurement of the instantenous photosystem II quantum yield following exposure to actinic light at 300 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	unitless
QY_L6	The sixth measurement of the instantenous photosystem II quantum yield following exposure to actinic light at 500 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	unitless
QY_L7	The seventh measurement of the instantenous photosystem II quantum yield following exposure to actinic light at 1000 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	unitless

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	Multicultivator MC-1000 OD (Photon Systems Instruments, Drasov, Czech Republic)
Generic Instrument Name	Cell Cultivator
Dataset-specific Description	Used for incubation of TP1014 cultures.
Generic Instrument Description	An instrument used for the purpose of culturing small cells such as algae or bacteria. May provide temperature and light control and bubbled gas introduction.

Dataset-specific Instrument Name	Aquapen-C AP-C 100 (Photon Systems Instruments)
Generic Instrument Name	Fluorometer
Dataset-specific Description	Used for assessment of photochemistry.
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	Spectrophotometer (Genesys 10SVIS)
Generic Instrument Name	Spectrophotometer
Dataset-specific Description	Used for measurement of pH.
Generic Instrument Description	An instrument used to measure the relative absorption of electromagnetic radiation of different wavelengths in the near infra-red, visible and ultraviolet wavebands by samples.

[table of contents | back to top]

Project Information

Collaborative Research: Effects of multiple stressors on Marine Phytoplankton (Stressors on Marine Phytoplankton)

The overarching goal of this project is to develop a framework for understanding the response of phytoplankton to multiple environmental stresses. Marine phytoplankton, which are tiny algae, produce as much oxygen as terrestrial plants and provide food, directly or indirectly, to all marine animals. Their productivity is thus important both for global elemental cycles of oxygen and carbon, as well as for the productivity of the ocean. Globally the productivity of marine phytoplankton appears to be changing, but while we have some understanding of the response of phytoplankton to shifts in one environmental parameter at a time, like temperature, there is very little knowledge of their response to simultaneous changes in several parameters. Increased atmospheric carbon dioxide concentrations result in both ocean acidification and increased surface water temperatures. The latter in turn leads to greater ocean stratification and associated changes in light exposure and nutrient availability for the plankton. Recently it has become apparent that the response of phytoplankton to simultaneous changes in these growth parameters is not additive. For example, the effect of ocean acidification may be severe at one temperature-light combination and negligible at another. The researchers of this project will carry out experiments that will provide a theoretical understanding of the relevant interactions so that the impact of climate change on marine phytoplankton can be predicted in an informed way. This project will engage high schools students through training of a teacher and the development of a teaching unit. Undergraduate and graduate students will work directly on the research. A cartoon journalist will create a cartoon story on the research results to translate the findings to a broader general public audience.

Each phytoplankton species has the capability to acclimatize to changes in temperature, light, pCO2, and nutrient availability - at least within a finite range. However, the response of phytoplankton to multiple simultaneous stressors is frequently complex, because the effects on physiological responses are interactive. To date, no datasets exist for even a single species that could fully test the assumptions and implications of existing models of phytoplankton acclimation to multiple environmental stressors. The investigators will combine modeling analysis with laboratory experiments to investigate the combined influences of changes in pCO2, temperature, light, and nitrate availability on phytoplankton growth using cultures of open ocean and coastal diatom strains (Thalassiosira pseudonana) and an open ocean cyanobacteria species (Synechococcus sp.). The planned experiments represent ideal case studies of the complex and interactive effects of environmental conditions on organisms, and results will provide the basis for predictive modeling of the response of phytoplankton taxa to multiple environmental stresses.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1538602

[table of contents | back to top]