Series 4A: Multiple stressor experiments on the cyanobacteria Synechococcus elongatus CCMP1629 - raw fluorescence readings for photophysiology computations

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

Contributors	Affiliation	Role
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Abstract
The experiments were designed to test the combined effects of two CO2 concentrations, four temperatures, and three light intensities on growth and photophysiology of the cyanobacteria S. elongatus CCMP1629 in a multifactorial design. This dataset contains raw fluorescence measurements for computing photophysiology using the Light curve (LC3) protocol of the Aquapen-C AP-C 100 fluorometer.

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Coverage

Temporal Extent: 2019-07 - 2019-08

Dataset Description

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Methods & Sampling

Experimental setup:

The experiments were designed to test the combined effects of two CO_2 concentrations, four temperatures, and three light intensities on growth and photophysiology of the cyanobacterium S. elongatus CCMP1629 in a multifactorial design. Two CO_2 concentrations were tested: 410 ppm, and 1000 ppm. For each CO_2 concentration, four temperatures were tested: 20°C, 28°C, 36°C, and 44°C. Within each temperature, three light levels were tested: sub-optimum irradiance (SOI) intensity of 50 umol photons · m-2 · s-1, optimum irradiance (OI) intensity of 230 umol photons · m-2 · s-1 and extreme Irradiance (EI) intensity of 600 umol 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, with all light treatments at all four temperatures running simultaneously. This was repeated for each CO_2 concentration.

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 0.2um filtered CO_2-air mix (Praxair Distribution Inc.) was bubbled through sterile artificial seawater, and the humidified gas mix was supplied to each tube via gentle sparging through a 2um stainless steel diffuser. Flow rates were gradually increased over the course of the incubation to compensate for the DIC uptake of actively growing cells, and ranged from <0.04 Liters per minute (LPM) at the start of the incubations to 0.08 LPM in each tube after 2 days. For each CO_2 and temperature level, replication was achieved by incubating three tubes at sub-optimum light intensities, two tubes at optimum light intensity, and three tubes at extreme light intensities. 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 3-day experimental phase during which assessments of growth, photophysiology, and nutrient cycling were carried out daily. All sampling started 5 hours into the daily light cycle to minimize effects of diurnal cycles.

Experiments were conducted with artificial seawater (ASW) prepared using previously described methods (Kester et. al 1967), and enriched with nitrate (NO_3), and phosphate (PO_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 expected DIC concentration, and pH of the growth media was determined for the different pCO_2 and temperatures using the CO2SYS calculator (Pierrot et al. 2006), with constants from Mehrbach et al. (1973, refit by Dickson & Millero 1987), and inputs of temperature, salinity, total alkalinity (2376.5 umol · kg−1), pCO_2, phosphate, and silicic acid. DIC levels in ASW at the start of each phase of the experiments were manipulated by the addition of $NaHCO_3$, and was then maintained by bubbling a CO_2-Air mix through the cultures over the course of the experiments. The pH of the growth media was measured spectrophometrically using the m-cresol purple method (Dickson 1993), and adjusted using 0.1N HCl or 0.1M NaOH. The media was distributed into 75 ml aliquots and each aliquot was inoculated with the S. elongatus CCMP 1629 (SE1629) stock culture at the start of the experiments.

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 tube, 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 umol photons m-2 · s-1. Red light (620 nm) was used as actinic light in these experiments, and measurements were made at measuring illumination (f-pulse) intensity of 0.03 umol photons m-2 · s-1, and saturating (F-pulse) illumination of 2700 umol 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 umol photons m-2 · s-1 (for each 60-second phase).

Data Processing Description

BCO-DMO Processing Notes:
- added conventional header with dataset name, PI name, version date
- modified parameter names to conform with BCO-DMO naming conventions
- changed "NA" to "nd", no data

- unpivoted the top 6 header rows to create a flat table
- concatenated the 410 and 1000 pCO2 tables

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Data Files

File	
4a_photophys_raw.csv	(Comma Separated Values (.csv), 540.92 KB) MD5:a15b3f0d79ecf0a17420b7004bebf4fe
Primary data file for dataset ID 808174	

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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

Dickson, A.G., Sabine, C.L. and Christian, J.R. (Eds.) 2007. Guide to best practices for ocean CO2 measurements. PICES Special Publication 3, 191 pp. ISBN: 1-897176-07-4. URL: https://www.nodc.noaa.gov/ocads/oceans/Handbook_2007.html https://hdl.handle.net/11329/249
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

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Parameters

Parameter	Description	Units
CO2	Indicates the concentration of CO_2 in the CO_2-Air mix that was bubbled through the samples over the course of the experiment	parts per million
Time	Time elapsed	microseconds
Temp	Indicates the temperature at which the samples were incubated.	degrees Celsius
Phase	Indicates whether the sample was collected during the acclimation phase or the experiment phase of the experiment.	unitless
Day	Indicates the timepoint (day) of sampling. D0 = day 0; D1 = day 1; etc.	day
Tube	Indicates the tube number in the multicultivator. The tube numbers Indicate replication within a treatment: T1-T3 = suboptimum irradiance; T4-T5 = optimum irradiance; and T6-T8 = extreme irradiance	unitless
Irradiance	Indicates the irradiance at which the samples were incubated: SOI = sub-optimum irradiance intensity of 50 umol photons · m^{-2} · s^{-1}; OI = optimum irradiance intensity of 230 umol photons · m^{-2} · s^{-1}; and EI = extreme irradiance intensity of 600 umol photons · m^{-2} · s^{-1}.	unitless
fluor	fluorescence measurements of each treatment	relative fluorescence units

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Instruments

Dataset-specific Instrument Name	Multicultivator MC-1000 OD (Qubit Systems)
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	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	Genesys 10SVIS
Generic Instrument Name	Spectrophotometer
Dataset-specific Description	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.

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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.

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Funding

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

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