Series 4A: Multiple stressor experiments on the cyanobacteria Synechococcus elongatus CCMP1629 - Chlorophyll, particulate organic carbon and particulate organic nitrogen.

Website: https://www.bco-dmo.org/dataset/807402
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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 Synechococcus elongatus CCMP1629 in a multifactorial design. This dataset contains measurements of extracted chlorophyll, particulate organic carbon (POC), and particulate organic nitrogen (PON) made over the course of the experiments.

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Coverage

Temporal Extent: 2019-07 - 2019-08

Dataset Description

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 *Synechococcus elongatus* CCMP1629 in a multifactorial design. This dataset contains measurements of extracted chlorophyll, particulate organic carbon (POC), and particulate organic nitrogen (PON) made over the course of the experiments.

Methods & Sampling

Experimental setup:

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 cyanobacterium S. elongatus CCMP1629 in a multifactorial design. Two CO2 concentrations were tested: 410 ppm, and 1000 ppm. For each CO2 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 CO2 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 CO2-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 CO2 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 (NO3), and phosphate (PO4), 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 pCO2 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), pCO2, phosphate, and silicic acid. DIC levels in ASW at the start of each phase of the experiments were manipulated by the addition of NaHCO3, and was then maintained by bubbling a CO2-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.

Organic Carbon and Nitrogen concentrations

Samples were filtered onto pre-combusted GF/F filters, dried at 60°C, and stored at room temperature until analyses of particulate organic carbon (POC), and particulate organic nitrogen (PON). Between 3 and > 10 mL were filtered, with larger filtration volumes used on the final day of the experiment. Samples were analyzed using an elemental analyzer (CEC 44OHA; Control Equipment). Samples where C or N concentrations were below instrument detection limits were flagged.

Chlorophyll

Daily subsamples from each treatment were filtered onto 0.45 um polycarbonate filters and stored at -20°C. Filters were placed in 90% acetone (v/v) overnight at -20°C, and the extracted chlorophyll was measured fluorometrically on a Turner 700 fluorometer (Strickland 1972). Chlorophyll-a liquid standards in 90% acetone (Turner Designs Inc.), and adjustable solid secondary standards (Turner Designs Inc. P/N 8000-952) were used for calibrations, and to calculate the chlorophyll content of the samples.

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

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Data Files

File
4A_CHN_Chl.csv (Comma Separated Values (.csv), 16.16 KB) MD5:d7c9b2e902af824b28725da290acf179 Primary data file for dataset ID 807402

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Related Publications

Clayton, T. D., & Byrne, R. H. (1993). Spectrophotometric seawater pH measurements: total hydrogen ion concentration scale calibration of m-cresol purple and at-sea results. Deep Sea Research Part I: Oceanographic Research Papers, 40(10), 2115–2129. doi:10.1016/0967-0637(93)90048-8
Methods

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

Fangue, N. A., O'Donnell, M. J., Sewell, M. A., Matson, P. G., MacPherson, A. C., & Hofmann, G. E. (2010). A laboratory-based, experimental system for the study of ocean acidification effects on marine invertebrate larvae. Limnology and Oceanography: Methods, 8(8), 441–452. doi:10.4319/lom.2010.8.441
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
Phase	Indicates whether the sample was collected during the acclimation phase or the experiment phase of the experiment.	unitless
CO2	Indicates the concentration of CO2 in the CO2-Air mix that was bubbled through the samples over the course of the experiment	parts per million (ppm)
Temp	Indicates the temperature at which the samples were incubated.	degrees Celsius
Day	Indicates the timepoint (day) of sampling. 0 = day 0; 1 = day 1; etc.	day
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.	micromol photons/meter^2/second
Tube	Indicates the tube number in the multicultivator. The tube numbers indicate replication within a treatment: T1-T3 = suboptimum irradiance; T4-T5 = optimum irradiance; T6-T8 = extreme irradiance	unitless
Vol_filtered_CHN	Indicates the volume of sample (in ml) filtered for CHN analyses	milliliters
C_ug	Organic carbon concentration in sample	micrograms
N_ug	Organic Nitrogen concentration in sample	micrograms
C_N	Ratio of organic carbon to organic nitrogen in a sample	unitless
C_detect_lim	Indicates the organic carbon detection limit (DL) of the instrument at the time each sample was analyzed	micrograms
N_detect_lim	Indicates the organic carbon detection limit (DL) of the instrument at the time each sample was analyzed	micrograms
Vol_filtered_Chl	Volume of the sample that was filtered for chlorophyll analyses	milliliters
Chl_ug_L	Chlorophyll concentration	micrograms/liter

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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	Elemental analyzer (CEC 44OHA; Control Equipment)
Generic Instrument Name	CHN Elemental Analyzer
Dataset-specific Description	Used for analysis of total organic carbon content.
Generic Instrument Description	A CHN Elemental Analyzer is used for the determination of carbon, hydrogen, and nitrogen content in organic and other types of materials, including solids, liquids, volatile, and viscous samples.

Dataset-specific Instrument Name	Turner 700 fluorometer
Generic Instrument Name	Fluorometer
Dataset-specific Description	Used for fluorometric analyses of extracted chlorophyll.
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

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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