Series 4A: Multiple stressor experiments on Synechococcus elongatus (CCMP1629) – Dissolved Inorganic Carbon (DIC)

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

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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 Dissolved Inorganic Carbon (DIC) made over the course of the experiments.

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Coverage

Temporal Extent: 2019-07 - 2019-08

Dataset Description

The experiments in Series 4A 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 reports the Dissolved Inorganic Carbon (DIC) levels measured during the experiments.

Methods & Sampling

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 *Synechococcus 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 $\cdot$ m^{-2} $\cdot$ s^{-1}, optimum irradiance (OI) intensity of 230 umol photons $\cdot$ m^{-2} $\cdot$ s^{-1} and extreme Irradiance (EI) intensity of 600 umol photons $\cdot$ m^{-2} $\cdot$ 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 $\cdot$ 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.

Dissolved Inorganic Carbon (DIC) measurements:

DIC was measured in freshly prepared media, and at the end of the experiment phase. 25 ml of the sample was siphoned into clean glass serum vials, fixed with HgCL2 (0.035 % final conc. v/v), and sealed with butyl rubber septa. Samples were stored at 4°C until analyzed. Prior experiments had confirmed that no gas exchange, and/or change in DIC occurred during sample storage for up to 30 days using this method. Total dissolved inorganic carbon (TCO2) samples were analyzed using an automated infrared inorganic carbon analyzer (AIRICA). The AIRICA-23 (MARIANDA, Kiel, Germany), is a high precision instrument used to measure total dissolved inorganic carbon in seawater. The system uses a high precision syringe and a mass flow controller to deliver a known volume of sample into a stripper where it is then acidified, converting the inorganic carbon species into CO2 and delivered under constant flow to nondispersive infrared detector. The CO2 is then carried using an inert reference gas (N2) into a LICOR-7000 that measures pCO2 using the difference in infrared absorbance between a sample and reference cell. The pCO2 is recorded over time and integrated by the AIRICA software. This integrated value is proportional to the amount of dissolved inorganic carbon evolved from the sample and converted to carbon units using a conversion factor (CT Factor). The CT Factor is determined by calibration of the system against a certified reference material of known value (Dickson et al. 2007. Guide to Best Practices for Ocean CO2 Measurements). The value is converted to gravimetric units (umol/kg) using the volume, temperature and salinity of the sample. In order to check for analytical stability of the system throughout a run, a certified reference material is used in between every 5 samples. Replicate DIC measurements were averaged.

Problem Report:
Target pH calculations were accidentally made for 25°C, so that the actual carbonate system in temperature treatments other than 25°C were vastly different from target.

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

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Data Files

File
4A_DIC.csv (Comma Separated Values (.csv), 2.58 KB) MD5:147844ab950ab13816baebe9afef2241 Primary data file for dataset ID 807010

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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 CO2 in the CO2-Air mix that was bubbled through the samples over the course of the experiment	parts per million (ppm)
Temperature	Indicates the temperature at which the samples were incubated.	degrees Celsius
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
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. 0 = day 0; 1 = day 1; etc.	day
DIC	Dissolved inorganic carbon concentration in each sample	micromole/kilogram

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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	AIRICA-23 (MARIANDA, Kiel, Germany)
Generic Instrument Name	Inorganic Carbon Analyzer
Dataset-specific Description	An Automated infrared inorganic carbon analyzer (AIRICA) for analysis of dissolved inorganic carbon.
Generic Instrument Description	Instruments measuring carbonate in sediments and inorganic carbon (including DIC) in the water column.

Dataset-specific Instrument Name	LICOR-7000
Generic Instrument Name	Inorganic Carbon Analyzer
Dataset-specific Description	A CO2/H2O Analyzer for analysis of dissolved inorganic carbon.
Generic Instrument Description	Instruments measuring carbonate in sediments and inorganic carbon (including DIC) in the water column.

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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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