# Series 4A: Multiple stressor experiments on the cyanobacteria Synechococcus elongatus CCMP1629 – cell abundance and size by flow cytometry

**Website**: https://www.bco-dmo.org/dataset/807327
**Data Type**: experimental
**Version**: 1
**Version Date**: 2020-04-01

**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 cell abundances and cell size expressed as forward scatter (FSC) as well as in equivalent spherical diameter (ESD) in um.

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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 cell abundances and cell size expressed as forward scatter (FSC) as well as in equivalent spherical diameter (ESD) in um.

### 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 Synechococcus 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.

**Flow cytometry:**

Samples were fixed in Hexamethylenetetramine-buffered formaldehyde (final concentration 1% v/v) and stored at 4°C in the dark for a maximum of 4 days. Cell counts were confirmed to be unaffected over storage for up to a week. Samples were analyzed on a Guava easyCyte HT Benchtop Flow Cytometer (Millipore-Sigma, USA). All data acquisitions were done with logarithmic signal amplification. Cytometer sample flow rates were kept low (0.24 uL · s-1) to accommodate high cell concentrations. Cyanobacteria were identified based on size and chlorophyll autofluorescence using the forward scatter channel (FSC) and Red-FL (695/50 nm) channel respectively. Growth rates were derived by fitting an exponential curve to cell concentrations vs. time for a 48-hour period during which cells exhibited maximum exponential growth in the experimental phase. Growth rates in treatments where cells did not grow, or declined in abundance were listed as 0. Particle sizes (equivalent spherical diameter in um, ESD) were derived from FSC using size-calibration beads of known diameters ranging from 2 um to 10 um (Particle Size standard kit, Spherotech Inc.).

**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" for no data

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## Data Files

| File |
| --- |
| **4A_size_abund.csv** (Comma Separated Values (.csv), 31.45 KB) MD5:f0a721dd37821db0ac35b4b4231052b5 |
| Primary data file for dataset ID 807327 |

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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*

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 |
| Gated_Count | Indicates the number of 'events' counted in the gate representative of Synechococcus elongatus cells (based on FSC and red fluorescence). | events |
| Concentration | Cell abundance. Treatments where abundances were not available (single sample; due to instrument error) are listed as NC (i.e. 'not counted'). | cells/milliliter |
| Mean_FSC | Mean Forward Scatter of cells | forward scatter channel relative units |
| Median_FSC | Median Forward Scatter of cells | forward scatter channel relative units |
| Coeff_Var_FSC | Coefficient of variation for mean Forward Scatter of cells (%) | unitless |
| Mean_cell_size | Mean cell size of cells; (Estimated Spherical Diameter; derived from forward scatter FSC) | microns |

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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** | Guava easyCyte HT Benchtop Flow Cytometer (Millipore-Sigma, USA) |
| **Generic Instrument Name** | Flow Cytometer |
| **Dataset-specific Description** | Used to measure abundance and forward scatter (proxy for cell size). |
| **Generic Instrument Description** | Flow cytometers (FC or FCM) are automated instruments that quantitate properties of single cells, one cell at a time. They can measure cell size, cell granularity, the amounts of cell components such as total DNA, newly synthesized DNA, gene expression as the amount messenger RNA for a particular gene, amounts of specific surface receptors, amounts of intracellular proteins, or transient signalling events in living cells. Description from: http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm |

| | |
|---|---|
| **Dataset-specific Instrument Name** | Genesys 10SVIS spectrophotometer |
| **Generic Instrument Name** | Spectrophotometer |
| **Dataset-specific Description** | Used to measure 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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