Series 2A-1: Multiple stressor experiments on T. pseudonana (CCMP1014) – cell abundance and cell size in experiments

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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
These experiments were designed to test the combined effects of temperatures and light intensity on the growth growth rate (mu) and photophysiology of the diatom Thalassiosira pseudonana CCMP 1014 in a multifactorial design. Experiments were conducted in artificial seawater supplemented with 5% sterilized seawater. Six temperatures (13.5°C, 20°C, 25°C, 29°C, 31°C, and 32.5°C), and eight light intensities (25, 50, 80, 115,190, 300, 400 and 600 umol photons · m-2 · s-1) were tested during the course of these experiments. This dataset contains measurements of cell abundances and cell size expressed as forward scatter (FSC) as well as in equivalent spherical diameter (ESD) in microns.

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Coverage

Temporal Extent: 2018-01-22 - 2018-02-28

Methods & Sampling

Experiments were conducted in the lab at the University of California Santa Barbara.

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 CCMP1014 in a multifactorial design. Six temperatures were tested: 13.5°C, 20°C, 25°C, 29°C, 31°C, and 32.5°C. Within each temperature, eight light levels were tested: 25, 50, 80, 115,190, 300, 400 and 600 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 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.

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 µL · s-1) to accommodate high cell concentrations. Diatoms 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 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 µm, ESD) were derived from FSC using size-calibration beads of known diameters ranging from 2 µm to 10 µm (Particle Size standard kit, Spherotech Inc.).

Problem report:
- No data was collected from treatments grown at 32.5°C as this extreme temperature inhibited growth. Growth rate was zero.
- Samples were lost from several treatments incubated at 20 and 25°C

Data Processing Description

BCO-DMO Processing Notes:
- data submitted in Excel file "2a_cell_abund_size: BCODMO_Series 2A - 1_cellabund_size.xlsx" sheets "Abundance_ExptPhase" and "CellSize_ExptPhase1" extracted to csv
- the two sheets were joined into a single table
- added conventional header with dataset name, PI name, version date
- renamed columns to conform with BCO-DMO naming conventions (removed units and special characters)

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Data Files

File
2A_cellabund_size.csv (Comma Separated Values (.csv), 7.85 KB) MD5:9e3cc90f95958d07f10697f56953b94f Primary data file for dataset ID 829675

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Related Publications

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

Sweet, J. A. (2020). A tale of two drivers: Exploring the response of the marine diatom, Thalassiosira pseudonana to changes in temperature and irradiance (Order No. 28092894). Available from ProQuest Dissertations & Theses A&I; ProQuest Dissertations & Theses Global. (2455969153). Sweet, J. A. (2020). A tale of two drivers: exploring the response of the marine diatom, Thalassiosira pseudonana to changes in temperature and irradiance. UC Santa Barbara. ProQuest ID: Sweet_ucsb_0035N_14840. Merritt ID: ark:/13030/m5pw207f. Retrieved from https://escholarship.org/uc/item/28m0d4h6
Results

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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
Temp	Indicates the temperature at which the samples were incubated.	degrees Celsius
Irradiance	Indicated light level at which the samples were incubated units of µmol photons m-2 s-1	micromol photons/meter^2/second
Day	Indicates the timepoint (day) of sampling. D0 = day 0; D1 = day 1; etc.	unitless
Replicate	Indicates replication within a treatment if applicable (not applicable).	unitless
abundance_cells_mL	cell abundance	cells/milliliter
FSC	Forward Scatter for cells	FSC relative units
ESD	Equivalent Spherical Diameter for cells	ESD microMolar

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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	Guava easyCyte HT Benchtop Flow Cytometer (Millipore-Sigma, USA)
Generic Instrument Name	Flow Cytometer
Dataset-specific Description	Used for measuring 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. (from: http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm)

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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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