Series 3A: Multiple stressor experiments on T. pseudonana (CCMP1014) - photophysiology measurements

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

Contributors	Affiliation	Role
Passow, Uta	University of California-Santa Barbara (UCSB-MSI)	Principal Investigator
Laws, Edward	Louisiana State University (LSU-CC&E [formerly SC&E])	Co-Principal Investigator
D'Souza, Nigel	University of California-Santa Barbara (UCSB-MSI)	Scientist, Contact
Copley, Nancy	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
The experiments were designed to test the combined effects of CO2, temperatures, and light on the growth of the diatom T. pseudonana CCMP1014 in a multifactorial design. This dataset contains measurements of photophysiology using the Light curve (LC3) protocol of the Aquapen-C AP-C 100 fluorometer.

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Coverage

Temporal Extent: 2018-07-01 - 2018-10-31

Dataset Description

The experiments in Series 3A were designed to test the combined effects of three CO2 concentrations, four temperatures, and three light intensities on growth and photophysiology of the diatom T. pseudonana CCMP1014 in a multifactorial design. This dataset contains measurements of photophysiology using the Light curve (LC3) protocol of the Aquapen-C AP-C 100 fluorometer.

The raw fluorescence data file are availble as Supplemental Data via this landing page.

Methods & Sampling

Three CO2 concentrations were tested: 410 ppm, 750 ppm, and 1000 ppm respectively. For each CO2 concentration, four temperatures were tested: 15 degrees-C, 20 degrees-C, 25 degrees-C, and 30 degrees-C. Within each temperature, three light levels were tested: a sub-optimum light (SOL) intensity of 60 umol

photons · m-2 · s-1, an optimum light (OL) intensity of 400 umol photons · m-2 · s-1 and an extreme light (EL) intensity of 800 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 two temperatures (either 15 degrees-C and 25 degrees-C or 20 degrees-C and 30 degrees-C) 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 4 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 the 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), 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 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 5 ml of the T. pseudonana CCMP 1014 (TP1014) 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 light treatment for each, 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 µmol photons m-2 · s-1. Blue light (455 nm) was used as actinic light in these experiments, and measurements were made at measuring illumination (f-pulse) intensity of 0.03 µmol photons m-2 · s-1, and saturating (F-pulse) illumination of 2100 micro-mol 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 micro-mol 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 "NA"
- combined all CO2 (410, 750 and 1000 ppm) into a single table
- added column 'CO2_expt'; removed column 'Computed_component' (no data present)

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Data Files

File	
3A_photophysiology.csv Primary data file for dataset ID 771461	(Comma Separated Values (.csv), 29.23 KB) MD5:07e580a073f74ee511df0e662edab9bc
Raw Fluorescence Data filename: Passow_DSouza_Series_3A_2_photophys_RawData.xlsx Raw data: fluorescence measurements from the LC3 protocol for samples at 410, 750, and 1000 ppm CO2, at 4 temperatures, and 3 light levels	(Octet Stream, 83.68 KB) MD5:e777234b257219e36037b3e5e8cd7412

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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_expt	Indicates the experiment CO2 level: 410/750/1000 ppm	unitless
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)

Temperature	Indicates the temperature at which the samples were incubated.	degrees Celsius
Day	Indicates the timepoint (day) of sampling. D0 = day 0; D1 = day 1; etc.	unitless
Replicate	Indicates replication within a treatment. "NA" indicates "not applicable"	unitless
Irradiance	Irradiance level: SOL = sub-optimum light; OL = optimum light; EL = extreme light	unitless
Fo	minimum fluorescence in dark-adapted state.	RFU (Relative Fluorscence Units)
Fm	the maximum fluorescence in dark-adapted state; measured during the first saturation flash after dark adaptation	RFU (Relative Fluorscence Units)
QY_max	The maximum Quantum yield. A measure of the Photosystem II efficiency. In a dark-adapted sample this is equivalent to Fv/Fm. In a light-adapted sample it is equivalent to Fv'/Fm'.	unitless
Fm_L1	The first measurement of the maximum fluorescence following exposure to actinic light at 10 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	RFU (Relative Fluorscence Units)
Fm_L2	The second measurement of the maximum fluorescence following exposure to actinic light at 20 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	RFU (Relative Fluorscence Units)
Fm_L3	The third measurement of the maximum fluorescence following exposure to actinic light at 50 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	RFU (Relative Fluorscence Units)
Fm_L4	The fourth measurement of the maximum fluorescence following exposure to actinic light at 100 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	RFU (Relative Fluorscence Units)
Fm_L5	The fifth measurement of the maximum fluorescence following exposure to actinic light at 300 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	RFU (Relative Fluorscence Units)
Fm_L6	The sixth measurement of the maximum fluorescence following exposure to actinic light at 500 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	RFU (Relative Fluorscence Units)
Fm_L7	The seventh measurement of the maximum fluorescence following exposure to actinic light at 1000 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	RFU (Relative Fluorscence Units)
Ft_L1	The first measurement of the maximum fluorescence following exposure to actinic light at 10 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	RFU (Relative Fluorscence Units)
Ft_L2	The second measurement of the maximum fluorescence following exposure to actinic light at 20 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	RFU (Relative Fluorscence Units)

Ft_L3	The third measurement of the maximum fluorescence following exposure to actinic light at 50 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	RFU (Relative Fluorscence Units)
Ft_L4	The fourth measurement of the maximum fluorescence following exposure to actinic light at 100 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	RFU (Relative Fluorscence Units)
Ft_L5	The fifth measurement of the maximum fluorescence following exposure to actinic light at 300 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	RFU (Relative Fluorscence Units)
Ft_L6	The sixth measurement of the maximum fluorescence following exposure to actinic light at 500 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	RFU (Relative Fluorscence Units)
Ft_L7	The seventh measurement of the maximum fluorescence following exposure to actinic light at 1000 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	RFU (Relative Fluorscence Units)
QY_L1	The first measurement of the instantenous photosystem II quantum yield following exposure to actinic light at 10 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	unitless
QY_L2	The second measurement of the instantenous photosystem II quantum yield following exposure to actinic light at 20 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	unitless
QY_L3	The third measurement of the instantenous photosystem II quantum yield following exposure to actinic light at 50 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	unitless
QY_L4	The fourth measurement of the instantenous photosystem II quantum yield following exposure to actinic light at 100 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	unitless
QY_L5	The fifth measurement of the instantenous photosystem II quantum yield following exposure to actinic light at 300 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	unitless
QY_L6	The sixth measurement of the instantenous photosystem II quantum yield following exposure to actinic light at 500 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	unitless
QY_L7	The seventh measurement of the instantenous photosystem II quantum yield following exposure to actinic light at 1000 micro-mol photons·m-2·sec-1 for 60 seconds (L1 indicates the first measurement in the "light" phase)	unitless

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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	hand-held Aquapen-C AP-C 100 (Photon Systems Instruments)
Generic Instrument Name	Fluorometer
Dataset-specific Description	Used 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 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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