

# Series 2A-3: Multiple stressor experiments on *T. pseudonana* (CCMP1014) – Chlorophyll, particulate organic carbon and particulate organic nitrogen

**Website:** <https://www.bco-dmo.org/dataset/829707>

**Data Type:** experimental

**Version:** 1

**Version Date:** 2020-11-18

## 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 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  $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) were tested during the course of these experiments. 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:** 2018-01-22 - 2018-02-28

## Acquisition Description

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, 450 and 600  $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ . 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 0.2 $\mu\text{m}$  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 ( $\text{NO}_3$ ), phosphate ( $\text{PO}_4$ ), silicic acid ( $\text{Si}[\text{OH}]_4$ ), 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.

### **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). Samples were analyzed using an elemental analyzer (CEC 440HA; Control Equipment). Samples where C or N concentrations were below instrument detection limits (columns J and K) were flagged (column I).

### **Chlorophyll**

Daily subsamples from each treatment were filtered onto 0.45  $\mu\text{m}$  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 (Column M)

Problem report: No data was collected from treatments grown at 32.5°C as this extreme temperature inhibited growth. Growth rate was zero.

### **Processing Description**

#### **BCO-DMO Processing Notes:**

- data submitted in Excel file "BCODMO\_Series 2A - 3\_CHN\_Chla.xlsx" sheet "C N and Chl" extracted to csv
- 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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### **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](https://doi.org/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](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](https://doi.org/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 CO<sub>2</sub> Measurements. PICES Special Publication 3, 191 pp <https://isbnsearch.org/isbn/1-897176-07-4>  
*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](https://doi.org/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](https://doi.org/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](https://doi.org/10.4319/lo.1967.12.1.0176)  
*Methods*

Mehrbach, C., Culberson, C. H., Hawley, J. E., & Pytkowicz, 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](https://doi.org/10.4319/lo.1973.18.6.0897)  
*Methods*

Pierrot, D. E. Lewis, and D. W. R. Wallace. 2006. MS Excel Program Developed for CO<sub>2</sub> 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](https://doi.org/10.3334/CDIAC/otg.CO2SYS_XLS_CDIAC105a).  
*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
Day	Indicates the timepoint (day) of sampling. D0 = day 0; D1 = day 1; etc.	unitless
Irradiance	Indicates the light intensity	micromol photons/meter <sup>2</sup> /second
Replicate	Indicates replication within a treatment	unitless
Reference_Label	Reference label (used internally for verifying sample identity)	unitless
C_ug	Organic carbon concentration (in µg) in sample	micrograms
N_ug	Organic Nitrogen concentration (in µg) in sample	micrograms
Flags	Indicates if C or N levels were below detection limits (DL) of the instrument	unitless
C_detection_limit_ug	Instrument detection limit for organic carbon concentration (in µg)	micrograms
N_detection_limit_ug	Instrument detection limit for organic carbon concentration (in µg)	micrograms
Volume_filtered	Volume of the sample that was filtered	?
Chl_a_ug_L	Chlorophyll concentration	micrograms/liter

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## Instruments

<b>Dataset-specific Instrument Name</b>	Turner 700 fluorometer
<b>Generic Instrument Name</b>	Fluorometer
<b>Dataset-specific Description</b>	Used for analysis of chlorophyll a concentrations.
<b>Generic Instrument Description</b>	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.

<b>Dataset-specific Instrument Name</b>	Elemental analyzer (CEC 440HA; Control Equipment)
<b>Generic Instrument Name</b>	CHN Elemental Analyzer
<b>Dataset-specific Description</b>	Used for analysis of total organic carbon and nitrogen content.
<b>Generic Instrument Description</b>	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.

<b>Dataset-specific Instrument Name</b>	Multicultivator MC-1000 OD (Photon Systems Instruments, Drasov, Czech Republic)
<b>Generic Instrument Name</b>	Cell Cultivator
<b>Dataset-specific Description</b>	Used for incubation of TP1014 cultures.
<b>Generic Instrument Description</b>	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.

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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, pCO<sub>2</sub>, 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 pCO<sub>2</sub>, 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
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-1538602</a>

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