

# Series 3B: Supplemental experiments on *T. pseudonana* (CCMP1014) growth under bubbling stress: LC3 protocol raw fluorescence measurements for non-aerated samples and aerated samples

**Website:** <https://www.bco-dmo.org/dataset/749211>

**Data Type:** experimental

**Version:** 1

**Version Date:** 2018-10-31

## Project

» [Collaborative Research: Effects of multiple stressors on Marine Phytoplankton](#) (Stressors on Marine Phytoplankton)

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

## Abstract

Experiments were conducted to investigate the impact of bubbling on the growth yield of *Thalassiosira pseudonana* CCMP 1014 grown in 80 ml culture tubes. This dataset includes raw fluorescence measurements for non-aerated samples and aerated samples using LC3 protocol.

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## Coverage

**Temporal Extent:** 2018-07-03 - 2018-07-06

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## Dataset Description

Experiments were conducted to investigate the impact of bubbling on the growth yield of *Thalassiosira pseudonana* CCMP 1014 grown in 80 ml culture tubes. This dataset includes LC3 protocol (...) raw fluorescence results for non-aerated samples and aerated samples.

## Methods & Sampling

Experiments were conducted to investigate the impact of bubbling gas through cultures of *Thalassiosira pseudonana* CCMP 1014 grown in Multicultivator MC-1000 OD culture tubes. TP1014 stock cultures were maintained in artificial seawater (Kester et. al 1967), enriched as with f/2 media (Guillard 1975). For the

experiment, 5 ml of the TP1014 stock cultures were inoculated into 75 ml of ASW in eight tubes. The tubes were incubated in a Multicultivator MC-1000 OD unit (Qubit Systems), at 25 deg C and 400  $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{sec}^{-1}$ , set at a 12:12 day:night cycle for four days. Three tubes had no aeration (T1, T2, and T3); three tubes were bubbled with air at 60  $\text{ml} \cdot \text{min}^{-1}$  through a 0.2  $\mu\text{m}$  stainless steel “carbonating stone” (T4, T5, and T6); and two tubes were bubbled with air at 120  $\text{ml} \cdot \text{min}^{-1}$  through a 0.2  $\mu\text{m}$  stainless steel “carbonating stone” (T7 and T8). Samples were collected from each tube at the start of the experiment (day-0), and 5 hours after the start of the light phase each day (i.e. at 24-hour intervals) after that for four days. Samples were always collected 5 hours after the start of the light phase.

Three ml of samples from the non-aerated (T1, T2, and T3) and the 60  $\text{ml} \cdot \text{min}^{-1}$  aeration tubes (T4, T5, and T6) were used for assessment of photochemistry using the Aquapen-C AP-C 100 (Photon Systems Instruments). Samples were placed in the dark at 25 deg C for a minimum of 30 minutes prior to measuring photochemistry. The NPQ1 protocol on the instrument was used for assessment of non-photochemical quenching in samples, and the LC3 protocol was used to generate light curves that provide measurements of photosynthesis rates. The NPQ1 protocol administers 5 light pulses over 60 seconds during actinic light exposure, followed by 3 light pulses over 88 seconds during recovery in the dark. The LC3 protocol involves measurements of baseline fluorescence and maximal fluorescence during 7 phases of 60 seconds each, with each phase representing a light intensity from 10 to 1000  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ . Blue light (455 nm) was used as actinic light in these experiments. Baseline measurements were made at 0.03  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ , Saturating pulses were set at 2100  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ , and actinic light pulses (for the NPQ1 protocol only) were set at 1000  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ .

## 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
<b>3B_bubble_LC_raw.csv</b> (Comma Separated Values (.csv), 2.20 KB) MD5:93c716c6f7d0fe9cbef4402f5eb0510a
Primary data file for dataset ID 749211

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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](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*

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## Parameters

Parameter	Description	Units
time_usec	time elapsed since beginning of run	microseconds
Non_Aerated_T1	fluorescence measurements from the NPQ1 protocol of the AquaPen for un-aerated treatment T1	relative fluorescence units (RFU)
Non_Aerated_T2	fluorescence measurements from the NPQ1 protocol of the AquaPen for un-aerated treatment T2	relative fluorescence units (RFU)
Non_Aerated_T3	fluorescence measurements from the NPQ1 protocol of the AquaPen for un-aerated treatment T3	relative fluorescence units (RFU)
Aerated_T4	fluorescence measurements from the NPQ1 protocol of the AquaPen for aerated treatment T1	relative fluorescence units (RFU)
Aerated_T5	fluorescence measurements from the NPQ1 protocol of the AquaPen for aerated treatment T2	relative fluorescence units (RFU)
Aerated_T6	fluorescence measurements from the NPQ1 protocol of the AquaPen for aerated treatment T3	relative fluorescence units (RFU)

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## Instruments

<b>Dataset-specific Instrument Name</b>	Multicultivator MC-1000 OD (Qubit Systems)
<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.

<b>Dataset-specific Instrument Name</b>	Guava easyCyte HT Sampling Flow Cytometer
<b>Generic Instrument Name</b>	Flow Cytometer
<b>Dataset-specific Description</b>	Used to count cells.
<b>Generic Instrument Description</b>	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: <a href="http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm">http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm</a> )

<b>Dataset-specific Instrument Name</b>	Aquapen-C AP-C 100 (Photon Systems Instruments)
<b>Generic Instrument Name</b>	Fluorometer
<b>Dataset-specific Description</b>	Used to measure fluorescence. A hand-held cuvette version of the FluorPen fluorometer equipped with a blue and red LED emitter. Blue excitation light (455 nm) is intended for chlorophyll excitation, i.e., for measuring chlorophyll fluorescence in algal cultures. Red-orange excitation light (620 nm) is intended for excitation through phycobilins and is suitable for measuring in cyanobacteria.
<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.

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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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