Series 3B: Supplemental experiments on T. pseudonana (CCMP1014) - growth under bubbling stress: flow cytometry measurements

Website: https://www.bco-dmo.org/dataset/749109
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
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
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 the flow cytometry results.

Table of Contents

Coverage

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

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 the flow cytometry results.

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 µmol photons * m-2 * 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 ml·min-1 through a 0.2 µm stainless steel “carbonating stone” (T4, T5, and T6); and two tubes were bubbled with air at 120 ml·min-1 through a 0.2 µm 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.

1 ml of each sample was fixed with buffered formalin (1% final conc. v/v), and stored at 4 deg C. At the end of the experiment, all fixed samples were run through a Guava easyCyte HT Sampling Flow Cytometer. TP1014 cells were identified based on their chlorophyll content (red fluorescence * cell-1), and size (forward scatter). A minimum of 300 cells was counted in each sample (except in one tube, where the culture did not grow well).

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

[table of contents | back to top]

Data Files

File	
3B_bubble_flowcytometry.csv	(Comma Separated Values (.csv), 3.83 KB) MD5:3516d7aa59cd89253465033e8fe6bd29
Primary data file for dataset ID 749109	

[table of contents | back to top]

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

[table of contents | back to top]

Parameters

Parameter	Description	Units
Sample_ID	sample identifier	unitless
Day	the day on which measurements were made. (range = 0 to 4)	day
Treatment	bubbling regime: includes samples with no bubbling; samples bubbled without a diffuser; and samples bubbled with an aquarium diffuser	unitless
Replicate	replicate sample identifier for each treatment	unitless
Concentration_cells_mL	cell concentration	cells/milliliter
FSC_Count	the number of events (cells) counted to generate forward scatter values	cells
FSC_Mean	the mean forward scatter value of TP1014 cells	relative units
FSC_pcnt_CV	the coefficient of variation for FSC expressed as a percentage	unitless
SSC_Count	the number of events (cells) counted to generate forward scatter values	cells
SSC_Mean	the mean side scatter value of TP1014 cells	relative units
SSCpcnt_CV	the coefficient of variation for SSC expressed as a percentage.	unitless
Red_FL_Count	the number of events (cells) counted to generate forward scatter values	cells
Red_FL_Mean	the mean red fluorescence per cell of the TP1014 cells counted	fluorescence/cell
Red_FL_pcnt_CV	the coefficient of variation for red FL expressed as a percentage	unitless

[table of contents | back to top]

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 Sampling Flow Cytometer
Generic Instrument Name	Flow Cytometer
Dataset-specific Description	Used to count cells.
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)

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

[table of contents | back to top]

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.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1538602

[table of contents | back to top]