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

Website: https://www.bco-dmo.org/dataset/749239

**Data Type**: experimental

Version: 1

Version Date: 2018-10-31

#### **Project**

» <u>Collaborative Research: Effects of multiple stressors on Marine Phytoplankton</u> (Stressors on Marine Phytoplankton)

| Contributors          | Affiliation  | Role                         |
|-----------------------|--|------------------------------|
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#### **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 LC3 protocol computed photochemical measurements for non-aerated samples and aerated samples.

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#### 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 LC3 protocol computed photochemical measurements 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  $\mu$ mol 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  $\mu$ m stainless steel "carbonating stone" (T4, T5, and T6); and two tubes were bubbled with air at 120 ml·min-1 through a 0.2  $\mu$ 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 ml·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  $\mu$ mol \* m-2 \* s-1. Blue light (455 nm) was used as actinic light in these experiments. Baseline measurements were made at 0.03  $\mu$ mol \* m-2 \* s-1, Saturating pulses were set at 2100  $\mu$ mol \* m-2 \* s-1, and actinic light pulses (for the NPQ1 protocol only) were set at 1000  $\mu$ mol \* m-2 \* s-1.

#### **Data Processing Description**

**BCO-DMO Processing Notes:** 

- added conventional header with dataset name, PI name, version date
- transformed rows and columns to conform to database best practices (each column has single description and units)
- modified parameter names to conform with BCO-DMO naming conventions
- replaced blank cells with 'nd' for 'no data'

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#### **Data Files**

#### File

**3B\_bubble\_LC\_computed\_trans.csv**(Comma Separated Values (.csv), 2.07 KB)

MD5:1e186bd5abb2ca2d2ead86d37a94185d

Primary data file for dataset ID 749239

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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

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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

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## **Parameters**

| Parameter | Description  | Units                                     |
|-----------|--|---|
| treatment | Treatment formatted as Tn_Apn: a culture grown at a specific light intensity (as described by T(n)) and each of these samples was tested at different A-pulse settings (described as (AP(n)).  | unitless                                  |
| Fo        | minimum fluorescence in dark-adapted state.  | RFU<br>(Relative<br>Fluorscence<br>Units) |
| Fm        | the maximum fluorescence in dark-adapted state; measured during the first saturation flash after dark adaptation   | RFU<br>(Relative<br>Fluorscence<br>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 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase)     | RFU<br>(Relative<br>Fluorscence<br>Units) |
| Fm_L2     | The second measurement of the maximum fluorescence following exposure to actinic light at 20 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase)    | RFU<br>(Relative<br>Fluorscence<br>Units) |
| Fm_L3     | The third measurement of the maximum fluorescence following exposure to actinic light at 50 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase)     | RFU<br>(Relative<br>Fluorscence<br>Units) |
| Fm_L4     | The fourth measurement of the maximum fluorescence following exposure to actinic light at 100 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase)   | RFU<br>(Relative<br>Fluorscence<br>Units) |
| Fm_L5     | The fifth measurement of the maximum fluorescence following exposure to actinic light at 300 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase)    | RFU<br>(Relative<br>Fluorscence<br>Units) |
| Fm_L6     | The sixth measurement of the maximum fluorescence following exposure to actinic light at 500 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase)    | RFU<br>(Relative<br>Fluorscence<br>Units) |
| Fm_L7     | The seventh measurement of the maximum fluorescence following exposure to actinic light at 1000 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase) | RFU<br>(Relative<br>Fluorscence<br>Units) |

| Ft_L1 | The first measurement of the maximum fluorescence following exposure to actinic light at 10 $\mu$ mol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase)                  | RFU<br>(Relative<br>Fluorscence<br>Units) |
|-------|--|---|
| Ft_L2 | The second measurement of the maximum fluorescence following exposure to actinic light at 20 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase)                      | RFU<br>(Relative<br>Fluorscence<br>Units) |
| Ft_L3 | The third measurement of the maximum fluorescence following exposure to actinic light at 50 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase)                       | RFU<br>(Relative<br>Fluorscence<br>Units) |
| Ft_L4 | The fourth measurement of the maximum fluorescence following exposure to actinic light at 100 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase)                     | RFU<br>(Relative<br>Fluorscence<br>Units) |
| Ft_L5 | The fifth measurement of the maximum fluorescence following exposure to actinic light at 300 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase)                      | RFU<br>(Relative<br>Fluorscence<br>Units) |
| Ft_L6 | The sixth measurement of the maximum fluorescence following exposure to actinic light at 500 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase)                      | RFU<br>(Relative<br>Fluorscence<br>Units) |
| Ft_L7 | The seventh measurement of the maximum fluorescence following exposure to actinic light at 1000 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase)                   | RFU<br>(Relative<br>Fluorscence<br>Units) |
| QY_L1 | The first measurement of the instantenous photosystem II quantum yield following exposure to actinic light at 10 µmol photons·m-2·sec-1 for60 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 µmol photons·m-2·sec-1 for60 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 µmol photons·m-2·sec-1 for60 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 µmol photons·m-2·sec-1 for60 seconds  | unitless                                  |

| QY_L5   | The fifth measurement of the instantenous photosystem II quantum yield following exposure to actinic light at 300 µmol photons·m-2·sec-1 for60 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 µmol photons·m-2·sec-1 for60 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 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase) | unitless |
| rETR_L1 | The (calculated) relative Electron Transport Rate following exposure to actinic light at 10 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase)                          | unitless |
| rETR_L2 | The (calculated) relative Electron Transport Rate following exposure to actinic light at 20 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase)                          | unitless |
| rETR_L3 | The (calculated) relative Electron Transport Rate following exposure to actinic light at 50 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase)                          | unitless |
| rETR_L4 | The (calculated) relative Electron Transport Rate following exposure to actinic light at 100 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase)                         | unitless |
| rETR_L5 | The (calculated) relative Electron Transport Rate following exposure to actinic light at 300 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase)                         | unitless |
| rETR_L6 | The (calculated) relative Electron Transport Rate following exposure to actinic light at 500 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase)                         | unitless |
| rETR_L7 | The (calculated) relative Electron Transport Rate following exposure to actinic light at 1000 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase)                        | unitless |
| NPQ_L1  | The (calculated) non photochemical chlorophyll fluorscence quenching following exposure to actinic light at 10 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase)       | unitless |
| NPQ_L2  | The (calculated) non photochemical chlorophyll fluorscence quenching following exposure to actinic light at 20 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase)       | unitless |

| NPQ_L3 | The (calculated) non photochemical chlorophyll fluorscence quenching following exposure to actinic light at 50 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase)   | unitless |
|--------|---|----------|
| NPQ_L4 | The (calculated) non photochemical chlorophyll fluorscence quenching following exposure to actinic light at 100 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase)  | unitless |
| NPQ_L5 | The (calculated) non photochemical chlorophyll fluorscence quenching following exposure to actinic light at 300 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase)  | unitless |
| NPQ_L6 | The (calculated) non photochemical chlorophyll fluorscence quenching following exposure to actinic light at 500 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase)  | unitless |
| NPQ_L7 | The (calculated) non photochemical chlorophyll fluorscence quenching following exposure to actinic light at 1000 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase) | unitless |
| QP_L1  | The (calculated) coefficient of photochemical quenching following exposure to actinic light at 10 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase)                | unitless |
| QP_L2  | The (calculated) coefficient of photochemical quenching following exposure to actinic light at 20 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase)                | unitless |
| QP_L3  | The (calculated) coefficient of photochemical quenching following exposure to actinic light at 50 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase)                | unitless |
| QP_L4  | The (calculated) coefficient of photochemical quenching following exposure to actinic light at 100 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase)               | unitless |
| QP_L5  | The (calculated) coefficient of photochemical quenching following exposure to actinic light at 300 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase)               | unitless |
| QP_L6  | The sixth coefficient of photochemical quenching following exposure to actinic light at 500 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase)                      | unitless |
| QP_L7  | The seventh coefficient of photochemical quenching following exposure to actinic light at 1000 µmol photons·m-2·sec-1 for60 seconds (L1 indicates the first measurement in the "light" phase)                   | unitless |

## Instruments

| Dataset-specific Instrument Name  Multicultivator MC-1000 OD (Qubit Systems) |  |
|--|--|
| Generic<br>Instrument Name   | Cell Cultivator  |
| Dataset-specific<br>Description  | Used for incubation of TP1014 cultures.  |
| Generic<br>Instrument<br>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-<br>specific<br>Instrument<br>Name | Guava easyCyte HT Sampling Flow Cytometer |
|--|---|
| Generic<br>Instrument<br>Name              | Flow Cytometer                            |
| Dataset-<br>specific<br>Description        | Used to count cells.                      |
| Generic<br>Instrument<br>Description       |   |

| Dataset-<br>specific<br>Instrument<br>Name | Aquapen-C AP-C 100 (Photon Systems Instruments)  |
|--|--|
| Generic<br>Instrument<br>Name              | Fluorometer  |
| Dataset-<br>specific<br>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. |
|  | 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, 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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