# SEM based morphology of Emiliania huxleyi, 2011-2012 (E Hux Response to pCO2 project)

**Website**: https://www.bco-dmo.org/dataset/520664
**Data Type**: experimental
**Version**: 2014-07-03

**Project**
» Planktonic interactions in a changing ocean: Biological responses of Emiliania huxleyi to elevated pCO2 and their effects on microzooplankton (E Hux Response to pCO2)

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|---|---|---|
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## Dataset Description

**Culturing**:

Cultures of *E. huxleyi* Strain CCMP2668, were innoculated at low cell density into media prepared from autoclaved filtered seawater with nutrient amendments based on F/2 medium kit from the National Center for Marine Algae and Microbiota, with a 1:25 reduction in nutrient additions. These were allowed to acclimate for approximately five generations, until cell density neared levels likely to significantly change the pH/pCO2. Daily dilutions of cultures with pre-equilibrated media kept cell density low ($<1x10^5$ cells/ml), ensured cells remained in exponential growth phase and prevented excessive drawdown of nutrients and $CO_2$. Cell density was determined by flow cytometry (Model) and each flask was diluted with media that was continuously sparged with air containing 400, 750 or 1000 ppm CO2. Air mixtures were created using CO2 free air (Powerex air compressor, and Twin Towers CO2 scrubber) and pure CO2 (Airgas) combined using a system of mass flow controllers (Sierra Instruments) and verified using a non-dispersive infrared CO2 sensor (Licor 820). Cultures were maintained in 1 liter polycarbonate flasks at $15^0$ C under a 12/12 light dark cycle. Replicates (n=5) were placed in Plexiglas chambers which were supplied with a flow of the appropriate air mixture for each treatment. Preliminary experiments showed that gas exchange across the air/water surface significantly helped to maintain the target pCO2 in cultures without the mechanical disturbance of bubbling. Sedimentation was minimized by gentle mixing of the cultures by rotation of the bottles twice a day, during sampling and dilution. Cell densities ranged between about 30,000 cells/ml after dilutions to 80,000 cells/ml on the following day. The culture volume that was removed was used for analyses, and replaced with pre-equilibrated media. Cultures were maintained in this fashion for about 8 days. Since the first dilution occurred on day 4 after innoculation, this gives a total of 12 to 14 days in culture at experimental conditions. This experiment was carried out twice, in 2011 and 2012.

**SEM**:

On days 1 and 8, a few small volume of culture from each replicate was dropped onto SEM stubs and allowed to dry. The stubs were sputter coated with Palladium gold for approximately three minutes (2012) or one minute (2011). Five images of each replicate with three or more cells in each image were taken at 5000x magnification (Smith et al. 2012) using an FEI Quanta 450 Scanning Electron Microscope. To compose each image the field was zoomed out to about 200x magnification to reduce bias in finding clusters of three or more cells. When a cluster of three of more cells was found, the magnification was changed to 5000x magnification and the image was focused and captured.

Parameters including cell size, coccolith size, coccoliths per cell, and percentage of malformed coccoliths were measured from the SEM images. Cell size (control: n= 235, moderate: n= 234, high: n= 200) and coccolith size (control: n= 75, moderate: n= 72, high: n= 67) were calculated using the free hand tool in Image J. To determine the number of coccoliths per cell and the percentage of malformed coccoliths, images were loaded into Windows Photo Viewer (control: n= 1453, moderate: n= 1409, high: n= 1215). Coccoliths malfomation was assessed by the scheme of DeBodt et al, 2010.

**Carbonate chemsitry: (THIS DATASET)**

pH was measured photometrically using 1 cm cuvettes, m-cresol dye and an Agilent 5480 UV-VIS spectrophotometer (+/- 0.02). Alkalinity was measured by gran titration using a Titrando 888, and 0.1 N HCl titrant, in a temperature controlled titration vessel (+/1 5 ueq/kg). Other parameters were calculated with CO2sys. pCO2 conditions were the same in the two experiments with the exception that the moderate concentration had slightly more elevated pCO2 in 2011 (662 ppm compared to 602 ppm). (When day 1 is included for 2012, there is no significant difference; the difference in the Moderate treatment is present when only day 12 and 14 are included.)

**Related dataset:** Emiliania huxleyi carbonate data

**Data Processing Description**

**BCO-DMO Processing notes:**

- original data submitted in Excel file 'Malformation_Data_Sheet_2011_2012_with pivot.xlsx'
- added conventional header with dataset name, PI name, version date, reference information
- changed parameter names to be BCO-DMO compatible
- changed date format from m_d_yy (3_28_11) to yyyy-mm-dd (2011-03-28)
- replaced 'Data' with '0' in incompleteness column, 3_28_s1_4, cell id #2 as per instructions from B. Love
- filled blank cells with 'nd'
- sorted data by date, treatment

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## Data Files

| File |
|---|
| **SEMlog.csv** (Comma Separated Values (.csv), 29.18 KB)<br>MD5:289de9adde026d26df7ad014569ccf11<br>Primary data file for dataset ID 520664 |

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## Parameters

| Parameter | Description | Units |
|---|---|---|
| year | year | yyyy |
| date | date | yyyy-mm-dd |
| month_local | month; local time | mm |
| day_local | day of month; local time | dd |
| yrday_local | local day and decimal time as 326.5 for the 326th day of the year or November 22 at 1200 hours (noon) | unitless |
| treatment | treatment: C=control (400 ppm CO2); M=medium (750 ppm); H=high (1000 ppm) | unitless |
| bottle | culture bottle from which sample was taken | unitless |
| image_id | Each SEM image includes the date, stub number, and image number from that stub. e.g. 3_28_s1_1 is March 28, stub one, image one. | unitless |
| stub | SEM stub number | unitless |
| cell_id | E. huxleyi cell id number. Some images have more than one cell which are designated by cell number | unitless |
| Coccolith_id | E. huxleyi coccolith id number; one coccolith per cell is analyzed | unitless |
| incompleteness | coccolith morphology: ranked 0-3 after the four level ranking system in DeBodt et al, 2010 | unitless |
| comments | comments on image | unitless |

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## Instruments

| | |
|---|---|
| **Dataset-specific Instrument Name** | Automatic titrator |
| **Generic Instrument Name** | Automatic titrator |
| **Dataset-specific Description** | Metrohm 888 Titrando |
| **Generic Instrument Description** | Instruments that incrementally add quantified aliquots of a reagent to a sample until the end-point of a chemical reaction is reached. |

| | |
|---|---|
| **Dataset-specific Instrument Name** | CO2 Analyzer |
| **Generic Instrument Name** | CO2 Analyzer |
| **Dataset-specific Description** | Licor 820: a non-dispersive infrared CO2 sensor |
| **Generic Instrument Description** | Measures atmospheric carbon dioxide (CO2) concentration. |

| | |
|---|---|
| **Dataset-specific Instrument Name** | SEM |
| **Generic Instrument Name** | Electron Microscope |
| **Dataset-specific Description** | FEI Quanta 450 Scanning Electron Microscope |
| **Generic Instrument Description** | Instruments that generate enlarged images of samples using the phenomena of reflection and absorption of electrons behaving as waves. |

| | |
|---|---|
| **Dataset-specific Instrument Name** | Flow Cytometer |
| **Generic Instrument Name** | Flow Cytometer |
| **Dataset-specific Description** | BD FACS Calibur flow cytometer |
| **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** | MFC |
| **Generic Instrument Name** | Mass Flow Controller |
| **Dataset-specific Description** | Sierra Instruments |
| **Generic Instrument Description** | Mass Flow Controller (MFC) - A device used to measure and control the flow of fluids and gases |

| | |
|---|---|
| **Dataset-specific Instrument Name** | spectrophotometer |
| **Generic Instrument Name** | Spectrophotometer |
| **Dataset-specific Description** | Agilent 5480 UV-VIS spectrophotometer (+/- 0.02) |
| **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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## Deployments

**Lab_Love**

| | |
|---|---|
| **Website** | https://www.bco-dmo.org/deployment/521422 |
| **Platform** | WWU |
| **Start Date** | 2011-03-23 |
| **End Date** | 2012-07-16 |
| **Description** | lab experiments |

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## Project Information

**Planktonic interactions in a changing ocean: Biological responses of Emiliania huxleyi to elevated pCO2 and their effects on microzooplankton (E Hux Response to pCO2)**

***Description from NSF award abstract:***
The calcifying Haptophyte *Emiliania huxleyi* appears to be acutely sensitive to the rising concentration of ocean pCO2. Documented responses by *E. huxleyi* to elevated pCO2 include modifications to their calcification rate and cell size, malformation of coccoliths, elevated growth rates, increased organic carbon production, lowering of PIC:POC ratios, and elevated production of the active climate gas DMS. Changes in these parameters are mechanisms known to elicit alterations in grazing behavior by microzooplankton, the oceans dominant grazer functional group. The investigators hypothesize that modifications to the physiology and biochemistry of calcifying and non-calcifying Haptophyte *Emiliania huxleyi* in response to elevated pCO2 will precipitate alterations in microzooplankton grazing dynamics. To test this hypothesis, they will conduct controlled laboratory experiments where several strains of *E. huxleyi* are grown at several CO2 concentrations. After careful characterization of the biochemical and physiological responses of the *E. huxleyi* strains to elevated pCO2, they will provide these strains as food to several ecologically-important microzooplankton and document grazing dynamics. *E. huxleyi* is an ideal organism for the study of phytoplankton and microzooplankton responses to rising anthropogenic CO2, the effects of which in the marine environment are called ocean acidification; *E. huxleyi* is biogeochemically important, is well studied, numerous strains are in culture that exhibit variation in the parameters described above, and they are readily fed upon by ecologically important microzooplankton.

The implications of changes in microzooplankton grazing for carbon cycling, specifically CaCO3 export, DMS production, nutrient regeneration in surface waters, and carbon transfer between trophic levels are profound, as this grazing, to a large degree, regulates all these processes. *E. huxleyi* is a model prey organism because it is one of the most biogeochemically influential global phytoplankton. It forms massive seasonal blooms, contributes significantly to marine inorganic and organic carbon cycles, is a large producer of the climatically active gas DMS, and is a source of organic matter for trophic levels both above and below itself. The planned controlled study will increase our knowledge of the mechanisms that drive patterns of change between trophic levels, thus providing a wider array of tools necessary to understand the complex nature of ocean acidification field studies, where competing variables can confound precise interpretation.

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## Funding


| Funding Source | Award |
|---|---|
| NSF Division of Ocean Sciences (NSF OCE) | OCE-0961229 |


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