

# Experimental results: *Emiliana huxleyi* growth rates under different pCO<sub>2</sub> levels from Bellingham, WA from 2011-2012 (E Hux Response to pCO<sub>2</sub> project)

**Website:** <https://www.bco-dmo.org/dataset/521279>

**Data Type:** experimental

**Version:** 2014-07-03

## Project

» [Planktonic interactions in a changing ocean: Biological responses of \*Emiliana huxleyi\* to elevated pCO<sub>2</sub> and their effects on microzooplankton](#) (E Hux Response to pCO<sub>2</sub>)

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## Table of Contents

- [Dataset Description](#)
  - [Methods & Sampling](#)
  - [Data Processing Description](#)
- [Data Files](#)
- [Parameters](#)
- [Instruments](#)
- [Deployments](#)
- [Project Information](#)
- [Funding](#)

## Dataset Description

These data show cellular characterizations of two strains of *Emiliana huxleyi* cultured semi-continuously over a period 13-14 days under three different pCO<sub>2</sub> concentrations (400 ppmv, 750 ppmv, and 1000 ppmv). Cellular characterization measurements were taken throughout the course of the experiments, resulting in a time course data set. CO<sub>2</sub> chemistry was also monitored over the course of the experiment. Cellular characterizations included: intrinsic growth rate, cell volume, cellular particulate organic carbon and nitrogen, cellular particulate inorganic carbon, cellular chlorophyll *a*, and cellular particulate dimethylsulfoniopropionate.

### *Emiliana huxleyi* strains:

Strain NCMA 2668, calcifying phenotype, isolated from Gulf of Maine 2002

Strain NCMA 374, non-calcifying phenotype, isolated from Gulf of Maine 1990

### Related Datasets:

[Emiliana huxleyi Chl-\*a\*, POC, cell volumes](#)

[Emiliana huxleyi CN content](#)

[Emiliana huxleyi DMSP](#)

[Emiliana huxleyi growth rates](#)

## Methods & Sampling

## **Culturing conditions:**

Cultures of *E. huxleyi* Strain NCMA 2668 and 374 were inoculated at low cell density into media prepared from autoclaved filtered seawater with f/50 nutrient amendment. Cell populations were allowed to acclimate for approximately five generations, until cell density neared levels likely to significantly change the pH/pCO<sub>2</sub>. Daily dilutions of cultures with pre-equilibrated media kept cell density low ( $<1 \times 10^5$  cells/ml), ensured cells remained in exponential growth phase and prevented excessive drawdown of nutrients and CO<sub>2</sub>. Cell density was determined by flow cytometry (model described below) and each flask was diluted with media that was continuously sparged with air containing 400, 750 or 1000 ppm CO<sub>2</sub>. Air mixtures were created using CO<sub>2</sub> free air (Powerex air compressor, and Twin Towers CO<sub>2</sub> scrubber) and pure CO<sub>2</sub> (Airgas) combined using a system of mass flow controllers (Sierra Instruments) and verified using a non-dispersive infrared CO<sub>2</sub> sensor (Licor 820). Cultures were maintained in 1 liter polycarbonate flasks at 15°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 pCO<sub>2</sub> 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 14 days. This experiment was carried out twice, in 2011 and 2012.

## **CO<sub>2</sub> chemistry:**

pCO<sub>2</sub> throughout the course of the experiment was calculated using CO<sub>2</sub>sys program, with pH and total alkalinity as variables and using Millero constants. pH was measured using a Metrohm 888 Titrand with a Metrohm Ecotrode combined electrode calibrated with TRIS and AMP buffers on the total H<sup>+</sup> ion pH scale.

Total alkalinity was measured with a Metrohm 888 Titrand with seawater buffers prepared by combining prepared sea salts and HCl with 2-amino-2-hydroxymethyl-1,3-propanediol and 2-aminopyridine.

## **Intrinsic growth rate:**

Daily cell counts were made using a BD FACSCalibur flow cytometer. Manual counts were done on select samples using a hemocytometer. Manual counts were consistently within 5% of flow cytometry counts. Intrinsic growth rate was calculated using exponential growth equation.

## **Additional results:**

[Stats testing differences in growth between strains: ANOVA](#)

## **Cell size:**

Live cells were imaged using an Olympus CH30 compound microscope networked to a Photometric CoolSNAP camera. Cell diameter was measured using ImageJ software, and cell volume was calculated using standard geometric equations.

## **Cellular chlorophyll a:**

Chlorophyll a samples were extracted for 24 h in acetone under -20 °C. Chlorophyll a was measured using a Turner Designs 10-AU fluorometer. The acidifying equations of Parsons were used to convert raw fluorescence into chlorophyll a concentration.

## **Cellular carbon and nitrogen:**

Samples for cellular particulate carbon and nitrogen were analyzed using a CE Elantech Flash EA 1112 elemental analyzer. In all analysis blanks were run, and internal standards were inserted between samples, and remained within 1% of standard curve. For the calcifying strain (2668), samples were acid fumed for 24 h to drive off PIC. Values of organic carbon were subtracted from total carbon to yield cellular particulate inorganic carbon.

## **Cellular particulate DMSP:**

A Shimadzu GC-14A gas chromatograph was used to measure cellular particulate DMSP. Standards were prepared using DMSP Cl.

## Data Processing Description

Data are compiled to show averages and standard deviations by day and treatment.

### Relevant References:

Wuori, Tristen, "The effects of elevated PCO<sub>2</sub> on the physiology of *Emiliana huxleyi*" (2012). Western Washington University Masters Thesis Collection. Paper 235 <http://cedar.wvu.edu/wwuet/235/>

[ [table of contents](#) | [back to top](#) ]

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## Data Files

File
<b>dilution.csv</b> (Comma Separated Values (.csv), 24.95 KB) MD5:644cb96122592fed9ee740a16d8b829a Primary data file for dataset ID 521279

[ [table of contents](#) | [back to top](#) ]

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## Parameters

Parameter	Description	Units
strain	Emiliana huxleyi strain id	unitless
date	date of analysis	yyyy-mm-dd
activity	sample growth or dilution	unitless
sample	sample bottle id	unitless
day1_concentration_init	initial concentration of Emiliana huxleyi on day 1	cells/ml
day1_vol_repl	volume replaced on day 1	ml
day1_concentration_dil	Emiliana huxleyi concentration after dilution on day 1	cells/ml
day2_concentration_init	initial concentration of Emiliana huxleyi on day 2	cells/ml
growth_rate	growth rate from day 1 to day 2	cells/day
vol_media_repl	volume of media to replace	ml
vol_media_remv	volume of media to remove	ml
time_double	doubling time of cells	unitless
comment	comments	unitless

[ [table of contents](#) | [back to top](#) ]

## Instruments

<b>Dataset-specific Instrument Name</b>	Automatic titrator
<b>Generic Instrument Name</b>	Automatic titrator
<b>Dataset-specific Description</b>	Metrohm 888 Titrande with a Metrohm Ecotrode combined electrode, calibrated with TRIS and AMP buffers on the total H <sup>+</sup> ion pH scale.
<b>Generic Instrument Description</b>	Instruments that incrementally add quantified aliquots of a reagent to a sample until the end-point of a chemical reaction is reached.

<b>Dataset-specific Instrument Name</b>	camera
<b>Generic Instrument Name</b>	Camera
<b>Dataset-specific Description</b>	Photometrics CoolSNAP camera, networked to microscope
<b>Generic Instrument Description</b>	All types of photographic equipment including stills, video, film and digital systems.

<b>Dataset-specific Instrument Name</b>	CHN_EA
<b>Generic Instrument Name</b>	CHN Elemental Analyzer
<b>Dataset-specific Description</b>	CE Elantech Flash EA 1112 elemental analyzer
<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>	CO2 Analyzer
<b>Generic Instrument Name</b>	CO2 Analyzer
<b>Dataset-specific Description</b>	Licor 820: a non-dispersive infrared CO2 sensor
<b>Generic Instrument Description</b>	Measures atmospheric carbon dioxide (CO2) concentration.

<b>Dataset-specific Instrument Name</b>	Flow Cytometer
<b>Generic Instrument Name</b>	Flow Cytometer
<b>Dataset-specific Description</b>	BD FACSCalibur flow cytometer
<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>	Fluorometer
<b>Generic Instrument Name</b>	Fluorometer
<b>Dataset-specific Description</b>	Turner Designs 10-AU fluorometer
<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>	Gas Chromatograph
<b>Generic Instrument Name</b>	Gas Chromatograph
<b>Dataset-specific Description</b>	Shimadzu GC-14A gas chromatograph
<b>Generic Instrument Description</b>	Instrument separating gases, volatile substances, or substances dissolved in a volatile solvent by transporting an inert gas through a column packed with a sorbent to a detector for assay. (from SeaDataNet, BODC)

<b>Dataset-specific Instrument Name</b>	MFC
<b>Generic Instrument Name</b>	Mass Flow Controller
<b>Dataset-specific Description</b>	Sierra Instruments
<b>Generic Instrument Description</b>	Mass Flow Controller (MFC) - A device used to measure and control the flow of fluids and gases

<b>Dataset-specific Instrument Name</b>	compound microscope
<b>Generic Instrument Name</b>	Microscope - Optical
<b>Dataset-specific Description</b>	Olympus CH30 compound microscope networked to a Photometric CoolSNAP camera
<b>Generic Instrument Description</b>	Instruments that generate enlarged images of samples using the phenomena of reflection and absorption of visible light. Includes conventional and inverted instruments. Also called a "light microscope".

<b>Dataset-specific Instrument Name</b>	spectrophotometer
<b>Generic Instrument Name</b>	Spectrophotometer
<b>Dataset-specific Description</b>	Agilent 5480 UV-VIS spectrophotometer (+/- 0.02)
<b>Generic Instrument Description</b>	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.

[ [table of contents](#) | [back to top](#) ]

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## Deployments

### Lab\_Olson\_B

<b>Website</b>	<a href="https://www.bco-dmo.org/deployment/521277">https://www.bco-dmo.org/deployment/521277</a>
<b>Platform</b>	WWU
<b>Start Date</b>	2011-03-31
<b>End Date</b>	2016-09-15
<b>Description</b>	laboratory experiments

[ [table of contents](#) | [back to top](#) ]

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## Project Information

### Planktonic interactions in a changing ocean: Biological responses of *Emiliana huxleyi* to elevated pCO<sub>2</sub> and their effects on microzooplankton (E Hux Response to pCO<sub>2</sub>)

#### **Description from NSF award abstract:**

The calcifying Haptophyte *Emiliana huxleyi* appears to be acutely sensitive to the rising concentration of ocean pCO<sub>2</sub>. Documented responses by *E. huxleyi* to elevated pCO<sub>2</sub> 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 *Emiliana huxleyi* in response to elevated pCO<sub>2</sub> 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 CO<sub>2</sub> concentrations. After careful characterization of the biochemical and physiological responses of the *E. huxleyi* strains to elevated pCO<sub>2</sub>, 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 CO<sub>2</sub>, 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 CaCO<sub>3</sub> 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.

[ [table of contents](#) | [back to top](#) ]

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## Funding

Funding Source	Award
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-0961229</a>

[ [table of contents](#) | [back to top](#) ]