Experimental results: Emiliania huxleyi growth rates under different pCO2 levels from Bellingham, WA from 2011-2012 (E Hux Response to pCO2 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 Emiliania huxleyi to elevated pCO2 and their effects on microzooplankton (E Hux Response to pCO2)

Contributors	Affiliation	Role
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Dataset Description

These data show cellular characterizations of two strains of *Emiliania huxleyi* cultured semi-continuously over a period 13-14 days under three different pCO2 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. CO2 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.

***Emiliania 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:
Emiliania huxleyi Chl-a, POC, cell volumes
Emiliania huxleyi CN content
Emiliania huxleyi DMSP
Emiliania huxleyi growth rates

Methods & Sampling

Culturing conditions:

Cultures of *E. huxleyi* StrainNCMA 2668 and 374 were innoculated at low cell density into media prepared from autoclaved filtered seawater with f/50 nutrient ammendment. Cell populations were allowed to acclimate for approximately five generations, until cell density neared levels likely to significantly change the pH/pCO_2. 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 described below) and each flask was diluted with media that was continuously sparged with air containing 400, 750 or 1000 ppm CO_2. Air mixtures were created using CO_2 free air (Powerex air compressor, and Twin Towers CO2 scrubber) and pure CO_2 (Airgas) combined using a system of mass flow controllers (Sierra Instruments) and verified using a non-dispersive infrared CO_2 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 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 maintianed in this fashion for 14 days. This experiment was carried out twice, in 2011 and 2012.

CO_2 chemistry:

pCO_2 throughout the course of the experiment was calculated using CO2sys program, with pH and total alkalinity as variables and using Millero constants. pH was measured using a Metrohm 888 Titrando with a Metrohm Ecotrode combined electrode calibrated with TRIS and AMP buffers on the total H^+ ion pH scale.

Total alkalinity was measured with a Metrohm 888 Titrando 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 PCO2 on the physiology of Emiliania huxleyi" (2012). Western Washington University Masters Thesis Collection. Paper 235 http://cedar.wwu.edu/wwuet/235/

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Data Files

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

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Parameters

Parameter	Description	Units
strain	Emiliania 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 Emiliania huxleyi on day 1	cells/ml
day1_vol_repl	volume replaced on day 1	ml
day1_concentration_dil	Emiliania huxleyi concentration after dilution on day 1	cells/ml
day2_concentration_init	initial concentration of Emiliania 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

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Instruments

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

Dataset-specific Instrument Name	CHN_EA
Generic Instrument Name	CHN Elemental Analyzer
Dataset-specific Description	CE Elantech Flash EA 1112 elemental analyzer
Generic Instrument Description	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.

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	Flow Cytometer
Generic Instrument Name	Flow Cytometer
Dataset-specific Description	BD FACSCalibur 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	Fluorometer
Generic Instrument Name	Fluorometer
Dataset-specific Description	Turner Designs 10-AU fluorometer
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.

Dataset-specific Instrument Name	Gas Chromatograph
Generic Instrument Name	Gas Chromatograph
Dataset-specific Description	Shimadzu GC-14A gas chromatograph
Generic Instrument Description	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)

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	compound microscope
Generic Instrument Name	Microscope - Optical
Dataset-specific Description	Olympus CH30 compound microscope networked to a Photometric CoolSNAP camera
Generic Instrument Description	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".

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.

Dataset-specific Instrument Name	Automatic titrator
Generic Instrument Name	Titrator
Dataset-specific Description	Metrohm 888 Titrando with a Metrohm Ecotrode combined electrode, calibrated with TRIS and AMP buffers on the total H+ ion pH scale.
Generic Instrument Description	Titrators are instruments that incrementally add quantified aliquots of a reagent to a sample until the end-point of a chemical reaction is reached.

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Deployments

Lab_Olson_B

Website	https://www.bco-dmo.org/deployment/521277
Platform	WWU
Start Date	2011-03-31
End Date	2016-09-15
Description	laboratory 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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