

# Laboratory experiment analyzing the photosynthetic and calcification rates of *Pleurochrysis carterae* (Ocean acidification effects on copes and coccoliths project)

**Website:** <https://www.bco-dmo.org/dataset/651520>

**Data Type:** experimental

**Version:** Final

**Version Date:** 2016-07-11

## Project

» [Effects of ocean acidification on \*Emiliana huxleyi\* and \*Calanus finmarchicus\*; insights into the oceanic alkalinity and biological carbon pumps](#) (OA\_Copes\_Coccoliths)

## Program

» [Science, Engineering and Education for Sustainability NSF-Wide Investment \(SEES\): Ocean Acidification \(formerly CRI-OA\)](#) (SEES-OA)

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## Dataset Description

Photosynthetic and calcification rates of *Pleurochrysis carterae* at three pCO<sub>2</sub> levels.

## Methods & Sampling

**Cultures:** *Pleurochrysis carterae* cultures were maintained in exponential growth phase under axenic conditions in semi-continuous batch culture using L1-Si media prepared on 0.2 um-filtered, UV-sterilized, autoclaved seawater. Cultures were acclimated to one of three pCO<sub>2</sub> treatments for over 9 generations before experiments were performed.

**pCO<sub>2</sub>:** Carbonate chemistry was manipulated by bubbling cultures and prepared media with 500 mL/min<sup>-1</sup> with 0.2 um-filtered 280, 380, or 750 ppm pCO<sub>2</sub> air. The pCO<sub>2</sub> levels of the treatment air were established using two mass flow controllers (Aalborg, Orangeburg, NY, USA) for each treatment to precisely mix in-house compressed air and pure CO<sub>2</sub> (Maine Oxy, Auburn, ME, USA). The in-house compressed air was stripped of CO<sub>2</sub> to less than 10 ppm CO<sub>2</sub> using a Puregas VCD CO<sub>2</sub> Adsorber (Puregas, LLC, Broomfield, CO, USA). The pCO<sub>2</sub> of the gas mixtures was stable to 8 ppm.

**Length of Incubation (hours):** Number of hours that algae were incubated with  $^{14}\text{C}$ -bicarbonate.

**Cell density (cells/mL-1):** Culture density measured using a Moxi Z mini automated cell counter (ORFLO Technologies, Ketchum, ID, USA), which has a coefficient of variation of 4%.

**Photosynthetic rate ( $\mu\text{g C/L}^{-1} \text{d}^{-1}$ ) and Calcification rate ( $\mu\text{g C/L}^{-1} \text{d}^{-1}$ ):** Triplicate samples from each  $p\text{CO}_2$  treatment were spiked with  $^{14}\text{C}$ - $\text{HCO}_3^-$  and incubated at  $16.5 \pm 0.5$  degrees Celsius and  $415 \mu\text{mol photons/m}^{-2}/\text{s}^{-1}$  PAR for 3 h. At the end of the incubation, the cells from each replicate, along with triplicate formalin-killed blanks for each  $p\text{CO}_2$  treatment, were filtered onto  $0.4 \mu\text{m}$  polycarbonate filters and their carbon was partitioned into organic and inorganic fractions by acidification and subsequent capture of  $^{14}\text{CO}_2$  (from PIC) in a trap containing a Whatman GFA filter presoaked with  $0.2 \text{ mL}$  phenethylamine (see Balch et al. 2000 for detailed methodology). The radioactivity of each fraction was measured on a Packard Tri-Carb 2750 LL scintillation counter (acquired by Perkin Elmer, Waltham, MA, USA) and the photosynthetic rate and calcification rate were calculated from the organic carbon and inorganic carbon fractions, respectively, using the equation on p. 118 from Parsons et al. (1984) and applying appropriate unit conversions:

$$\text{Photosynthesis OR Calcification (mg C} \cdot (\text{m}^3)^{-1} \text{d}^{-1}) = [(R_s - R_b) \cdot W \cdot 1.05] / (R \cdot t)$$

Where  $R$  is the total activity (dpm) of bicarbonate added;  $t$  is the length of incubation (in days);  $R_s$  is the sample count (dpm),  $R_b$  is the formalin blank count (dpm), and  $W$  is the weight of total carbon dioxide present in  $\text{mg C} \cdot (\text{m}^3)^{-1}$ , determined from the expression  $W = 12,000 \cdot TC$ , where  $TC$  is the total carbon dioxide, which can be approximated from salinity, as described in Parsons et al. (1984). The factor of 1.05 accounts for preferential uptake of  $^{12}\text{C}$ . The calculation is the same for photosynthetic rate and calcification rate – the difference is using the sample count from either the organic fraction or the inorganic fraction, respectively.

## Data Processing Description

**Photosynthetic rate corrected to 14:10 L:D cycle ( $\mu\text{g C} / \text{L}^{-1} \text{d}^{-1}$ ):** Because our cultures are maintained in an incubator with a 14-10 h light-dark cycle, and our photosynthetic rate ( $P_3$ ) was extrapolated to a per day rate based on a 3 h light incubation, we corrected for our 14 h day to obtain a true 24 h day photosynthetic rate ( $P_{24}$ ) as follows:

$$P_{24} = P_3 \cdot \left( \frac{14 \text{ h}}{24 \text{ h}} \right)$$

**Calcification rate corrected to 14:10 L:D cycle ( $\mu\text{g C} / \text{L}^{-1} \text{d}^{-1}$ ):** Because our cultures are maintained in an incubator with a 14-10 h light-dark cycle, and our calcification rate ( $C_3$ ) was extrapolated to a per day rate based on a 3 h light incubation, we corrected for our 14 h day to obtain a true 24 h day calcification rate ( $C_{24}$ ). In a separate experiment we determined that calcification in *P. carterae* is somewhat, but not entirely, light dependent, so we calculated  $x$ , the proportion of light calcification that is representative of dark calcification for each  $p\text{CO}_2$  treatment as follows:

$$x(280) = 0.0092$$

$$x(380) = 0.1983$$

$$x(750) = 0.2191$$

We then corrected  $C_3$  to  $C_{24}$  as follows:

$$C_{24} = C_3 \cdot \left( \frac{14 \text{ h}}{24 \text{ h}} \right) + C_3 \cdot x \cdot \left( \frac{10 \text{ h}}{24 \text{ h}} \right)$$

**Photosynthetic and calcification rates corrected to cell density ( $\mu\text{g C} / \text{cell}^{-1} / \text{d}^{-1}$ ):** To obtain  $P_{24}$  and  $C_{24}$  rates on a per cell basis, rates were divided by cell density and then by a volume conversion factor.

**Photosynthetic and calcification rates corrected to cell density ( $\text{pmol C} / \text{cell}^{-1} / \text{d}^{-1}$ ):** Standard unit conversion factors were applied to get photosynthetic and calcification rates in terms of  $\text{pmol organic C}$  or  $\text{pmol CaCO}_3 \text{ cell}^{-1} \text{ day}^{-1}$ , respectively.

**Calcification/Photosynthesis:** To obtain a particulate inorganic carbon to particulate organic carbon ratio (PIC / POC<sup>-1</sup>), the calcification rate was divided by the photosynthetic rate.

**DMO notes:**

- added underscores and removed spaces and units from column names
- changed column names to comply with BCO-DMO standards.
- added "na" to blank cells in columns where SD and mean were calculated

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## Data Files

File
<b>photo_calc_rates.csv</b> (Comma Separated Values (.csv), 1.19 KB) MD5:b8a6e44a6f1dd2b2f9597048d73457fa Primary data file for dataset ID 651520

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## Parameters

Parameter	Description	Units
replicate	Denotes the three replicates for each pCO <sub>2</sub> treatment.	unitless
CO <sub>2</sub> _treatment	The independent variable; one of three pCO <sub>2</sub> levels: 280 ppm; 380 ppm; 750 ppm	parts per million
cell_density	Density of cells in each replicate.	cells per milliliter
photo_rate	Rate of organic carbon production by Pleurochrysis based on a 3 h incubation in the light; divided by 0.125 (3 h/24 h) to give a rate per day.	micrograms per liter per day
calcification_rate	Rate of inorganic carbon production by Pleurochrysis based on a 3 h incubation in the light, but divided by 0.125 (3 h/24 h) to give a rate per day.	micrograms per liter per day
photoRate_LDcycle	Rate of organic carbon production by Pleurochrysis corrected to a 14-10 h light-dark cycle.	micrograms per liter per day
calcRate_LDcycle	Rate of inorganic carbon production by Pleurochrysis corrected to a 14-10 h light-dark cycle taking into consideration calcification in the dark.	micrograms per liter per day
photoRate_cellDensity_ugC	P24 corrected to the cell density of the replicate to give a per cell photosynthetic rate.	micrograms per cell per day
photoRate_cellDensity_pmolC	Unit conversion to give photosynthetic rate in pmol units.	picomoles per cell per day
calcRate_cellDensity_ugC	C24 corrected to the cell density of the replicate to give a per cell calcification rate.	micrograms per cell per day
calcRate_cellDensity_pmolC	Unit conversion to give calcification rate in pmol units.	picomoles per cell per day
POC_ratio	Calculation of the particulate inorganic (PIC) to particulate organic (POC) ratio of <i>P. carterae</i> .	dimensionless
photoRate_mean	Mean of the photosynthetic rate. Three replicates per mean.	picomoles per cell per day
photoRate_SD	Standard deviation of the photosynthetic rate. Three replicates per standard deviation.	picomoles per cell per day
calcRate_mean	Mean of the calcification rate. Three replicates per mean.	picomoles per cell per day
calcRate_SD	Standard deviation of the calcification rate. Three replicates per standard deviation.	picomoles per cell per day
POC_ratio_mean	Mean of PIC to POC ratio. Three replicates per mean.	dimensionless
POC_ratio_SD	Standard deviation of the PIC to POC ratio. Three replicates per standard deviation.	dimensionless

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## Instruments

<b>Dataset-specific Instrument Name</b>	Moxi Z Mini Automated Cell Counter
<b>Generic Instrument Name</b>	Automated Cell Counter
<b>Dataset-specific Description</b>	measures culture density
<b>Generic Instrument Description</b>	An instrument that determines the numbers, types or viability of cells present in a sample.

<b>Dataset-specific Instrument Name</b>	Puregas VCD CO2 Adsorber
<b>Generic Instrument Name</b>	CO2 Adsorber
<b>Dataset-specific Description</b>	instrument stripped compressed air of CO2
<b>Generic Instrument Description</b>	CO2 Adsorber - an instrument designed to remove CO2 and moisture from compressed air.

<b>Dataset-specific Instrument Name</b>	Packard Tri-Carb 2750 LL
<b>Generic Instrument Name</b>	Liquid Scintillation Counter
<b>Dataset-specific Description</b>	measures radioactivity
<b>Generic Instrument Description</b>	Liquid scintillation counting is an analytical technique which is defined by the incorporation of the radiolabeled analyte into uniform distribution with a liquid chemical medium capable of converting the kinetic energy of nuclear emissions into light energy. Although the liquid scintillation counter is a sophisticated laboratory counting system used to quantify the activity of particulate emitting ( $\beta$ and $\alpha$ ) radioactive samples, it can also detect the Auger electrons emitted from $^{51}\text{Cr}$ and $^{125}\text{I}$ samples.

<b>Dataset-specific Instrument Name</b>	Aalborg Mass Flow Controller
<b>Generic Instrument Name</b>	Mass Flow Controller
<b>Dataset-specific Description</b>	Indicate and control set flow rates of gases. Manufactured in Orangeburg, NY USA.
<b>Generic Instrument Description</b>	Mass Flow Controller (MFC) - A device used to measure and control the flow of fluids and gases

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## Project Information

**Effects of ocean acidification on *Emiliana huxleyi* and *Calanus finmarchicus*; insights into the oceanic alkalinity and biological carbon pumps (OA\_Copes\_Coccoliths)**

**Coverage:** Laboratory experiments; East Boothbay, Maine

*(Extracted from the NSF award abstract)*

Ocean acidification is one of the most pressing marine science issues of our time, with potential biological impacts spanning all marine phyla and potential societal impacts affecting man's relationship to the sea. Rising

levels of atmospheric pCO<sub>2</sub> are increasing the acidity of the world oceans. It is generally held that average surface ocean pH has already declined by 0.1 pH units relative to the pre-industrial level (Orr et al., 2005), and is projected to decrease 0.3 to 0.46 units by the end of this century, depending on CO<sub>2</sub> emission scenarios (Caldeira and Wickett, 2005). The overall goal of this research is to parameterize how changes in pCO<sub>2</sub> levels could alter the biological and alkalinity pumps of the world ocean. Specifically, the direct and indirect effects of ocean acidification will be examined within a simple, controlled predator/prey system containing a single prey phytoplankton species (the coccolithophore, *Emiliana huxleyi*) and a single predator (the oceanic metazoan grazer, *Calanus finmarchicus*). The experiments are designed to elucidate both direct effects (i.e. effects of ocean acidification on the individual organisms only) and interactive effects (i.e. effects on the combined predator/prey system). Interactive experiments with phytoplankton prey and zooplankton predator are a critical starting point for predicting the overall impact of ocean acidification in marine ecosystems. To meet these goals, a state-of-the-art facility will be constructed with growth chambers that are calibrated and have highly-controlled pH and alkalinity levels. The strength of this approach lies in meticulous calibration and redundant measurements that will be made to ensure that conditions within the chambers are well described and tightly monitored for DIC levels. Growth and calcification rates in coccolithophores and the developmental rates, morphological and behavioral effects on copepods will be measured. The PIC and POC in the algae and the excreted fecal pellets will be monitored for changes in the PIC/POC ratio, a key parameter for modeling feedback mechanisms for rising pCO<sub>2</sub> levels. In addition, <sup>14</sup>C experiments are planned to measure calcification rates in coccolithophores and dissolution rates as a result of grazing. These key experiments will verify closure in the mass balance of PIC, allowing the determination of actual dissolution rates of PIC within the guts of copepod grazers.

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## Program Information

### Science, Engineering and Education for Sustainability NSF-Wide Investment (SEES): Ocean Acidification (formerly CRI-OA) (SEES-OA)

**Website:** [https://www.nsf.gov/funding/pgm\\_summ.jsp?pims\\_id=503477](https://www.nsf.gov/funding/pgm_summ.jsp?pims_id=503477)

**Coverage:** global

NSF Climate Research Investment (CRI) activities that were initiated in 2010 are now included under Science, Engineering and Education for Sustainability NSF-Wide Investment (SEES). SEES is a portfolio of activities that highlights NSF's unique role in helping society address the challenge(s) of achieving sustainability. Detailed information about the SEES program is available from NSF ([https://www.nsf.gov/funding/pgm\\_summ.jsp?pims\\_id=504707](https://www.nsf.gov/funding/pgm_summ.jsp?pims_id=504707)).

In recognition of the need for basic research concerning the nature, extent and impact of ocean acidification on oceanic environments in the past, present and future, the goal of the SEES: OA program is to understand (a) the chemistry and physical chemistry of ocean acidification; (b) how ocean acidification interacts with processes at the organismal level; and (c) how the earth system history informs our understanding of the effects of ocean acidification on the present day and future ocean.

#### Solicitations issued under this program:

[NSF 10-530](#), FY 2010-FY2011

[NSF 12-500](#), FY 2012

[NSF 12-600](#), FY 2013

[NSF 13-586](#), FY 2014

NSF 13-586 was the final solicitation that will be released for this program.

#### PI Meetings:

[1st U.S. Ocean Acidification PI Meeting](#) (March 22-24, 2011, Woods Hole, MA)

[2nd U.S. Ocean Acidification PI Meeting](#) (Sept. 18-20, 2013, Washington, DC)

3rd U.S. Ocean Acidification PI Meeting (June 9-11, 2015, Woods Hole, MA – Tentative)

#### NSF media releases for the Ocean Acidification Program:

[Press Release 10-186 NSF Awards Grants to Study Effects of Ocean Acidification](#)

[Discovery Blue Mussels "Hang On" Along Rocky Shores: For How Long?](#)

[Discovery nsf.gov - National Science Foundation \(NSF\) Discoveries - Trouble in Paradise: Ocean Acidification This Way Comes - US National Science Foundation \(NSF\)](#)

[Press Release 12-179 nsf.gov - National Science Foundation \(NSF\) News - Ocean Acidification: Finding New Answers Through National Science Foundation Research Grants - US National Science Foundation \(NSF\)](#)

[Press Release 13-102 World Oceans Month Brings Mixed News for Oysters](#)

[Press Release 13-108 nsf.gov - National Science Foundation \(NSF\) News - Natural Underwater Springs Show How Coral Reefs Respond to Ocean Acidification - US National Science Foundation \(NSF\)](#)

[Press Release 13-148 Ocean acidification: Making new discoveries through National Science Foundation research grants](#)

[Press Release 13-148 - Video nsf.gov - News - Video - NSF Ocean Sciences Division Director David Conover answers questions about ocean acidification. - US National Science Foundation \(NSF\)](#)

[Press Release 14-010 nsf.gov - National Science Foundation \(NSF\) News - Palau's coral reefs surprisingly resistant to ocean acidification - US National Science Foundation \(NSF\)](#)

[Press Release 14-116 nsf.gov - National Science Foundation \(NSF\) News - Ocean Acidification: NSF awards \\$11.4 million in new grants to study effects on marine ecosystems - US National Science Foundation \(NSF\)](#)

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## Funding

Funding Source	Award
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-1220068</a>

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