Light-dark calcification rates of Pleurochrysis carterae analyzed at Bigelow Laboratory in 2013 (OA Copes Coccoliths project)

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Project

» Effects of ocean acidification on Emiliania 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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Abstract

Light-dark calcification rates of Pleurochrysis carterae analyzed at Bigelow Laboratory in 2013 (OA Copes Coccoliths project)

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Coverage

Temporal Extent: 2013 - 2013

Dataset Description

Laboratory experiment located at Bigelow Laboratory, East Boothbay, ME using *Pleurochrysis carterae* (NCMA strain 645), which was isolated from 41.525 deg N 70.6736 deg W (Woods Hole, Massachusetts USA), but has been maintained in culture since 1958.

Acquisition Description

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 pCO2 treatments for > 9 generations before experiments were performed. Cultures were maintained in an incubator at 16.5 +/- 0.5 deg C and 470 umol photons/m-2/s PAR.

pCO2: Carbonate chemistry was manipulated by bubbling cultures and prepared media with 500 mL/min with 0.2 um-filtered 280, 380, or 750 ppm pCO2 air. The pCO2 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 CO2 (Maine Oxy, Auburn, ME, USA). The in-house compressed air was stripped of CO2 to

less than 10 ppm CO2 using a Puregas VCD CO2 Adsorber (Puregas, LLC, Broomfield, CO, USA). The pCO2 of the gas mixtures was stable to +/- 8 ppm. pCO2 values of the cultures may be different than the target levels due to biological activity.

Dissolution of existing coccoliths: Coccoliths were dissolved by 1.75 M HCl to drop the pH to 5.5 for 2 min. Following the dissolution (de-lithing), 1.75 M NaOH was added to bring the pH back to the respective starting pH. Dissolution of coccoliths was immediately confirmed by looking at the cells under cross-polarized light microscopy to verify the absence of birefringence indicative of CaCO3. Dissolution was further confirmed by filtering the acidified/neutralized sample onto a 0.4 um polycarbonate filter. Filters were mounted on stubs, sputter-coated with gold using a Denton Desk IV sputter coater (Denton Vacuum, Moorestown, NJ, USA), and imaged on a Zeiss Supra25 field emission scanning electron microscope (Carl Zeiss Microscopy, LLC, Thornwood, NY, USA). At least 15 cells per sample were imaged and the number of coccoliths/cell was manually counted to determine the number of coccoliths that remained after the acidification/neutralization dissolution step.

24 h incubation in either light or dark conditions: To determine the number of coccoliths formed (as a proxy for calcification rate) in 24 h in either light or dark conditions, for each pCO2 level, 15 mL of de-lithed culture were added to 8 scintillation vials. Three vials were 'light' replicates, three vials were 'dark' replicates, and two vials were poisoned with buffered formalin to serve as a 'light' blank and a 'dark' blank. Dark replicate and blank vials were covered in black aluminum foil and all vials were incubated together for 24 h in an incubator set at 16.5 +/- 0.5 deg C and 470 umol photons/m-2/s PAR on a 14-10 h light-dark cycle. The experiment was timed to start when the lights in the incubator turned on in the morning, thus the 'light' replicates were exposed to light for 14 of 24 h.

Determination of attached coccoliths: Coccolith formation was assessed by counting the number of coccoliths formed during the incubation period. After the 24 h incubation period, each replicate and blank vial was filtered onto a 0.4 um polycarbonate filters. Filters were mounted on stubs, sputter-coated with gold using a Denton Desk IV sputter coater (Denton Vacuum, Moorestown, NJ, USA), and imaged on a Zeiss Supra25 field emission SEM (Carl Zeiss Microscopy, LLC, Thornwood, NY, USA). At least 15 cells per replicate were imaged and the number of coccoliths/cell was manually counted. The counted coccoliths represented calcification during the 24 h incubation period and the average number of coccoliths per cell for each replicate and blank is reported.

Processing Description

Blank correction: For each replicate, the number of coccoliths per cell determined from the corresponding blank sample (same pCO2 and light conditions) was subtracted from the number of coccoliths per cell determined from the replicate. If the blank-correction resulted in a negative value, the value was set to 0 coccoliths per cell.

Verification of de-lithing: The number of coccoliths per cell immediately after the de-lithing was not explicitly used in any calculations. However, because so few liths were formed in the 'dark' replicates, we performed a 2-way ANOVA to compare the number of coccoliths per cell immediately after the de-lithing with the number of coccoliths per cell in the dark incubation treatments. This test showed that there were significantly more coccoliths per cell for the dark incubation replicates, relative to the samples collected immediately after the de-lithing, which supports the claim that there was calcification in the dark. The results of this statistical test are presented in our paper, so those values are not included in the dataset.

BCO-DMO Processing Notes:
- added underscores and removed spaces and units from column names
- changed column names to comply with BCO-DMO standards.
- replaced all "n/a" with "nd," and filled in blank cells with "nd"

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Parameters

Parameter	Description	Units
pCO2_treatment	The independent variable - one of three pCO2 levels (280 ppm, 380 ppm, or 750 ppm) These treatment levels are nominal values as they represent the target pCO2 for each treatment.	parts per million (ppm)

sample	For each pCO2 treatment there are three types of samples; After de-lithing: These samples were taken immediately after the coccolithophores were acidified to dissolve their coccoliths and then neutralized to return the culture pH to the original pH. These samples were taken before the 24 h incubation period and therefore do not have a Light or Dark treatment. Replicate: These represent the three replicates for each light condition and each CO2 treatment. These samples were taken after the cells incubated in either light or dark conditions for 24 h. Blank: These samples were killed by the addition of formalin but were allowed to incubate in either light or dark conditions for 24 h.	unitless
light_conditions	This identifies whether the sample was incubated for 24 h in light (470 umol photons/m-2/s PAR) or dark (0 umol photons/m-2/s PAR) conditions.	unitless
attached	This is the number of attached coccoliths observed per cell. This number represents an average of counts from at least 15 cells per replicate or sample.	coccoliths per cell
attached_blankCorrected	This is the number of attached coccoliths observed per cell with the number of coccoliths observed per cell from the blank samples subtracted to account for any coccoliths that might have remained after cells were de-lithed.	coccoliths per cell
mean_attached_blankCorrected	The average value for each set of three replicates.	coccoliths per cell
stdev_attached_blankCorrected	The standard deviation for each set of three replicates.	coccoliths per cell

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Instruments

Dataset-specific Instrument Name	Incubator
Generic Instrument Name	In-situ incubator
Dataset-specific Description	Dark replicate and blank vials were covered in black aluminum foil and all vials were incubated together for 24 h in an incubator set at 16.5 +/- 0.5 deg C and 470 umol photons/m-2/s PAR on a 14-10 h light-dark cycle.
Generic Instrument Description	A device on shipboard or in the laboratory that holds water samples under controlled conditions of temperature and possibly illumination.

Dataset-specific Instrument Name	Orion ROSS electrode
Generic Instrument Name	pH Sensor
Dataset-specific Description	Orion ROSS electrode was connected to an Orion Star A211 Benchtop pH meter (ThermoFisher Scientific, Waltham, MA, USA)
Generic Instrument Description	General term for an instrument that measures the pH or how acidic or basic a solution is.

Dataset-specific Instrument Name	Zeiss Supra25 field emission SEM
Generic Instrument Name	Microscope-Electron
Dataset-specific Description	Carl Zeiss Microscopy, LLC, Thornwood, NY, USA
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	Aalborg Mass Flow Controller
Generic Instrument Name	Mass Flow Controller
Dataset-specific Description	Indicate and control set flow rates of gases. Manufactured in Orangeburg, NY USA.
Generic Instrument Description	Mass Flow Controller (MFC) - A device used to measure and control the flow of fluids and gases

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

Dataset-specific Instrument Name	Denton Desk IV sputter coater
Generic Instrument Name	Sputter Coater
Dataset-specific Description	Filters were mounted on stubs, sputter-coated with gold using a Denton Desk IV sputter coater (Denton Vacuum, Moorestown, NJ, USA).
Generic Instrument Description	Sputter coating is the standard method for preparing non-conducting or poorly conducting specimens prior to observation in a scanning electron microscope (SEM)

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Deployments

Balch_2013

Website	https://www.bco-dmo.org/deployment/660148
Platform	lab Bigelow
Start Date	2013-07-07
Description	Laboratory located at Bigelow Laboratory for Ocean Sciences

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Project Information

Effects of ocean acidification on Emiliania 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 pCO2 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 CO2 emission scenarios (Caldeira and Wickett, 2005). The overall goal of this research is to parameterize how changes in pCO2 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, Emiliania 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 pCO2 levels. In addition, 14C 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: http://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 (http://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-FY2011NSF 12-500, FY 2012NSF 12-600, FY 2013NSF 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
NSF Division of Ocean Sciences (NSF OCE)	OCE-1220068

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