Pleurochrysis carterae diel culture dynamics analyzed at Bigelow Laboratory from 2013 (OA Copes Coccoliths project)

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Data Type: experimental
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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)

Contributors	Affiliation	Role
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Abstract
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Coverage

Temporal Extent: 2013-07-17 - 2013-07-18

Dataset Description

Diel (24 hour) culture dynamics of *Pleurochrysis carterae* (NCMA strain 645). It was isolated from 41.525 degrees North, 70.6736 degrees West (Woods Hole, Massachusetts USA), but has been maintained in culture since 1958.

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 pCO2 treatments for > 9 generations before experiments were performed. Cultures were maintained in an incubator at 16.5 +/- 0.5 degrees C and 470 umol photons/m-2/s PAR on a 14-10 light-dark cycle where the lights turned on at 6 am and turned off at 8 pm.

pCO2 Treatments: 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.

24 h Culture Dynamics Monitoring: To understand the chemical and biological culture dynamics over a 24 h period, the investigators took measurements of one culture from each pCO2 treatment every hour for 24 h. To reduce analytical costs, some parameters were not measured each hour (PIC, nutrients, total alkalinity). During the dark period, a red lamp was used to limit the light that might influence the cultures.

pH Measurements: The pH of the cultures was measured using an OrionTM ROSSTM electrode connected to an Orion StarTM A211 Benchtop pH meter (ThermoFisher Scientific, Waltham, MA, USA), calibrated with NBS buffers (EK Industries, Inc., Joliet, IL, USA) and corrected to the total scale using spectrophotometric pH measurements of culture samples. Spectrophotometric pH measurements of 0.2 um-filtered culture samples were made with 20 mM m-Cresol purple sodium salt indicator dye (Alfa Aesar, Ward Hill, MA, USA) using a Hitachi U-3010 spectrophotometer (Hitachi High-Technologies, Clarksburg, MD, USA) equipped with a water circulated cell holder connected to a VWR 1160 water bath (VWR, Radnor, PA, USA) set at 16.5 degrees C, holding a 1 cm quartz cell. The method followed the procedure described by Clayton and Byrne (1993) and Dickson et al. (2007), using the refit equation of Liu et al. (2011), resulting in a resolution of +/-0.004 pH units.

Temperature: Temperature measurements were made with an OrionTM ROSSTM electrode connected to an Orion StarTM A211 Benchtop pH meter (ThermoFisher Scientific, Waltham, MA, USA).

Salinity: Salinity was measured using an Acorn SALT 6 handheld salinity meter (Oakton Instruments, Vernon Hills, IL, USA) with a resolution of +/- 0.1 ppt.

in vivo Fluorescence: Fluoresence was measured using a Turner 10-AU fluorometer (Turner Designs, Sunnyvale, CA, USA).

Cell density and cell diameter: Culture density and mean cell diameter were measured using a Moxi Z mini automated cell counter (ORFLO Technologies, Ketchum, ID, USA), which has a coefficient of variation of 4%.

Particulate Inorganic Carbon: Bulk culture PIC analyses followed the technique of Fernandez et al. (1993): 10 mL culture samples were filtered onto 0.4 um polycarbonate filters and rinsed with potassium borate buffer with the pH adjusted to 8.0 to remove seawater calcium chloride. Filters were carefully moved to trace-metal free centrifuge tubes and digested with 5 mL of 5% nitric acid. The calcium concentration was measured using a Jobin Yvon Ultima C inductively coupled plasma-atomic emission spectrometer (ICP-AES, HORIBA, Ltd., Kyoto, Japan). Bulk culture PIC measurements were corrected to PIC/cell-1 using the corresponding cell density measurements.

Nutrients (measured at University of California Santa Barbara): Culture samples were filtered to 0.2 um to remove all algal cells and coccoliths, and samples were frozen prior to analysis. Total N (nitrate + nitrite), nitrate, phosphate, and silicate were measured by Flow Injection Analysis at the University of California, Santa Barbara, Marine Science Institute's Analytical Lab using a QuikChem 8000 (Lachat Instruments, Loveland, CO, USA).

Nutrients (measured by Bigelow Analytical Services): Culture samples were filtered to 0.2 um to remove all algal cells and coccoliths, and samples were frozen prior to analysis. Nitrate and phosphate were measured by Continuous Flow Analysis by Bigelow Analytical Services using a SEAL AutoAnalyzer 3 HR (SEAL Analytical Inc., Mequon, WI, USA).

Total Alkalinity: Culture samples were filtered to 0.2 um to remove all algal cells and coccoliths. Total alkalinity was measured via titration with 0.01 N HCl using a Metrohm Titrando 888 controlled by Tiamo software (Metrohm, Riverview, FL, USA) to perform automated Gran titrations of 4 mL samples. Titrations

were corrected to Certified Reference Materials (supplied by the laboratory of Andrew Dickson, Scripps Institution of Oceanography, La Jolla, CA, USA).

Data Processing Description

Carbonate Chemistry Calculations: Using the measured values of pH (total scale), total alkalinity, temperature, salinity, phosphate, and silicate, the investigators used CO2SYS software (Pierrot et al. 2006) to calculate dissolved inorganic carbon (DIC), pCO2, [HCO3-], [CO32-], [CO2], and calcite using the first and second dissociation constants (K1 and K2) of carbonic acid in seawater from Mehrbach et al. (1973), refit by Dickson and Millero (1987); KHSO4 from Dickson (1990); and [B]T from Uppstrom et al. (1974). Full carbonate chemistry could only be calculated for timepoints at which both pH and alkalinity were measured (~every 3 h).

DMO notes:
- added underscores and removed spaces and units from column names
- changed column names to comply with BCO-DMO standards.
- replaced all "na" with "nd"

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Data Files

File	
24h_cultureDynamics.csv	(Comma Separated Values (.csv), 12.48 KB) MD5:b67cc279d884687d2448f25056225440
Primary data file for dataset ID 660194	

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Related Publications

Clayton, T. D., & Byrne, R. H. (1993). Spectrophotometric seawater pH measurements: total hydrogen ion concentration scale calibration of m-cresol purple and at-sea results. Deep Sea Research Part I: Oceanographic Research Papers, 40(10), 2115–2129. doi:10.1016/0967-0637(93)90048-8
Methods

Dickson, A. G. (1990). Standard potential of the reaction: AgCl(s) + 1/2 H2(g) = Ag(s) + HCl(aq) and the standard acidity constant of the ion HSO4− in synthetic sea water from 273.15 to 318.15 K. The Journal of Chemical Thermodynamics, 22(2), 113–127. doi:10.1016/0021-9614(90)90074-z https://doi.org/10.1016/0021-9614(90)90074-Z
Methods

Dickson, A. G., & Millero, F. J. (1987). A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep Sea Research Part A. Oceanographic Research Papers, 34(10), 1733–1743. doi:10.1016/0198-0149(87)90021-5
Methods

Dickson, A.G., Sabine, C.L. and Christian, J.R. (Eds.) 2007. Guide to best practices for ocean CO2 measurements. PICES Special Publication 3, 191 pp. ISBN: 1-897176-07-4. URL: https://www.nodc.noaa.gov/ocads/oceans/Handbook_2007.html https://hdl.handle.net/11329/249
Methods

Fernández, E., Boyd, P., Holligan, P., & Harbour, D. (1993). Production of organic and inorganic carbon within a large-scale coccolithophore bloom in the northeast Atlantic Ocean. Marine Ecology Progress Series, 97, 271–285. doi:10.3354/meps097271
Methods

Liu, X., Patsavas, M. C., & Byrne, R. H. (2011). Purification and Characterization of meta-Cresol Purple for Spectrophotometric Seawater pH Measurements. Environmental Science & Technology, 45(11), 4862–4868. doi:10.1021/es200665d

Methods

Mehrbach, C., Culberson, C. H., Hawley, J. E., & Pytkowicx, R. M. (1973). Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnology and Oceanography, 18(6), 897–907. doi:10.4319/lo.1973.18.6.0897
Methods

Pierrot, D. E. Lewis,and D. W. R. Wallace. 2006. MS Excel Program Developed for CO2 System Calculations. ORNL/CDIAC-105a. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee. doi: 10.3334/CDIAC/otg.CO2SYS_XLS_CDIAC105a.
Methods

Uppström, L. R. (1974). The boron/chlorinity ratio of deep-sea water from the Pacific Ocean. Deep Sea Research and Oceanographic Abstracts, 21(2), 161–162. doi:10.1016/0011-7471(74)90074-6
Methods

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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. Within each pCO2 treatment there are 14 days worth of culture measurements for the algal cultures. Measurements of the media (without algae) were made on days 0 and 14. Only chemical measurements were made on the media not biological measurements.	parts per million (ppm)
approx_time	The approximate time of the given measurement; HH:MM	unitless
elapsed_time	The time elapsed since the beginning of the 24 h monitoring.	hours
date	The date the measurement was taken; YYYY/mm/dd	unitless
time	The actual time that the measurements for that timepoint began; HH:MM	unitless
light	Indicates whether the lights in the incubator were turned on or off. When the lights were on the intensity was 470 umol photons/m-2/s PAR. When the lights were off the intensity was 0 umol photons/m-2/s PAR and the investigators used a red light when in the incubator to take samples.	unitless
pH	pH measured on the total scale.	pH
temperature	Temperature	celsius
salinity	Salinity	practical salinity units (PSU)
inVivo_fluorescence	fluorescence to indicate relative chlorophyll-a concentration.	relative fluorescence units
cell_density	cell density of the culture as measured by the Moxi Z Automated Cell Counter.	cells/mL
mean_cellDiameter	mean cell diameter as measured by the Moxi Z Automated Cell Counter.	microns (um)
fluorescencePerCellDensity	Fluorescence divided by cell density to give an estimate of fluorescence per cell.	fluorescence/cell/mL
PIC_ugCPerL	Particulate inorganic carbon concentration	ugC/L

PIC_pgCPerCell	Particulate inorganic carbon concentration with unit conversion to pg/mL-1 and divided by the cell density to give pg C per cell.	pgC/cell
NO3_NO2	Nitrate + Nitrite concentration measured at the University of California Santa Barbara.	umol/kg
NO3_UCSB	Nitrate concentration, measured at the University of California Santa Barbara	umol/kg
NO3_Bigelow	Nitrate concentration, measured by Bigelow Analytical Services	umol/kg
PO4_UCSB	Phosphate concentration measured at the University of California Santa Barbara	umol/kg
PO4_Bigelow	Phosphate concentration measured by Bigelow Analytical Services	umol/kg
SiO4	Silicate concentration measured at the University of California Santa Barbara	umol/kg
mean_SiO4	Silicate concentration estimated as the average of the values from the first and last timepoint. Biologically there should be no change in silicate concentration because coccolithophores do not take up silicate. However silicate values are needed to calculate the carbonate chemistry parameters in CO2sys so the average was used for timepoints when carbonate chemistry values were calculated.	umol/kg
TA	Total alkalinity	ueq/kg
DIC	Dissolved inorganic carbon concentration calculated using CO2sys	umol/kg
pCO2	Partial pressure of carbon dioxide in the water calculated using CO2sys. This calculated value more accurately represents the pCO2 of the culture than the nominal treatment value, which represents the pCO2 of the air bubbled into the culture.	uatm
HCO3	Bicarbonate concentration calculated using CO2sys	umol/kg
CO3	Carbonate concentration calculated using CO2sys	umol/kg
CO2	Carbon dioxide concentration calculated using CO2sys	umol/kg
omega_calcite	Saturation state of calcium carbonate with respect to calcite calculated using CO2sys	unitless
ISO_DateTime_UTC	Date/Time (UTC) ISO formatted; YYYY/mm/dd;HH:MM:SS	unitless

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Instruments

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

Dataset-specific Instrument Name	Metrohm Titrando 888
Generic Instrument Name	Automatic titrator
Dataset-specific Description	Automatic titrations controlled by Tiamo software (Metrohm, Riverview, FL, USA).
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	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	Jobin Yvon Ultima C
Generic Instrument Name	Inductively Coupled Plasma Optical Emission Spectrometer
Dataset-specific Description	Calcium concentration measured (ICP-AES, HORIBA, Ltd., Kyoto, Japan).
Generic Instrument Description	Also referred to as an Inductively coupled plasma atomic emission spectroscope (ICP-AES). These instruments pass nebulised samples into an inductively-coupled gas plasma (8-10000 K) where they are atomised and excited. The de-excitation optical emissions at characteristic wavelengths are spectroscopically analysed. It is often used in the detection of trace metals.

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	SEAL AutoAnalyzer 3 HR
Generic Instrument Name	Nutrient Autoanalyzer
Dataset-specific Description	Continuous flow analysis performed by Bigelow Analytical Services (SEAL Analytical Inc., Mequon, WI, USA).
Generic Instrument Description	Nutrient Autoanalyzer is a generic term used when specific type, make and model were not specified. In general, a Nutrient Autoanalyzer is an automated flow-thru system for doing nutrient analysis (nitrate, ammonium, orthophosphate, and silicate) on seawater samples.

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	An instrument that measures the hydrogen ion activity in solutions. The overall concentration of hydrogen ions is inversely related to its pH. The pH scale ranges from 0 to 14 and indicates whether acidic (more H+) or basic (less H+).

Dataset-specific Instrument Name	Acorn SALT 6 handheld salinity meter
Generic Instrument Name	Salinity Sensor
Dataset-specific Description	Salinity measured using this instrument with a resolution of +/- 0.1 ppt (Oakton Instruments, Vernon Hills, IL, USA)
Generic Instrument Description	Category of instrument that simultaneously measures electrical conductivity and temperature in the water column to provide temperature and salinity data.

Dataset-specific Instrument Name	Hitachi U-3010 spectrophotometer
Generic Instrument Name	Spectrophotometer
Dataset-specific Description	Spectrophotometric pH measurements were taken of culture samples
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

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: 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).

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
NSF Division of Ocean Sciences (NSF OCE)	OCE-1220068

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