Experimental results from a study of pPeudo-nitzschia multiseries domoic acid production, C-fixation, mean growth, and element composition under varying pCO2 and phosphate levels (PhytoTM_in_HighCO2 project)

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Project
» Changing Phytoplankton Trace Metal Requirements in a High CO2 Ocean (PhytoTM_in_HighCO2)

Contributors	Affiliation	Role
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Dataset Description

Experimental data examining *Pseudo-nitzschia multiseries* (CCMP 2708) domic acid production, Carbon fixation, growth, and elemental composition under varying pCO2 and phosphate treatments. Values reported are the means of triplicate samples.

Data and methods are described in:
Sun J., Hutchins D. A., Feng Y., Seubert E. L., Caron D. A., & Fu F.-X., 2011. Effects of changing pCO2 and phosphate availability on domoic acid production and physiology of the marine harmful bloom diatom Pseudo-nitzschia multiseries. Limnology and Oceanography 56(3):829-840. DOI: 10.4319/lo.2011.56.3.0829

Methods & Sampling

The methods below are described in Sun et al. 2011.

Cultures and growth conditions
Stock cultures of marine diatom *Pseudo-nitzschia multiseries* (Hasle) (CCMP 2708, originally isolated from Eastern Canada) were maintained at 17 degrees C in 0.2 um-filtered, microwave-sterilized natural seawater, enriched with levels of phosphate, nitrate, silicate, vitamins, and trace nutrients as in Price et al. (1988). Light was provided on a 12 h dark:12 h light cycle using cool white fluorescent bulbs at 120 umol photons per square meter per second. Irradiance was measured with a biospherical LICOR sensor (model LI-250).

Experimental design and determination of growth rates
Semi-continuous culturing methods were used in order to measure the effects of P availability and/or pCO2 levels during acclimated, steady-state growth. Cultures were diluted daily with medium that was previously adjusted to the appropriate temperature and pCO2. Each bottle was diluted back to the same cell density present in that bottle directly after the previous day's dilution. Cultures were harvested following approximately 4 to 6 weeks of semi-continuous incubation when they were fully acclimated to the experimental conditions, after statistically invariant growth rates were recorded for at least 4 to 6 consecutive dilutions.

Samples from each culture bottle were always taken at the same time in the diel cycle, between 09:00 h and 10:00 h in the morning, to measure cell density and thus determine changes in growth rate. Dilutions were done in real time using biomass estimates made by in vivo fluorescence, and were subsequently validated using preserved cell count samples. Growth rates were calculated based on the equation:

$$u = (\ln Nb - \ln Na) / (tb - ta),$$

where Na and Nb are the average cell density at times ta (directly after a dilution) and tb (directly before the next day's dilution). For cell counts, whole-culture samples were fixed with glutaraldehyde (2.5% v to v final concentration) and counted in triplicate. About 1000 cells per replicate were enumerated in a 1-mL Sedgewick-Rafter counting chamber, using an Olympus BX51 epifluorescence microscope at 100-fold magnification.

Triplicate bottles at two conditions of phosphate availability were equilibrated at three different CO2 concentrations by gentle bubbling with commercially prepared certified standard air and CO2 gas mixtures (Praxair Gas). CO2 concentrations examined included preindustrial atmospheric levels (~22 Pa), near-present day concentrations (~41 Pa), and values predicted to occur before the end of this century (~74 Pa, IPCC 2007). In-line high efficiency particulate air (HEPA) filters were used to avoid contamination from particles in the gas tanks or lines. Phosphate levels used were 20 umol per liter (P replete) and 0.5 umol per liter (P limited). A total of six different phosphate and CO2 conditions were used in this study: 20 umol per liter P and ~22 Pa CO2; 20 umol per liter P and ~41 Pa CO2; 20 umol per liter P and ~74 Pa CO2; 0.5 umol per liter P and ~22 Pa CO2; 0.5 umol per liter P and ~41 Pa CO2; and 0.5 umol per liter P and ~74 Pa CO2.

Carbonate buffer system measurements and pCO2 treatments
The pH in each bottle was monitored daily using a high sensitivity microprocessor pH-meter (Orion EA 940), calibrated with pH 4, 7 and 10 buffer solutions. The relative precision of this instrument is ~0.01 and accuracy is ~0.03 pH units. For the analysis of total dissolved inorganic carbon (DIC), DIC samples were stored in 2 mL capped borosilicate vials free of air bubbles and were preserved with 20 uL saturated HgCl2 per liter, and stored at 4 degrees C until analyzed. Total DIC was measured by acidifying 2-mL 10% of H3PO4 and quantifying the CO2 trapped in an acid sparging column (model CM 5230) with a carbon coulometer (model CM 140, UIC). Certified reference materials obtained from Andrew Dickson (University of California, San Diego, http://andrew.ucsd.edu/co2qc/index.html) were measured periodically during the run and used for calibration. pH values remained invariant before and after the dilution, suggesting that bubbling rates were sufficient to maintain the target CO2 equilibration levels in the medium, regardless of diel changes in photosynthesis and respiration. Based in the daily measurements of pH and DIC, pCO2 stabilized during the early part of the semi-continuous growth period and then remained steady throughout the latter part of the incubation period. Calculated pCO2 values (using CO2SYS; http://www.cdiac.ornl.gov/ftp/co2sys/CO2SYS_calc_XLS) for the three CO2 treatments in both P treatments ranged from 22-23 Pa, 39-42 Pa, and 73-75 Pa (see table below where the numbers in parenthenses are the standard deviations of triplicate samples), very close to the certified standard gas mixture values. For convenience, these values were averaged and rounded to 22 Pa, 41 Pa, and 74 Pa when referring to the three pCO2 treatments throughout the dataset and paper (Sun et al. 2011).

Treatment conditions and calculated pCO2:

Treatment	Measured pH (sd)	Measured DIC (sd); umol/L	Calculated CO2 (sd); umol/L	Calculated pCO2 (sd); Pa
P-limited, 22 Pa	8.38 (0.05)	1917 (38)	7.4 (0.6)	23 (2)
P-limited, 41 Pa	8.15 (0.02)	2029 (8)	13.9 (0.7)	42 (2)
P-limited, 74 Pa	7.94 (0.01)	2145 (9)	24.5 (0.6)	75 (2)
P-replete, 22 Pa	8.40 (0.03)	1970 (4)	7.1 (0.5)	22 (2)
P-replete, 41 Pa	8.19 (0.02)	2066 (11)	12.8 (0.8)	40 (3)
P-replete, 74 Pa	7.96 (0.01)	2177 (6)	23.9 (0.4)	73 (1)

Analysis of POC, PON, POP and BSi
Samples for the analysis of particulate organic carbon (POC) and particulate organic nitrogen (PON) were collected on precombusted GF/F glass fiber filters (450 degrees C for 5 hr) under low vacuum and dried at 55 degrees C. The samples were then analyzed on an Elemental Analyzer (Costech Instruments, model 4010). Particulate organic phosphorus (POP) was measured followed by the protocol in Fu et al. (2005). Cellular biogenic silica (BSi) was analyzed according to the spectrophotometric method of Brzezinski and Nelson (1995).

Analysis of domoic acid concentrations
Particulate and dissolved domoic acid was measured using amnesic shellfish poison (ASP) enzyme-linked immunosorbent assay (ELISA) kits available from Biosense Laboratories. Particulate domoic acid samples were collected on uncombusted Whatman GF/F filters and frozen at –20 degrees C until analyzed. The filtrate from each sample was also collected, frozen and later analyzed for dissolved DA. Sample preparation and ELISA tests were carried out following the protocol of Biosense Laboratories (2005 version). The limit of detection for the ELISA method for particulate DA is 6.8 ng per liter. Total DA produced per cell (including the sum of both particulate and dissolved DA) was calculated by dividing the DA content of the whole-culture sample by the cell density.

References:
Brzezinski, M. A., and D. M. Nelson. 1995. The annual silica cycle in the Sargasso Sea near Bermuda. Deep-Sea Res. I. 42: 1215-1237, doi:10.1016/0967-0637(95)93592-3
Fu, F-X., Y. Zhang, K. Leblanc, S. A. Sañudo-Wilhelmy, and D.A. Hutchins. 2005. The biological and biogeochemical consequences of phosphate scavenging onto phytoplankton cell surfaces. Limnol. Oceanogr. 50: 1459-1472, doi: 10.4319/lo.2005.50.5.1459
Platt, T., C. L. Gallegos, and W. G. Harrison. 1980. Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. J. Mar. Res. 38: 687-701.

Data Processing Description

Values reported are the means of triplicate samples.

BCO-DMO merged data submitted as 3 separate tables into one dataset. Parameter names were modified to conform with BCO-DMO conventions.

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Data Files

File
P_multiseries_exp.csv (Comma Separated Values (.csv), 1.02 KB) MD5:360d660e7c8cd0b9ddebb59389588d14 Primary data file for dataset ID 3779

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Parameters

Parameter	Description	Units
condition	Phosphate treatment/condition. Limited = 0.5 umol per liter P; Replete = 20 umol per liter P.	text
pCO2	Calculated partial pressure of CO2. See Acquisition Description for information about how these values were calculated.	pa
DA_cellular	Cellular particulate domoic acid content.	pg/cell
DA_cellular_sd	Standard deviation of DA_cellular.	pg/cell
DA_dissolved	Dissolved domic acid level.	pg/cell
DA_dissolved_sd	Standard deviation of DA_dissolved.	pg/cell
DA_total	Total domic acid level (particulate + dissolved).	pg/cell
DA_total_sd	Standard deviation of DA_total.	pg/cell
C_fix_rate	Carbon fixation rate of Pseudo-nitzschia multiseries CCMP 2708.	mg C per mg Chl-a per hour
C_fix_rate_sd	Standard deviation of C_fix_rate	mg C per mg Chl-a per hour
sp_growth_rate	Specific growth rates of Pseudo-nitzschia mutliseries CCMP 2708.	specific growth per day
sp_growth_rate_sd	Standard deviation of sp_growth_rate.	specific growth per day
Si	Cellular quota of Silicon.	pmol/cell
Si_sd	Standard deviation of Si.	pmol/cell
Si_to_C	Ratio of cellular Si to Carbon.	mol:mol
Si_to_C_sd	Standard deviation of Si_to_C.	mol:mol
C_to_P	Ratio of cellular Carbon to P.	mol:mol
C_to_P_sd	Standard deviation of C_to_P.	mol:mol
C_to_N	Ratio of cellular Carbon to N.	mol:mol
C_to_N_sd	Standard deviation of C_to_N.	mol:mol

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Instruments

Dataset-specific Instrument Name	CHN Elemental Analyzer
Generic Instrument Name	CHN Elemental Analyzer
Dataset-specific Description	A Costech Instruments model 4010 elemental analyzer was used in measuring POC and PON.
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 Coulometer
Generic Instrument Name	CO2 Coulometer
Dataset-specific Description	A model CM 140 (UIC) carbon coulometer was used to measure total DIC.
Generic Instrument Description	A CO2 coulometer semi-automatically controls the sample handling and extraction of CO2 from seawater samples. Samples are acidified and the CO2 gas is bubbled into a titration cell where CO2 is converted to hydroxyethylcarbonic acid which is then automatically titrated with a coulometrically-generated base to a colorimetric endpoint.

Dataset-specific Instrument Name	Fluorescence Microscope Image Analysis System
Generic Instrument Name	Fluorescence Microscope Image Analysis System
Dataset-specific Description	Cells were enumerated using an Olympus BX51 epifluorescence microscope at 100-fold magnification.
Generic Instrument Description	A Fluorescence (or Epifluorescence) Microscope Image Analysis System uses semi-automated color image analysis to determine cell abundance, dimensions and biovolumes from an Epifluorescence Microscope. An Epifluorescence Microscope (conventional and inverted) includes a camera system that generates enlarged images of prepared samples. The microscope uses excitation ultraviolet light and the phenomena of fluorescence and phosphorescence instead of, or in addition to, reflection and absorption of visible light.

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Deployments

lab_Fu

Website	https://www.bco-dmo.org/deployment/58877
Platform	USC
Start Date	2009-08-01
End Date	2012-07-01
Description	Laboratory experiments carried out by Feixue Fu et al. of the University of Southern California (USC) for the project "Changing Phytoplankton Trace Metal Requirements in a High CO2 Ocean".

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Project Information

Changing Phytoplankton Trace Metal Requirements in a High CO2 Ocean (PhytoTM_in_HighCO2)

Coverage: Laboratory

This award is funded under the American Recovery and Reinvestment Act of 2009 (Public Law 111-5). The award is also associated with the NSF Integrative Computing Education and Research (ICER) initiative.

Over the past two decades, the fundamental importance of iron and other bioactive trace metals in structuring marine food webs and biogeochemical cycles has been realized. Even more recently, over the past several years, the international ocean science community has begun to mobilize in an urgent effort to understand the ecosystem-level consequences of rising anthropogenic CO2 and acidification of the global ocean. This project examines the intersection of these two major research themes, by asking the question: **How will the trace element requirements of marine phytoplankton change in response to future increases in atmospheric pCO2?**

Preliminary data generated by the investigators suggests that changing pCO2 can indeed profoundly affect the cellular quotas of Fe, Mo, Zn, Cd, Co and Mn in both prokaryotic and eukaryotic phytoplankton. Trace metals play critical roles as enzymatic co-factors for processes that are closely linked to the availability of CO2 such as carbon and nitrogen fixation, photosynthetic electron transport, and nutrient acquisition. Therefore, it is important to develop methods to quantitatively predict how algal metal requirements will change in tomorrow's rapidly changing ocean.

The investigators will take a three-pronged approach to addressing this overarching question:
(1) Laboratory experiments will measure the trace metal quotas of steady-state cultures of key phytoplankton functional groups like diatoms, coccolithophores, Phaeocystis, and diazotrophic and pico-cyanobacteria while varying pCO2 both alone, and together with other limiting factors such as iron, temperature, and light.
(2) Field work in the Southern California bight will provide measurements in trace metal stoichiometry of natural phytoplankton communities over a seasonal cycle in relation to pCO2 and other environmental variables -- this region is already experiencing some of the largest increases in acidic upwelled water along the entire West Coast.
(3) This observational and correlative study will be coupled with manipulative experiments at the USC Catalina Island facility in which trace metal quotas of the same natural phytoplankton communities can be measured in relation to pCO2 shifts under controlled incubation conditions.

Together, these three complementary approaches will enable the investigators to determine over a variety of temporal and spatial scales how phytoplankton-driven trace element biogeochemistry is likely to change in a future high-CO2 ocean.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-0850730

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