Experimental results from a study of photosynthesis rates of the diatom Pseudo-nitzschia multiseries under varying pCO2, phosphate, and light 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
Fu, Feixue	University of Southern California (USC)	Principal Investigator
Hutchins, David A.	University of Southern California (USC-HIMS)	Co-Principal Investigator
Sanudo-Wilhelmy, Sergio A.	University of Southern California (USC)	Co-Principal Investigator
Rauch, Shannon	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Table of Contents

Dataset Description

Photosynthesis rates of the diatom *Pseudo-nitzschia multiseries* (CCMP 2708) measured under 6 different pCO2 and phosphate treatments, at a range of light intensities.

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

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.

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)

Determination of P-E curves and primary production
Photosynthesis vs. irradiance (P-E) curves were performed by measuring 14C fixation rates at a range of light intensities using a photosynthetron (Composite High Pressure Technologies). Five mL of scintillation cocktail was added and the filters were stored in the dark overnight, and then counted using a Wallac System 1400 liquid scintillation counter.

All 14C uptake rates were corrected for dark uptake and carbon assimilation values were subsequently normalized to Chl-a. The initial slope of the P vs. E curve, i.e., the photosynthetic efficiency alpha [(mg C per

mg Chl a per hour per (umol quanta per square meter per second)] and the maximum chlorophyll specific carbon fixation rate PBmax [mg C per mg Chl a per hour] were calculated from least-squares nonlinear regression using the exponential function of Platt et al. (1980). Ek (umol quanta per square meter per second), the light saturation point and index of light adaptation, was calculated as PBmax:alpha. All carbon fixation rates for PE curves were calculated using measured initial experimental DIC and Chl a concentrations for each treatment.

References:

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

Phtosynthetic rates (photosyn) reported are means of the triplicate samples.

Parameter names were modified to conform with BCO-DMO conventions.

[table of contents | back to top]

Data Files

File
P_multiseries_photosyn.csv (Comma Separated Values (.csv), 2.42 KB) MD5:fb2262451237aefb3080b4d6631fd8a9 Primary data file for dataset ID 3771

[table of contents | back to top]

Parameters

Parameter	Description	Units
condition	Phosphate treatment/condition. Limited = 0.5 umol phosphate per liter; Replete = 20 umol phosphate per liter.	text
pCO2	Calculated partial pressure of CO2 measured in pascals. See Acquisition Description for description of how pCO2 was measured.	Pa
irradiance	Light level (irradiance) measured as umol photons per square meter per second.	umol photons per m^2 per second
photosyn	Chlorophyll-a specific carbon fixation rate (PB).	mg C per mg Chl-a per hour
photosyn_sd	Standard deviation of photosyn.	mg C per mg Chl-a per hour

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	CO2 Coulometer
Generic Instrument Name	CO2 Coulometer
Dataset-specific Description	A model CM 140 (UIC) coulometer was used to measure 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.

[table of contents | back to top]

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".

[table of contents | back to top]

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.

[table of contents | back to top]

Funding

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

[table of contents | back to top]