Results from experiments on Pseudo-nitzschia fraudulenta growth and domic acid productionunder varying CO2 and silicate levels (PhytoTM_in_HighCO2 project)

Website: https://www.bco-dmo.org/dataset/3769
Version: 02 Nov 2012
Version Date: 2012-11-02

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 measuring domoic acid (DA) production and growth rate of the diatom *Pseudo-nitzschia fraudulenta* under changing pCO2 and silicate conditions. Growth under projected future CO2 levels (765 ppm) was tested in addition to modern (360 ppm) and pre-industrial (200 ppm) levels, under silicate replete and limited conditions.

Data and methods are described in:
Tatters AO, Fu F-X, Hutchins DA (2012) High CO2 and Silicate Limitation Synergistically Increase the Toxicity of Pseudo-nitzschia fraudulenta. PLoS ONE 7(2): e32116. doi:10.1371/journal.pone.0032116

Methods & Sampling

All methods below are described in Tatters et al. 2012**.**

Culture, Media, and Sampling
Pseudo-nitzschia fraudulenta was isolated from public nearshore water collected at 34.08 N, 119.05 W in Ventura County, California in March, 2010. The culture was grown at 16 degrees C on a 12-h light:12-h dark cycle in modified f/2 enriched seawater growth media (Guillard 1975) under 90 photons per square meter per second of cool white fluorescent illumination. Silicate (Si(OH)4) final concentrations in the medium were 10.6 uM for the Si(OH)4-limited treatment and 106.1 uM for the nutrient replete treatment. Dissolved CO2 concentrations were controlled by gentle bubbling with commercially prepared air/CO2 mixtures (Praxair Gas).

Cell Counts and Growth Rates
Steady state semi-continuous culture methodology was employed to maintain cultures in exponential state and specific growth rates were calculated as the equation:

u = (lnNb - lnNa) / (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). Growth rate as determined by bi-weekly microscopic cell counts and in vivo fluorescence determined the dilution rate of each bottle. Cells of *P. fraudulenta* preserved in acidified Lugol's solution were vortexed and enumerated by direct counts using an Accu-Scope 3032 inverted microscope according to the Utermohl method (1931). A minimum of 300 cells were counted to guarantee a 95% confidence interval with +/- 11.5% accuracy (Guillard 1973). Cultures were acclimated for a period of three months to the respective experimental conditions prior to splitting into triplicates, which were then further acclimated for one month (5 to 17 cell divisions) prior to final sampling.

Domoic acidy by high performance liquid chromatography
High performance liquid chromatography with ultraviolet detection (HPLC-UV) of domoic acid (DA) was performed using a SCL-10ADVP controlled system (Shimadzu). The UV detector was programmed for sample and reference wavelengths of 242 and 280 nm respectively and the system was operated by EZ START software version 7.4 SP1 (Shimadzu). Cellular concentrations of domoic acid were determined according to Mafra et al., (2009) with slight modifications (Mafra et al 2009). Culture subsamples of 10 to 20 ml were carefully measured and cells were collected by gentle filtration on 25 mm GF/F filters (Whatman). The filters were stored in the dark at -20 degrees C. Filters were subsequently subject to sonication in 10% aqueous methanol for 2 min. at 40W in a water bath. The cell extracts were then clarified by centrifugation at 3000 x g for 10 min at 4 degrees C. The pellet was discarded and the clarified extracts were transferred to 300 ul polyspring inserts (National Scientific) placed inside clean 2.0 ml Target DPTM vials (National Scientific). Prior to analysis, all samples were treated with 0.15% trifluoroacetic acid (TFA).

The chromatographic separation was carried out on a reversed phase Luna C18 (2) column (3 um, 2x100 mm, Phenomenex) at 25 degrees C with a mobile phase system consisting of water with 0.1% TFA (A), and acetonitrile (MeCN) with 0.1% TFA (B). The elution gradient began with a 10–35% B transition over 10 min, then was held at 35% B until 15 min, followed by a subsequent decrease to 10% B at 16 min, and held at 10% B. The flow rate was 0.2 ml per min and the injection volume 5 to 10 ul. Quantification of domoic acid was determined using certified reference material CRM-domoic acid-e obtained from the National Resource Council, Canada at a range of concentrations. Calibration curves of CRM-domoic acid-e were determined by linear regressions (r squared values=0.99) for each sample treatment. Interpolation from the standard curves was used to calculate the amount of compound injected from the peak areas of each sample under the same experimental conditions. Domoic acid per cell concentrations were determined by the ratio of reconstituted volume (300 ul) to the volume injected (5 to 10 ul) and dividing by the total cell count in the original sample. Reported values represent means of the results (n= 3). Domoic acid production rates were calculated by multiplying the growth rate by toxin per cell.

Carbonate Buffer System analysis
Dissolved inorganic carbon analysis was performed using a CM5230 CO2 coulometer (UIC). pH was determined on freshly collected samples using a calibrated Orion 5-star plus pH meter using an NBS buffer system with three-point calibration. The pCO2 in the experimental media was calculated from these two parameters using CO2SYS software. For clarity, pCO2 treatments in the cultures are referred to in the data and text (Tatters et al. 2012) using rounded-off values of 200 ppm, pre-industrial atmospheric levels; 360 ppm, modern atmospheric levels: and 765 ppm, projected year 2100 levels.

Carbonate buffer system parameters:

Treatment	pH	DIC (umol/kg)	Calculated pCO2 (ppm)
190 ppm	8.43	1965	198
380 ppm	8.23	2107	357
750 ppm	7.95	2249	764

Chlorophyll a, POC, PON, and Biogenic Silica
For chlorophyll a (Chl a) measurements, samples were filtered in duplicate onto 25 mm GF/F filters. Five ml of 90% acetone was later added and each vial was allowed to extract overnight in the dark at -20 degrees C. After 24 hours, Chl a was determined using a Turner Designs 10-AU fluorometer. For particulate organic carbon

(POC) and nitrogen (PON), sample volumes of 20 ml were collected onto pre-combusted (450 degrees C for 5 h) GF/F glass fiber filters, stored at -20 degrees C, and dried at 55 degrees C before analysis. Molar POC and PON content was analyzed using a 4010 Costech Elemental Combustion System calibrated with methionine and atropine as reference materials. For measurement of biogenic silica, sample volumes of 20 ml were collected onto 0.6 uM polycarbonate filters until analysis. Cellular biogenic silica (BSi) was measured according to Brzezinski and Nelson (1995).

Data Processing Description

BCO-DMO merged data submitted as 2 separate tables into one dataset. Parameter names were changed to conform with BCO-DMO conventions.

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Data Files

File	
P_fraudulenta_exp.csv	(Comma Separated Values (.csv), 2.00 KB) MD5:21d9a196d7a2daf84ea11939086c61c6
Primary data file for dataset ID 3769	

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Parameters

Parameter	Description	Units
condition	Si availability. Replete = 106.1 uM Si; Limited = 10.6 uM Si.	text
pCO2	Carbon dioxide partial pressure (see Acquisition Description).	ppm
replicate	Replicate indentifier (e.g. 190A, 190B, and 190C are three replicates of the 190 ppm treatment.)	text
BSi	Cellular biogenic Silica.	umol/L
POC	Particulate organic Carbon concentration.	umol/L
PON	Particulate organic Nitrogen concentration.	umol/L
POP	Particulate organic Phosphate concentration.	umol/L
DIC	Dissolved inorganic Carbon in umol per kilogram seawater.	umol/kg
pH	pH.	unitless; pH scale
DA_per_cell	Total domoic acid content per cell.	pg per cell
DA_rate	Domoic acid production rate. Calculated by multiplying the growth rate by toxin (DA) per cell.	pg per cell per day
chl_per_cell	Chl-a per cell.	pg
growth_rate	Specific growth rate.	growth rate per day
counts	Number of cells.	per mL

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Instruments

Dataset-specific Instrument Name	Benchtop pH Meter
Generic Instrument Name	Benchtop pH Meter
Dataset-specific Description	pH of the carbonate buffer system was determined on freshly collected samples using a calibrated Orion 5-star plus pH meter.
Generic Instrument Description	An instrument consisting of an electronic voltmeter and pH-responsive electrode that gives a direct conversion of voltage differences to differences of pH at the measurement temperature. (McGraw-Hill Dictionary of Scientific and Technical Terms) This instrument does not map to the NERC instrument vocabulary term for 'pH Sensor' which measures values in the water column. Benchtop models are typically employed for stationary lab applications.

Dataset-specific Instrument Name	CHN Elemental Analyzer
Generic Instrument Name	CHN Elemental Analyzer
Dataset-specific Description	Molar POC and PON were analyzed using a 4010 Costech elemental analyzer (combustion system).
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	DIC analysis was perfomed using a CM5230 CO2 coulometer manufactured by UIC, Inc.
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	High Performance Liquid Chromatograph
Generic Instrument Name	High-Performance Liquid Chromatograph
Dataset-specific Description	High performance liquid chromatography with ultraviolet detection (HPLC-UV) of domoic acid was performed using a Shimadzu SCL-10ADVP controlled system.
Generic Instrument Description	A High-performance liquid chromatograph (HPLC) is a type of liquid chromatography used to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir of the mobile phase, a pump, an injector, a separation column, and a detector. Compounds are separated by high pressure pumping of the sample mixture onto a column packed with microspheres coated with the stationary phase. The different components in the mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase.

Dataset-specific Instrument Name	Turner Designs Fluorometer -10-AU
Generic Instrument Name	Turner Designs Fluorometer 10-AU
Dataset-specific Description	Turner Designs 10-AU fluorometer was used to determine chl-a.
Generic Instrument Description	The Turner Designs 10-AU Field Fluorometer is used to measure Chlorophyll fluorescence. The 10AU Fluorometer can be set up for continuous-flow monitoring or discrete sample analyses. A variety of compounds can be measured using application-specific optical filters available from the manufacturer (read more from Turner Designs, turnerdesigns.com, Sunnyvale, CA, USA).

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Deployments

nearshore_sampling_Fu

Website	https://www.bco-dmo.org/deployment/58878
Platform	Ventura_County_shore
Start Date	2010-03-15
End Date	2010-03-15
Description	Pseudo-nitzschia fraudulenta was isolated from public nearshore water collected at 34.08 N, 119.05 W in Ventura County, California in March, 2010 for the project "Changing Phytoplankton Trace Metal Requirements in a High CO2 Ocean" (PI: Feixue Fu, USC).

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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