Results from experiments on Attheya sp. growth, productivity, and trace metal use under varying pCO2 and B12 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 testing the effects of B12 and CO2 availability on specific growth rates, primary productivity, cellular trace metal quotas, and net use efficiencies (NUEs) of the subarctic diatom *Attheya sp.*

Data and methods are described in:
King, A, Sanudo-Wilhelmy, S.A., Leblanc, K., Hutchins, D.A., Fu, F-X. CO2 and vitamin B12 interactions determine bioactive trace metal requirements of a subarctic Pacific diatom. ISME J. 2011 August; 5(8): 1388–1396. doi: 10.1038/ismej.2010.211

Methods & Sampling

All methods below are described in King et al. 2011.

Organism source and culture conditions
Unialgal axenic *Attheya sp.* (CCMP207) stock cultures, isolated from the Bering Sea, were obtained from the Provasoli-Guillard Culture Collection of Marine Phytoplankton (Boothbay Harbor, ME) and were grown in microwave-sterilized media prepared from 0.2 mm-filtered natural seawater (collected using trace metal clean techniques). Media had Aquil concentrations of nitrate, phosphate and silicic acid with additions of 5 uM EDTA, 451 nM Fe, 80 nM Zn, 50 nM Co and no added Cd (Price et al., 1988/89). Stock cultures were incubated at 3 degrees C with a 12 h:12 h light–dark cycle and an incident photon flux density of 80 µmol photons per square meter per second.

Experimental treatments/conditions
Experimental treatments included trace metal clean vitamin B12 additions of 500 and 10 ng per liter (370 and 7 pM) for the B12-replete and B12-limited cultures, respectively. Vitamin and nutrient stocks were cleaned of trace metals before use by passing them through a column packed with Chelex-100 (Bio-Rad, Hercules, CA, USA; Price et al., 1988/89). For both B12 conditions, triplicate acid-washed polycarbonate bottles were equilibrated with commercially prepared air:CO2 mixtures at three different CO2 concentrations: 200, 370 and 670 ppm pCO2. In-line HEPA filters were used to avoid trace metal and bacterial contamination from the gas tanks or lines.

Trace metal clean semi-continuous culturing methods were used during acclimation periods and steady-state growth. Final sampling was carried out following 4 to 8 weeks of semi-continuous incubation after statistically invariant growth rates were recorded for at least three consecutive transfers.

Seawater medium pCO2 in the experimental bottles was calculated throughout the experiment using measurements of pH and total dissolved inorganic carbon according to Dickson and Goyet (1994). To ensure that CO2 levels remained constant during growth, the pH in each bottle was monitored daily using a microprocessor pH meter, calibrated with pH 4, 7 and 10 buffer solutions. Total dissolved inorganic carbon was measured via coulometry (model CM 140, UIC, Joliet, IL, USA). The calculated pCO2 values (using CO2SYS; http://www.cdiac.ornl.gov/ftp/co2sys/CO2SYS_calc_XLS) from the final day of the experiment were 201±13, 367±21 and 671±31 ppm (see table below); these treatments are referred to in the dataset and the literature using rounded-off values of 200, 370 and 670 ppm pCO2.

Seawater carbonate buffer system conditions (n=3):

Treatment	Measured pH (sd)	Measured DIC (sd); uM	Calculated pCO2 (sd); ppm
B12-limited, 208 p.p.m	8.36 (0.03)	1975 (27)	208 (15)
B12-limited, 380 p.p.m	8.13 (0.02)	2070 (20)	380 (18)
B12-limited, 680 p.p.m	7.91 (0.02)	2208 (13)	680 (32)
B12-replete, 195 p.p.m	8.38 (0.02)	1946 (16)	195 (10)
B12-replete, 353 p.p.m	8.15 (0.02)	2020 (8)	353 (16)
B12-replete, 661 p.p.m	7.92 (0.01)	2197 (12)	661 (31)

Primary production and trace metal measurements
Primary production was measured in triplicate using 24 h incubations with 3.7 kBq per mL H14CO3 under the appropriate experimental growth conditions of light and temperature for each treatment, followed by filtration and scintillation counting. Aliquots for analysis of particulate organic C and N were filtered onto pre-combusted 25mm GF/F filters (Whatman, Maidstone, UK) and analyzed using a 4010 CHNS Elemental Combustion System (Costech, Valencia, CA, USA). Samples for particulate organic phosphorous were determined spectrophotometrically (Fu et al. (2005, 2007).

Particulate samples for trace metal analysis were filtered onto acid-washed 3-um-pore-size polycarbonate filters (Millipore, Billerica, MA, USA), rinsed with oxalate reagent to remove extracellular trace metals (Tovar-Sanchez et al., 2003), and Fe, Zn, Co and Cd were determined with a magnetic sectorfield high-resolution inductively coupled plasma mass spectrometer (ICPMS) (Element 2, Thermo, Waltham, MA, USA). Procedural filter blanks were also subjected to the same storage, digestion, dilution and analysis processes, and these blank values were subtracted from sample measurements (Sañudo-Wilhelmy et al., 2001). The digestion protocol consisted of sequential additions of ultrapure HCl, HNO3 and HF (Omnitrace Ultra; VWR, Westchester, PA, USA) and heating to 100 degrees C (Eggimann and Betzer, 1976).

The measured concentrations of particulate elements (C, N, Fe, Co, Zn and Cd) were normalized to P. Cellular elemental 'quota' or 'content' are used interchangeably with 'requirement'. Trace metal:C ratios were used in conjunction with specific growth rates to calculate net use efficiencies (NUEs; specific growth rate divided by trace metal:C ratio), representing how efficiently trace metals are used by cells for growth and C fixation (Raven, 1991).

Data Processing Description

BCO-DMO merged data submitted as 4 separate tables into one dataset. Parameter names were modified to conform with BCO-DMO conventions.

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Data Files

File
Attheya_sp_exp.csv (Comma Separated Values (.csv), 1.36 KB) MD5:046994bd3e4852569812ec033fbb9429 Primary data file for dataset ID 3770

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Parameters

Parameter	Description	Units
condition	B12 treatment/condition. Replete = 370 pM B12; Limited = 7 pM B12.	text
pCO2	Seawater pCO2 in the experimental bottles. Calculated using pH and total dissolved inorganic carbon (see Acquisition Description).	ppm CO2
sp_growth_rate	Specific growth rate per day.	unitless
sp_growth_rate_sd	Standard deviation of sp_growth_rate.	unitless
pr_productivity	14C based primary productivity in micrograms C per micorgrams chl per hour.	ug C / ug chl / h
pr_productivity_sd	Standard deviation of pr_productivity.	ug C / ug chl / h
C_to_P	Carbon concentration normalized to Phosophorus.	mol:mol
C_to_P_sd	Standard deviation of C_to_P.	mol:mol
N_to_P	Nitrogen concentration normalized to Phosphorus.	mol:mol
N_to_P_sd	Standard deviation of N_to_P.	mol:mol
Fe_to_P	Iron concentration normalized to Phosphorus.	mmol:mol
Fe_to_P_sd	Standard deviation of Fe_to_P.	mmol:mol
Co_to_P	Cobalt concentration normalized to Phosphorus.	mmol:mol
Co_to_P_sd	Standard deviation of Co_to_P.	mmol:mol
Zn_to_P	Zinc concentration normalized to Phosphorus.	mmol:mol
Zn_to_P_sd	Standard deviation of Zn_to_P.	mmol:mol
Cd_to_P	Cadmium concentration normalized to Phosphorus.	mmol:mol
Cd_to_P_sd	Standard deviation of Cd_to_P.	mmol:mol
Fe_NUE	Net use efficiency (NUE) of Iron. Specific growth rate divided by Fe:C ratio.	mol C fixed per mol Fe per day
Fe_NUE_sd	Standard deviation of Fe_NUE.	mol C fixed per mol Fe per day
Co_NUE	Net use efficiency (NUE) of Cobalt. Specific growth rate divided by Co:C ratio.	mol C fixed per mol Co per day
Co_NUE_sd	Standard deviation of Co_NUE.	mol C fixed per mol Co per day
Zn_NUE	Net use efficiency (NUE) of Zinc. Specific growth rate divided by Zn:C ratio.	mol C fixed per mol Zn per day
Zn_NUE_sd	Standard deviation of Zn_NUE.	mol C fixed per mol Zn per day
Cd_NUE	Net use efficiency (NUE) of Cadmium. Specific growth rate divided by Cd:C ratio.	mol C fixed per mol Cd per day
Cd_NUE_sd	Standard deviation of Cd_NUE.	mol C fixed per mol Cd per day

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Instruments

Dataset-specific Instrument Name	CHN Elemental Analyzer
Generic Instrument Name	CHN Elemental Analyzer
Dataset-specific Description	Particulate C and N were analyzed using a 4010 CHNS Elemental Combusion 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	Inductively Coupled Plasma Mass Spectrometer
Generic Instrument Name	Inductively Coupled Plasma Mass Spectrometer
Dataset-specific Description	Fe, Zn, Co, and Cd were determined with a magnetic sectorfield high-resolution inductively coupled plasma mass spectrometer (ICPMS) (Element 2, Thermo).
Generic Instrument Description	An ICP Mass Spec is an instrument that passes nebulized samples into an inductively-coupled gas plasma (8-10000 K) where they are atomized and ionized. Ions of specific mass-to-charge ratios are quantified in a quadrupole mass spectrometer.

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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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