Experimental results from a study of cellular quotas of carbon, nitrogen, and phosphorus in Trichodesmium erythraeum strain IMS101 under varying pCO2 and light conditions (PhytoTM_in_HighCO2 project)

Website: https://www.bco-dmo.org/dataset/3775
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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 on the effects of pCO2 and light on cellular carbon, nitrogen, and phosphorus quotas in the cyanobacteria *Trichodesmium erythraeum*.

Data and methods are described in:
Garcia, N. S., Fu, F.-X., Breene, C. L., Bernhardt, P. W., Mulholland, M. R., Sohm, J. A. and Hutchins, D. A. (2011), INTERACTIVE EFFECTS OF IRRADIANCE AND CO2 ON CO2 FIXATION AND N2 FIXATION IN THE DIAZOTROPH TRICHODESMIUM ERYTHRAEUM (CYANOBACTERIA). Journal of Phycology, 47: 1292–1303. doi: 10.1111/j.1529-8817.2011.01078.x

Methods & Sampling

The methods below are described in Garcia et al. (2011).

Culture conditions
Stock and experimental cultures of *Trichodesmium erythraeum* GBRTRL101 (GBR; from the Great Barrier Reef, Pacific Ocean) and IMS101 (IMS; from coastal North Carolina, Atlantic Ocean) were cultured at 24 degrees C (unless otherwise stated) in an artificial seawater medium without fixed N using a modified version of the YBCII recipe of Chen et al. (1996). Phosphate and trace metal solutions were filtered (0.2 um) and added in concentrations equivalent to the AQUIL recipe (Morel et al. 1979) to microwave- (experiment with GBR) or

autoclave-sterilized (experiments with IMS) seawater. Irradiance was supplied with cool white fluorescent bulbs on a 12:12 light:dark cycle. For all experiments, cultures were grown in triplicate using a semi-continuous batch culturing method to achieve steady state exponential growth for approximately 7-10 generations prior to sampling, in order to fully acclimatize cells to treatment pCO2 and irradiance conditions. We monitored cell density every 2-3 days using microscopic cell counts. When the biomass reached approximately 100-200 trichomes per mL (~100-200 nmol C per mL), we diluted cultures with fresh medium to 50-100 trichomes per mL (~50-100 nmol C per mL). In this semi-continuous culturing method, the growth rate determines the dilution rate; this culturing technique does not attempt to control the growth rate with the dilution rate, as continuous culturing methods do.

Experimental design: CO2/light experiments
Cultures of the IMS strain were grown in 18 1-L polycarbonate bottles at 38, 100 and 220 umol quanta per square m per second at two concentrations of CO2. The experiment was conducted on a 12:12 light:dark cycle at 24 degrees C. Within each irradiance treatment, cultures were bubbled with 0.2 um filtered lab air and elevated, 100-year predicted (750 ppm certified value for both CO2 experiments) atmospheric CO2 concentrations. The rate of bubbling was visually monitored daily to ensure that cultures were bubbled with sufficient positive gas flow to keep the pH of the cultures at an appropriate level for respective CO2 treatments. Based on rates of gas utilization from the supply cylinders, estimated gas flow rates were between 30-60 mL per min.

Seawater carbonate system estimates
Total dissolved inorganic carbon (DIC) was preserved in whole water samples (5-70 mL; stored at 4 degrees C) with a 5% HgCl2 solution (final concentration diluted to 0.5% HgCl2) as described in Fu et al. (2007), and estimated by acidifying 5 mL and quantifying the CO2 trapped in an acid sparging column (model: CM 5230) with a carbon coulometer (model: CM 140, UIC inc., Joliet, IL, USA). Reference material for the DIC analysis was prepared by Andrew Dickson at Scripps Institute of Oceanography. pH was measured with a pH meter (model: Orion 5 star Thermo Scientific, Beverly, MA, USA) and was monitored to ensure that perturbations of the seawater with the either air or certified pre-mixed air (Gilmore Liquid Air Company 750 ppm) resulted in the desired target pH of either ~8.2 or ~7.95. For the CO2/light manipulation experiment with IMS, measurements of pH were paired with total DIC samples 5-6 days prior to measuring rates of CO2- and N2 fixation and were used to calculate pCO2 at 24 degrees C using the CO2sys program provided by Lewis and Wallace (1998) with K1 and K2 constants from Mehrbach et al. (1973) refit by Dickson and Millero (1987).

Carbonate system parameters in the experiment:

Strain	pCO2 treatment	DIC (uM)	pH	Calculated pCO2 (ppm)
IMS	Present day	2018 ±27	8.23 ±0.01	435 ± 9
IMS	100-year projected	2116 ±15	8.00 ± 0	771 ± 8
GBR	Present-day	2037 ±9	n.d.	n.d.
GBR	100-year projected	2165 ±9	n.d.	n.d.

Cellular C, N and P quotas
Particulate N and C were estimated in cells filtered onto combusted GF/F filters. Samples were dried at 80-90 degrees C for 2 days, compressed into pellets and the amounts of C and N were determined using an elemental analyzer (model: 4010, Costech Analytical Technologies Inc., Valencia, CA, USA). Particulate P was estimated by filtering 20-30 mL of the cultures onto combusted GF/F filters. Filters were rinsed twice with 2 mL of a 0.17M sodium sulfate solution and dried in a combusted glass vial with 2 mL of a 0.017M magnesium sulfate solution. Samples were then combusted for 2 hours at 450 degrees C to volatilize organic compounds bound to P. Residual P was estimated using the spectrophotometric method with a spectrophotometer (model: SP-830, Barnstead/Turner, Dubuque, Iowa, USA) at a wavelength of 885 nm.

References:
Chen, Y. B., Zehr, J. P. & Mellon, M. 1996. Growth and nitrogen fixation of the diazotrophic filamentous nonheterocystous cyanobacterium Trichodesmium sp IMS 101 in defined media: Evidence for a circadian rhythm. J. Phycol. 32:916-923. DOI: 10.1111/j.0022-3646.1996.00916.x
Dickson, A. G., & Millero, F. J. 1987. A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep-Sea Res. 34:1733-1743. DOI: 10.1016/0198-0149(87)90021-5
Lewis, E. and D. W. R. Wallace (1998). Program Developed for CO2 System Calculations. ORNL/CDIAC-105. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tenessee. Available at: http://cdiac.ornl.gov/oceans/co2rprt.html.
Mehrbach, Y., Culberson, C., Hawley, J. & Pytkovicz, R. 1973. Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnol. Oceanogr. 18:897-907.

Morel, F. M. M., Rueter, J. G., Anderson, D. M. and Guillard, R. R. L. 1979. Aquil - chemically defined phytoplankton culture-medium for trace-metal studies. J. Phycol. 15:135-141. DOI: 10.1111/j.1529-8817.1979.tb02976.x

Data Processing Description

Parameter names have been changed to conform with BCO-DMO conventions.

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Data Files

File
tricho_CNP_quotas.csv (Comma Separated Values (.csv), 399 bytes) MD5:bbecf162b3daca742e7b6cceb522bc6f Primary data file for dataset ID 3775

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Parameters

Parameter	Description	Units
pCO2	Partial pressure of CO2. Present-day CO2 level = 380 ppm; 100-year predicted CO2 level = 750 ppm. See Acquisition Description for more detail on how actual values were determined.	ppm
irradiance	Light/irradiance level in terms of umol quanta per square meter per second.	umol quanta meter-2 sec-1
cellular_C	Carbon concentration per cell of T. erythraeum IMS101.	pmol C per cell
cellular_C_sd	Standard deviation of cellular_C based on the means of 3 triplicates.	pmol C per cell
cellular_N	Nitrogen concentration per cell of T. erythraeum IMS101.	pmol N per cell
cellular_N_sd	Standard deviation of cellular_N based on the means of 3 triplicates.	pmol N per cell
cellular_P	Phosphorus concentration per cell of T. erythraeum IMS101.	pmol P per cell
cellular_P_sd	Standard deviation of cellular_P based on the means of 3 triplicates.	pmol P per cell

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Instruments

Dataset-specific Instrument Name	Benchtop pH Meter
Generic Instrument Name	Benchtop pH Meter
Dataset-specific Description	pH was measured with an Orion 5 star Thermo Scientific (Beverly, MA, USA) 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	Amounts of C and N were determined using a Costech Model 4010 elemental analyzer manufactured by Costech Analytical Technologies Inc., Valencia, CA, USA.
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	Spectrophotometer
Generic Instrument Name	Spectrophotometer
Dataset-specific Description	Residual P was estimated using the spectrophotometric method with a Barnstead/Turner SP-830 spectrophotometer at wavelength of 885 nm.
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

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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