Results from experiments examining the cell diameter of Crocosphaera watsonii (WH0003) as a function of total P, light, and CO2; conducted in the Hutchins Laboratory, USC

Website: https://www.bco-dmo.org/dataset/4069
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Project
» CO2 control of oceanic nitrogen fixation and carbon flow through diazotrophs (Diaz N2-Fix in High CO2)

Contributors	Affiliation	Role
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Dataset Description

Results of a laboratory experiment examining growth of the WH0003 isolate of *Crocosphaera watsonii* as a function of total Phosphorus (P) concentration, under different light intensities and pCO2 levels. WH0003 was isolated near Sta. ALOHA (A Long Term Oligotrophic Habitat Assessment) in the North Pacific Ocean near Hawaii (22 deg 45' N, 158 deg 00' W).

Detailed methods and results are described in the following publication (see Figure 4, panel b):
Garcia, N.S., Fu, F.X., and Hutchins, D.A. (2013). Colimitation of the unicellular photosynthetic diazotroph Crocosphaera watsonii by phosphorus, light, and carbon dioxide. Limnology and Oceanography 58(4): 1501-1512. DOI: 10.4319/lo.2013.58.4.1501

Methods & Sampling

Culturing and experimental conditions
Experimental cultures were grown with a semi-continuous culturing method at 28 degrees C in autoclave-sterilized artificial seawater medium with nutrients added in concentrations equivalent to the recipe for the Aquil medium (except for NO3-), as in Garcia et al. (2011) and originally described by Morel et al. (1979).

P-light-CO2 experiment and cellular growth rates
In the P-light-CO2 experiment, triplicate cultures were diluted every two days to 5 x 103 cells per mL with medium that contained treatment concentrations of PO43- ranging from 0.1 - 4.0 umol per L. Cells were counted microscopically in each replicate culture with a hemocytometer at the end of each dilution period, and steady state growth rates were calculated from an increase in culture cell number per unit volume between 2-3 dilution periods (4-6 days) after cultures were acclimated to treatment conditions for 7-10 generations.

A low cell biomass was necessary to control CO2 concentrations in cultures and a consistent dilution period

reduced variations in growth rates between dilutions. In the P-light-CO2 experiment cultures were grown in 1 L polycarbonate bottles at 40 or 150 umol quanta per square meter per second and bubbled with 19 Pa or 81 Pa pCO2 pre-mixed air supplied and certified by Gilmore Liquid Air Company. Culture pH was measured with a pH meter using the National Bureau of Standards (NBS) scale for seawater pH measurements (model: Orion 5 star, Thermo Scientific). For the P-light-CO2 experiment seawater was bubbled and pre-equilibrated with treatment concentrations of pCO2 before measuring pH and adding nutrients. This was essential to maintain high pH values in the 19 Pa pCO2 treatments. The investigators excluded data from the high light, 19-Pa pCO2 treatment where the pH was >0.05 units lower than the expected pH range of 8.45-8.49 (specifically, the 0.4, 0.8, 2.0 umol total P per L treatments).

Light was supplied on a 12:12 light:dark cycle with cool white fluorescent bulbs. The investigators terminally sampled each replicate culture 24 hours after the last dilution for N2-fixation rates and CO2-fixation rates, and at this point they also sampled for P-uptake rate measurements and cellular P content from each replicate in the P-light-CO2 experiment. To acclimate cultures to low P conditions in the P-light-CO2 experiment, the investigators consecutively reduced the concentration of P by transferring cultures acclimated to neighboring P concentrations in the experimental matrix. Steady-state growth was not achievable in treatments with the lowest P concentrations because growth rates continuously declined when the concentration of P was reduced to those concentrations. In these cases, the investigators sampled cultures before growth rates became negative, except for the low-light, low-P, low-pCO2 treatment, which did have a negative growth rate.

Phosphorus-uptake rates
Phosphorus-uptake rates were determined with radioactive 33PO43- over 24 h. The investigators inoculated 200 mL culture samples from each treatment replicate with 0.46 KBq 33PO43- mL-1, yielding a final added concentration of 0.33 pmol 33PO43- mL-1. The investigators accounted for 33PO43- that was not incorporated into the cell by inoculating parallel 200 mL culture samples (pooled from 3 experimental replicates) with the same final activity and concentration of 33PO43- just before filtering at the end of the 24 h incubation period.

Cellular P
Near the end of the light period (9th-11th hour), samples were filtered for cellular P content (50 mL) from each replicate onto combusted (450 degrees C, 4 h) Whatman GF/F filters and measured them as in Fu et al. (2005). Filtered samples were rinsed 3 times with 2 mL 0.017 mol L-1 Na2SO4 and placed in 20 mL glass scintillation vials with 2 mL 0.017 MgSO4, which was evaporated at ~80 degrees C over a few days. Filters were combusted at 450 degrees C for 2 h to release P from organic compounds. After cooling, filters were reheated to 80 degrees C along with 5 mL 0.2 mol per liter HCl for 30 minutes and phosphate concentrations were estimated spectrophotometrically with the colorimetric assay described by Lebo and Sharp (1992).

References:
Garcia, N. S., F.-X. Fu, , C. L. Breene, P. W. Bernhardt, M. R. Mulholland, J. A. Sohm, and D. A. Hutchins. 2011. Interactive effects of irradiance and CO2 on CO2- and N2 fixation in the diazotroph Trichodesmium erythraeum (Cyanobacteria). J. Phycol. 47: 1292-1303. DOI: 10.1111/j.1529-8817.2011.01078.x

Lebo, M. E., and J. H. Sharp. 1992. Modeling phosphorus cycling in a well-mixed coastal plain estuary. Estuar. Coastal Shelf Sci. 35: 235-252. doi: 10.1016/S0272-7714(05)80046-0

Morel, F. M. M., J. G. Rueter, D. M. Anderson, 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

BCO-DMO re-arranged data formatted as separate tables into one dataset. Parameter names were changed to conform with BCO-DMO conventions.

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Data Files

File
C_watsonii_WH0003_cell_diam_figb.csv (Comma Separated Values (.csv), 899 bytes) MD5:0ce78248e77c7036039e0d95e6b042c5 Primary data file for dataset ID 4069

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Parameters

Parameter	Description	Units
light	Light intensity. (For more about light measurement see: Australian National Algae Culture Collection and Plant Physiology Online.)	micromoles quanta per square meter per second (umol quanta m-2 s-1)
pCO2	Partial pressure of carbon dioxide (pCO2) in the water body (19 or 81 Pa).	Pascals (Pa)
log_total_P	Log of total phosphate concentration.	micromoles per Liter (umol L-1)
cell_diameter	Cell diameter measured in micrometers (um).	micrometers (um)

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Instruments

Dataset-specific Instrument Name	Hemocytometer
Generic Instrument Name	Hemocytometer
Dataset-specific Description	Cells were counted microscopically in each replicate culture with a hemocytometer.
Generic Instrument Description	A hemocytometer is a small glass chamber, resembling a thick microscope slide, used for determining the number of cells per unit volume of a suspension. Originally used for performing blood cell counts, a hemocytometer can be used to count a variety of cell types in the laboratory. Also spelled as "haemocytometer". Description from: http://hlsweb.dmu.ac.uk/ahs/elearning/RITA/Haem1/Haem1.html.

Dataset-specific Instrument Name	Microscope-Optical
Generic Instrument Name	Microscope - Optical
Dataset-specific Description	Cell diameters were measured with an ocular micrometer.
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of reflection and absorption of visible light. Includes conventional and inverted instruments. Also called a "light microscope".

Dataset-specific Instrument Name	pH Sensor
Generic Instrument Name	pH Sensor
Dataset-specific Description	Culture pH was measured with an Orion 5 star (Thermo Scientific) pH meter using the National Bureau of Standards (NBS) scale for seawater pH measurements.
Generic Instrument Description	An instrument that measures the hydrogen ion activity in solutions. The overall concentration of hydrogen ions is inversely related to its pH. The pH scale ranges from 0 to 14 and indicates whether acidic (more H+) or basic (less H+).

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Deployments

lab_Hutchins_07-12_diazotrophs

Website	https://www.bco-dmo.org/deployment/59043
Platform	USC
Description	Laboratory experiments conducted as part of project titled, "CO2 control of oceanic nitrogen fixation and carbon flow through diazotrophs".

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Project Information

CO2 control of oceanic nitrogen fixation and carbon flow through diazotrophs (Diaz N2-Fix in High CO2)

Coverage: Laboratory

From NSF award abstract:

The importance of marine N2 fixation to present ocean productivity and global nutrient and carbon biogeochemistry is now universally recognized. Marine N2 fixation rates and oceanic N inventories are also thought to have varied over geological time due to climate variability and change. However, almost nothing is

known about the responses of dominant N2 fixers in the ocean such as Trichodesmium and unicellular N2 fixing cyanobacteria to past, present and future global atmospheric CO2 regimes. Our preliminary data demonstrate that N2 and CO2 fixation rates, growth rates, and elemental ratios of Atlantic and Pacific Trichodesmium isolates are controlled by the ambient CO2 concentration at which they are grown. At projected year 2100 pCO2 (750 ppm), N2 fixation rates of both strains increased 35-100%, with simultaneous increases in C fixation rates and cellular N:P and C:P ratios. Surprisingly, these increases in N2 and C fixation due to elevated CO2 were of similar relative magnitude regardless of the growth temperature or P availability. Thus, the influence of CO2 appears to be independent of other common growth-limiting factors. Equally important, Trichodesmium growth and N2 fixation were completely halted at low pCO2 levels (150 ppm), suggesting that diazotrophy by this genus may have been marginal at best at last glacial maximum pCO2 levels of ~190 ppm. Genetic evidence indicates that Trichodesmium diazotrophy is subject to CO2 control because this cyanobacterium lacks high-affinity dissolved inorganic carbon transport capabilities. These findings may force a re-evaluation of the hypothesized role of past marine N2 fixation in glacial/interglacial climate changes, as well as consideration of the potential for increased ocean diazotrophy and altered nutrient and carbon cycling in the future high-CO2 ocean.

We propose an interdisciplinary project to examine the relationship between ocean N2 fixing cyanobacteria and changing pCO2. A combined field and laboratory approach will incorporate in situ measurements with experimental manipulations using natural and cultured populations of Trichodesmium and unicellular N2 fixers over range of pCO2 spanning glacial era to future concentrations (150-1500 ppm). We will also examine how effects of pCO2 on N2 and C fixation and elemental stoichiometry are moderated by the availability of other potentially growth-limiting variables such as Fe, P, temperature, and light. We plan to obtain a detailed picture of the full range of responses of important oceanic diazotrophs to changing pCO2, including growth rates, N2 and CO2 fixation, cellular elemental ratios, fixed N release, photosynthetic physiology, and expression of key genes involved in carbon and nitrogen acquisition at both the transcript and protein level.

This research has the potential to evolutionize our understanding of controls on N2 fixation in the ocean. Many of our current ideas about the interactions between oceanic N2 fixation, atmospheric CO2, nutrient biogeochemistry, ocean productivity, and global climate change may need revision to take into account previously unrecognized feedback mechanisms between atmospheric composition and diazotrophs. Our findings could thus have major implications for human society, and its increasing dependence on ocean resources in an uncertain future. This project will take the first vital steps towards understanding how a biogeochemically-critical process, the fixation of N2 in the ocean, may respond to our rapidly changing world during the century to come.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-0722337

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