Results of experiments examining CO2 and gross N2 fixation rates by Crocosphaera watsonii (WH0003) under differing levels of phosphate, light, and pCO2; conducted in the Hutchins Laboratory, USC

Website: https://www.bco-dmo.org/dataset/4042
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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 gross N2 fixation and CO2 fixation by the WH0003 isolate of *Crocosphaera watsonii* in response to different levels of phosphate, light, and pCO2. 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 3):
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). The total P concentration was altered in the P-light-CO2 manipulation experiment by adding P as H2NaPO4 in aqueous solution.

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 CO_2 concentrations in cultures and a consistent dilution period reduced variations in growth rates between dilutions. In the P-light-CO_2 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 pCO_2 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-CO_2 experiment seawater was bubbled and pre-equilibrated with treatment concentrations of pCO_2 before measuring pH and adding nutrients. This was essential to maintain high pH values in the 19 Pa pCO_2 treatments. The investigators excluded data from the high light, 19-Pa pCO_2 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 N_2-fixation rates and CO_2-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-CO_2 experiment. To acclimate cultures to low P conditions in the P-light-CO_2 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-pCO_2 treatment, which did have a negative growth rate.

Nitrogen fixation rates

Nitrogen-fixation rates were determined with the acetylene reduction method as described in Garcia et al. (2013). Briefly, duplicate 50 mL culture samples were collected from experimental replicates and 4 mL of acetylene was injected into 30 mL headspace at the beginning of the dark period of the light cycle. Samples were gently agitated to equilibrate gas concentrations between the headspace and culture samples after injecting acetylene and before measuring ethylene concentrations. The investigators used a Bunsen coefficient for ethylene of 0.082 (Breitbarth et al. 2004) and an ethylene production:N_2 fixation rate ratio of 3:1 and they calculated N_2-fixation rates over 14 h (this included the 12 h dark cycle and the first 2 h of the light cycle).

Total CO_2

Samples for measurements of total CO_2 (TCO_2) were preserved with 0.05% mercuric chloride (final concentration) in glass bottles without headspace and determined using a carbon coulomb meter (model: CM 140, UIC inc.). For these analyses, the investigators acidified 5 mL with a 10% phosphoric acid solution (1-2% final concentration), quantified the CO_2 trapped in an acid sparging column, and calculated TCO_2 with reference material provided by Andrew Dickson's laboratory (batch 95). PCO_2 was calculated with the CO_2 System Calculations program using K1 and K2 constants from Mehrbach et al. (1973), refit by Dickson and Millero (1987) and the NBS pH scale (Lewis and Wallace 1998; see Table 1 of Garcia et al. (2013) for TCO_2 measurements and PCO_2 calculations in the P-light-CO_2 experiment).

CO_2 fixation rates

CO_2-fixation rates were determined using a Multi-purpose Scintillation Counter (model: LS-6500, Beckman Coulter) similar to the method described by Garcia et al. (2011). Briefly, the investigators inoculated 40 mL samples from each treatment replicate with 0.925 KBq mL^{-1} $H^{14}CO_3^-$. The concentration of $H^{14}CO_3^-$ added to the sample was negligible in comparison with the TCO_2 concentration of the sample. Samples were incubated for 12 h under treatment-specific conditions of irradiance and temperature, and then filtered onto Whatman GF/F filters and rinsed 3 times with ~5 mL filtered seawater to remove extracellular $H^{14}CO_3^-$. The incubation was initiated at the beginning of the light period and terminated at the end of the 12 h light period. Total CO_2 concentrations were multiplied by the ratio of radioactivity of cellular incorporation of ^{14}C to the total radioactivity of $H^{14}CO_3^-$. For CO_2-fixation rate calculations in the P-light-CO_2 experiment, the investigators pooled ~25 mL from each of 3 treatment replicates into one sample for TCO_2 measurements. Non-photosynthetically driven ^{14}C incorporation was determined by incubating replicate culture samples (40 mL) for 12 h during the same time period in opaque bottles at 28 degrees C with the same concentration of $H^{14}CO_3^-$; these values were subtracted from measured total ^{14}C incorporation to estimate photosynthetic incorporation. The total radioactivity of $H^{14}CO_3^-$ was determined by stabilizing 50 uL of the 37 MBq $H^{14}CO_3^-$ with 100 mL of a basic solution of phenylethylamine (99%) before adding 4 mL of Ultima Gold® XR (PerkinElmer).

Other measurements

The investigators calculated the light compensation point (Ec, where net rates are zero) and the minimum concentration (Cmin) of total P for growth, CO_2- and N_2-fixation rates using the hyperbolic function [$y=(a•x)/(b+x)$] with the software program Sigma Plot 10. All 3 replicates in the 0.15 umol total P per L low-light, low-PCO2 treatment had slightly negative growth rates, so the investigators assumed net growth rates of zero in those replicates as was done in a prior study of phytoplankton growth kinetics (Hutchins et al. 2007). Next, the values of 'a' (the maximum rate) and 'b' (the half-saturation concentration, K½) were calculated after aligning the data set as a whole along the x-axis, with respect to the origin, to yield the highest r2 value. The investigators then realigned the data to their original values along with the best-fit hyperbolic functions. The Cmin and Ec are equivalent to the origin before the realignment. This method yields realistic Monod hyperbolic maximum rates, K½, and Cmin or Ec values. The investigators also calculated 95% confidence bands on the hyperbolic functions using Sigma Plot 10. For the light experiment the hyperbolic function of CO2- and N2-fixation rates were fitted to irradiance without including the rates measured at 100 umol quanta per square meter per second due to problems with an altered light level for this treatment just prior to sampling for CO2- and N2-fixation rates.

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

Garcia, N. S., F.-X. Fu, C. L. Breene, E. K. Yu, P. W. Bernhardt, M. R. Mulholland, and D. A. Hutchins. 2013. Combined effects of CO2 and light on large and small isolates of the unicellular N2-fixing cyanobacterium Crocosphaera watsonii from the western tropical Atlantic Ocean. Eur. J. Phycol. 48: 128-139. DOI: 10.1080/09670262.2013.773383

Hutchins, D. A., F.-X. Fu, Y. Zhang, M. E. Warner, Y. Feng, K. Portune, P. W. Bernhardt, and M. R. Mulholland. 2007. CO2 control of Trichodesmium N2-fixation, photosynthesis, growth rates, and elemental ratios: Implications for past, present, and future ocean biogeochemistry. Limnol. Oceanogr. 52: 1293-1304. DOI: 10.4319/lo.2007.52.4.1293

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_fixation.csv (Comma Separated Values (.csv), 3.39 KB) MD5:346b1505146142832fdefe3f068d4433 Primary data file for dataset ID 4042

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Parameters

Parameter	Description	Units
compound	The fixation rate measured; either "Gross_N2" or "CO2".	text
light	Light intensity (either 150 or 40 umol quanta per square meter per second). (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).	Pa
log_total_P	Log of total phosphate concentration.	micromoles per liter (umol L-1)
fixation	Fixation rates of CO2 or gross N2 (units: fmol C cell-1 hour-1 OR fmol N cell-1 hour-1)	femtomoles per cell per hour

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Instruments

Dataset-specific Instrument Name	CO2 Coulometer
Generic Instrument Name	CO2 Coulometer
Dataset-specific Description	Samples for measurements of total CO2 (TCO2) were determined using a CM140 (UIC) carbon coulomb meter.
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	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	Liquid Scintillation Counter
Generic Instrument Name	Liquid Scintillation Counter
Dataset-specific Description	CO2-fixation rates were determined using a Beckman Coulter LS-6500 Multi-purpose Scintillation Counter.
Generic Instrument Description	Liquid scintillation counting is an analytical technique which is defined by the incorporation of the radiolabeled analyte into uniform distribution with a liquid chemical medium capable of converting the kinetic energy of nuclear emissions into light energy. Although the liquid scintillation counter is a sophisticated laboratory counting system used the quantify the activity of particulate emitting (ß and a) radioactive samples, it can also detect the auger electrons emitted from 51Cr and 125I samples.

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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