Results from experiments examining cellular growth, CO2- and N2-fixation by Crocosphaera watsonii (WH0003) as a function of light; conducted in the Hutchins Laboratory, USC

Website: https://www.bco-dmo.org/dataset/4039
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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, CO2- and NO2-fixation rates of the WH0003 isolate of *Crocosphaera watsonii* in response to different light intensities. 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 1):
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).

Light experiment and cellular growth rates
Triplicate cultures were grown in 800 mL polystyrene flasks under 5 irradiances (18, 40, 100, 180, 300 umol quanta per m^2 per second) and diluted every 2-3 days to 10-20 x 103 cells per mL. 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. To calculate growth rates, the investigators used the equation $N_T=N_0e^{\mu T}$, where N_0 and N_T are the initial and final culture cell densities, respectively and T is the amount of time in days between culture cell number estimates. With this method, the dilution rate is determined by the growth rate of the algae as determined by the

experimental treatments, rather than by controlling the growth rate through imposing a dilution rate, as one does for continuous cultures.

Cell diameters of ~12 cells from treatment replicates were measured with an ocular micrometer. In the light experiment, cells in one replicate from each light were measured treatment twice, once in the middle of the light period and once at the end of the light period on the same day.

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.

Nitrogen fixation
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:N2 fixation rate ratio of 3:1 and N2-fixation rates were calculated over 14 h (this included the 12 h dark cycle and the first 2 h of the light cycle).

CO2 fixation
CO2-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 H14CO3-. The concentration of H14CO3- added to the sample was negligible in comparison with the TCO2 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 H14CO3-. The incubation was initiated at the beginning of the light period and terminated at the end of the 12 h light period. Total CO2 concentrations were multiplied by the ratio of radioactivity of cellular incorporation of 14C to the total radioactivity of H14CO3-. To calculate CO2-fixation rates in the light experiment, the investigators used a mean concentration of 2061 umol L-1 TCO2 that was measured in identical non-bubbled experiments with other isolates of C. watsonii (Garcia et al. 2013). Non-photosynthetically driven 14C 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 H14CO3-; these values were subtracted from measured total 14C incorporation to estimate photosynthetic incorporation. The total radioactivity of H14CO3- was determined by stabilizing 50 uL of the 37 MBq H14CO3- with 100 mL of a basic solution of phenylethylamine (99%) before adding 4 mL of Ultima Gold® XR (PerkinElmer).

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

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_light_exp.csv (Comma Separated Values (.csv), 635 bytes) MD5:a749761ace8d16f22cc16cb385611aab Primary data file for dataset ID 4039

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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)
cellular_growth	Cellular growth rate (per day).	per day
CO2_fixation_rate	CO2 fixation rate.	femtomoles Carbon per cell per hour (fmol C cell-1 h-1)
gross_N2_fixation	Gross N2 fixation rate.	femtomoles Nitrogen per cell per hour (fmol N cell-1 h-1)

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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 at the end of each dilution period.
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	A Beckman Coulter LS-6500 Multi-purpose Scintillation Counter was used to determine CO2-fixation rates.
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.

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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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