

Results of experiments examining cellular growth by *Crocospaera watsonii* (WH0003) under differing levels of phosphate, light, and pCO₂; conducted in the Hutchins Laboratory, USC

Website: <https://www.bco-dmo.org/dataset/4041>

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Project

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

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Dataset Description

Results of a laboratory experiment examining cellular growth of the WH0003 isolate of *Crocospaera 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 2):

Garcia, N.S., Fu, F.X., and Hutchins, D.A. (2013). Colimitation of the unicellular photosynthetic diazotroph *Crocospaera watsonii* by phosphorus, light, and carbon dioxide. *Limnology and Oceanography* 58(4): 1501-1512. DOI: [10.4319/lo.2013.58.4.1501](https://doi.org/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. To

calculate growth rates, the investigators used the equation $N_T = N_0 e^{\mu T}$, 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.

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 $\mu\text{mol quanta per square meter per second}$ 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 $\mu\text{mol total P per L}$ 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.

Total CO₂

Samples for measurements of total CO₂ (TCO₂) 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 CO₂ trapped in an acid sparging column, and calculated TCO₂ with reference material provided by Andrew Dickson's laboratory (batch 95). PCO₂ was calculated with the CO₂ 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 TCO₂ measurements and PCO₂ calculations in the P-light-CO₂ experiment).

Other measurements

The investigators calculated the light compensation point (E_c , where net rates are zero) and the minimum concentration (C_{min}) of total P for growth, CO₂- and N₂-fixation rates using the hyperbolic function $[y = (a \cdot x) / (b + x)]$ with the software program Sigma Plot 10. All 3 replicates in the 0.15 $\mu\text{mol total P per L}$ 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, $K_{1/2}$) 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 C_{min} and E_c are equivalent to the origin before the realignment. This method yields realistic Monod hyperbolic maximum rates, $K_{1/2}$, and C_{min} or E_c 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 $\mu\text{mol quanta per square meter per second}$ 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](https://doi.org/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 *Crocospaera watsonii* from the western tropical Atlantic Ocean. Eur. J. Phycol. 48: 128-139. DOI: [10.1080/09670262.2013.773383](https://doi.org/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/lb.2007.52.4.1293](https://doi.org/10.4319/lb.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](https://doi.org/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_cellular_growth.csv (Comma Separated Values (.csv), 1.31 KB) MD5:92cb4f83374876859a6a5aa7399ef92c
Primary data file for dataset ID 4041

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Parameters

Parameter	Description	Units
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)
pCO ₂	Partial pressure of carbon dioxide (pCO ₂) in the water body (19 or 81 Pa).	Pa
log_total_P	Log of total phosphate concentration.	micromoles per liter (umol L ⁻¹)
cellular_growth_rate	Cellular growth rate of <i>Crocospaera watsonii</i> (WH0003).	per day (d ⁻¹)

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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 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	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".

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.

Funding

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

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