

# Results of laboratory experiment examining growth and N<sub>2</sub>-fixation of *Crocospaera watsonii* under differing pCO<sub>2</sub> levels; conducted in the Hutchins Laboratory, USC

**Website:** <https://www.bco-dmo.org/dataset/3964>

**Version:** 12 June 2013

**Version Date:** 2013-06-12

## Project

» [CO<sub>2</sub> control of oceanic nitrogen fixation and carbon flow through diazotrophs](#) (Diaz N<sub>2</sub>-Fix in High CO<sub>2</sub>)

Contributors	Affiliation	Role
<a href="#">Hutchins, David A.</a>	University of Southern California (USC)	Principal Investigator, Contact
<a href="#">Fu, Feixue</a>	University of Southern California (USC)	Contact
<a href="#">Rauch, Shannon</a>	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

## Table of Contents

- [Dataset Description](#)
  - [Methods & Sampling](#)
  - [Data Processing Description](#)
- [Data Files](#)
- [Parameters](#)
- [Instruments](#)
- [Deployments](#)
- [Project Information](#)
- [Funding](#)

## Dataset Description

Results of laboratory experiments examining growth and N<sub>2</sub>-fixation rate of two isolates of *Crocospaera watsonii*, WH0401 and WH0402, in response to three levels of CO<sub>2</sub> (190, air, and 750) at a light intensity of 155  $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ . Isolates of *C. watsonii*, a unicellular marine N<sub>2</sub>-fixing cyanobacterium, were obtained from the western tropical Atlantic Ocean and cultured in the laboratory.

Detailed methods and results are described in the following publication (see Figure 2):

Garcia, N.S., Fu, F.X., Breene, C.L., Yu, E., Bernhardt, P.W., Mulholland, M.R., and Hutchins, D.A. (2013).

Combined effects of CO<sub>2</sub> and irradiance on the unicellular N<sub>2</sub>-fixing cyanobacterium *Crocospaera watsonii*: a comparison of two isolates from the Western Tropical Atlantic Ocean. *European Journal of Phycology* 48: 128-139. DOI: [10.1080/09670262.2013.773383](https://doi.org/10.1080/09670262.2013.773383)

Related Datasets:

[C watsonii light experiment](#)

[C watsonii CO<sub>2</sub>-light experiment](#)

## Methods & Sampling

### Culturing and experimental conditions

Stock cultures of the two Atlantic *C. watsonii* isolates used in this study were provided courtesy of Dr. Eric Webb. Both isolates were collected in March 2002, WH0401 from 6° 58.78' N, 49° 19.70' W and WH0402 from 11° 42.125' S, 32° 00.64' W. Triplicate cultures were grown using a semi-continuous culturing technique (Garcia et al., 2011) at 28 degrees C in an artificial seawater medium (Chen et al., 1996). Nutrients were added to autoclaved seawater at the concentrations listed in the AQUIL recipe (Morel et al., 1979), except for nitrate, which was omitted. The growth rates of cultures were measured over 2–3 day intervals and were used to determine the dilution rate. Culture cell density was kept low (cells ml<sup>-1</sup> = 50–500 × 10<sup>3</sup> for experiments with

WH0401 and  $5.0\text{--}30 \times 10^3$  for WH0402) to prevent light limitation of photosynthesis and deviation from the expected pH values for respective pCO<sub>2</sub> culture treatments. Light was supplied with cool-white fluorescent lamps on a 12:12 h light:dark cycle and measured with a LI-250A light meter (LiCor Biosciences, light sensor serial# SPQA 4020). Because of large differences in cell size between WH0401 and WH0402, WH0401 was cultured at higher cell densities to maintain relatively equivalent levels of total culture biomass (0.1–2.5 mM particulate C for cultures of WH0401; 0.1–1.3 mM particulate C for WH0402). For CO<sub>2</sub> experiments, media and cultures were bubbled with filtered air from the room (0.2 µm filtered, present-day pCO<sub>2</sub> concentration of ~385 ppm) or premixed air prepared by Gilmore Liquid Air Company with certified values of 190 ppm pCO<sub>2</sub> (last glacial maximum levels: Petit et al., 1999) and 750 or 761 ppm pCO<sub>2</sub> (within the range predicted for the year 2100: Alley et al., 2007) for the entire term of the experiment. Cells were considered fully acclimated to treatment conditions after cultures had remained at steady-state growth for seven generations or more (unless stated otherwise). Fast growing cultures (i.e. high light cultures) were acclimated for more than ten generations while slow growing cultures (i.e. low light and low pCO<sub>2</sub> cultures) were acclimated over two months but for fewer generations. Cultures were sampled over the period between 24 and 48 h after the preceding dilution to measure growth rates, gross and net 15N<sub>2</sub>-fixation rates, CO<sub>2</sub>-fixation rates, particulate elemental composition, and carbonate system measurements (for CO<sub>2</sub> experiments).

### CO<sub>2</sub> experiments

To investigate variability in the effects of CO<sub>2</sub> on growth and N<sub>2</sub>-fixation rates between strains of *C. watsonii*, the investigators conducted experiments with cultures of WH0401 and WH0402. They measured growth and gross and net N<sub>2</sub>-fixation rates (see N<sub>2</sub>-fixation rates) in response to three levels of CO<sub>2</sub> (190, air, and 750 ppm) at a light intensity of 155 µmol quanta m<sup>-2</sup> s<sup>-1</sup>. This light intensity was chosen so that growth rates in these cultures would not be limited by light.

### Growth rate and cell density estimates

Growth rate was determined as an increase in culture cell density over time with the equation  $N_T = N_0 e^{\mu T}$ , where  $N_0$  and  $N_T$  are the initial and final culture cell densities, respectively,  $T$  is the time in days between culture cell density estimates, and  $\mu$  is the specific growth rate. Culture cell density was determined using a haemocytometer and an Olympus BX51 microscope. Cell diameter was measured using an ocular micrometer calibrated with the same microscope. Growth rates were fitted to a Monod linear hyperbolic function of light (Monod, 1949) using Sigma Plot 10 software program. The hyperbola was fit to the data without including the origin to yield the highest  $r^2$  value.

### Carbonate system measurements

Culture pH was measured intermittently during the CO<sub>2</sub> experiments with a pH meter using the NBS seawater scale (Orion 5 star Thermo Scientific, Beverly, MA, USA). Samples for total CO<sub>2</sub> (TCO<sub>2</sub>) measurements were preserved in unfiltered water collected from cultures (5–70 ml; stored at 4°C) with a 5% HgCl<sub>2</sub> solution (0.5% final concentration) until later analysis with a carbon coulomb meter (CM 140, UIC, Joliet, IL, USA). TCO<sub>2</sub> was measured by acidifying a 5 ml sample with phosphoric acid (1–2% final concentration) and quantifying the CO<sub>2</sub> trapped in an acid sparging column as described in Garcia et al. (2011). TCO<sub>2</sub> analyses were not available in the preliminary CO<sub>2</sub> experiments. pCO<sub>2</sub> was calculated with the CO<sub>2</sub>sys program (Lewis & Wallace, 1998) using the NBS pH scale and K<sub>1</sub> and K<sub>2</sub> constants from Mehrbach et al. (1973), refit by Dickson & Millero (1987).

### N<sub>2</sub> fixation

The acetylene reduction assay described by Capone et al. (1993) was used to estimate the gross N<sub>2</sub>-fixation rate. Rate measurements were initiated at the beginning of the 12-h dark period, when *C. watsonii* is known to fix N<sub>2</sub> (Mohr et al., 2010a; Saito et al., 2011). For the CO<sub>2</sub> experiments, the acetylene assay was initiated during the seventh hour of the 12-h dark period and continued for 4 h. For this assay, two 50 ml (light and CO<sub>2</sub>-light experiments) or 60 ml (CO<sub>2</sub> experiments) culture samples were collected from each replicate and incubated in 80-ml polycarbonate bottles at 28 degrees C. Four millilitres of acetylene were injected into the headspace ~1 h after the beginning of the dark period and samples were withdrawn from the headspace every 2–3 h to measure acetylene reduction. Gross N<sub>2</sub>-fixation rates were calculated in the same way as described in Garcia et al. (2011), using a Bunsen coefficient for ethylene of 0.082 (Breitbarth et al., 2004) and an ethylene production : N<sub>2</sub>-fixation ratio of 3:1.

Net N<sub>2</sub>-fixation rates were measured using the 15N<sub>2</sub> isotope tracer method (Mulholland & Bernhardt, 2005; Mulholland et al., 2004). Samples were prepared the same way as described in Garcia et al. (2011). Briefly, 169 ml of each experimental replicate was inoculated with 169 µl of 99% doubly labelled 15N<sub>2</sub> gas and incubated at 28 degrees C in complete darkness for 12 h during the dark period. The incubation was then terminated by filtering the entire volume onto precombusted (450 degrees C, 4 h) GF/F filters for the analysis of particulate 15N, total particulate N, and total particulate C. Filters were dried at 80–90 degrees C, pelleted, and combusted in a quartz column with chromium oxide and silver wool at 1000 degrees C. For this analysis, ammonium sulphate and sucrose were used as standards. At the time these experiments were conducted, the

investigators were not aware of the criticisms of the  $^{15}\text{N}_2$  uptake method that have been discussed by Mohr et al. (2010b). Thus, for another independent estimate of net  $\text{N}_2$  fixation, the investigators calculated a particulate N (PN) accumulation rate in cultures over time ( $\Delta\text{PN} = \text{PN}_{\text{final}} - \text{PN}_{\text{initial}}$ ) by using estimates of particulate N. Particulate N was measured in subsamples of experimental replicates that were incubated with  $^{15}\text{N}_2$  at the end of the dark period and used as the end-period PN measurement ( $\text{PN}_{\text{final}}$ ). Because only one sample of PN was collected, the investigators back-calculated an estimate of  $\text{PN}_{\text{initial}}$  based on their measurements of cellular growth rate using the equation:  $\text{growth rate (d}^{-1}) = [\ln(\text{PN}_{\text{final}}) - \ln(\text{PN}_{\text{initial}})] / (t_2 - t_1)$ , where  $t_1$  is the initial time and  $t_2$  is the final time. Based on their measurements of growth rates, the investigators assumed that PN per cell was in a daily steady state. The gross  $\text{N}_2$ -fixation rate:PN-accumulation rate ratio (hereafter the gross:PN accumulation ratio) was then calculated and compared with the ratio of gross  $\text{N}_2$ -fixation rate:net  $^{15}\text{N}_2$ -fixation rate ratio (gross:net), which is a proxy for cellular N retention (Mulholland et al., 2004; Mulholland, 2007).

## References:

- ALLEY, R.B., BERNTSEN, T., BINDOFF, N.L., CHEN, Z., CHIDTHAISONG, A., FRIEDLINGSTEIN, P., GREGORY, J.M., HEGERL, G.C., HEIMANN, M., HEWITSON, B., HOSKINS, B.J., JOOS, F., JOUZEL, J., KATTSOV, V., LOHMANN, U., MANNING, M., MATSUNO, T., MOLINA, M., NICHOLLS, N., OVERPECK, J., QIN, D., RAGA, G., RAMASWAMY, V., REN, J., RUSTICUCCI, M., SOLOMON, S., SOMERVILLE, R., STOCKER, T.F., STOTT, P.A., STOUFFER, R.J., WHETTON, P., WOOD, R.A. & WRATT, D. (2007). Summary for policymakers. In *Climate change 2007: The physical science basis. Contribution of Working Group I to the fourth assessment report of the Intergovernmental Panel on Climate Change* (Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M. & Miller, H.L., editors), 1-18. Cambridge University Press, Cambridge and New York.
- BREITBARTH, E., MILLS, M.M., FRIEDRICHS, G. & LAROCHE, J. (2004). The Bunsen gas solubility coefficient of ethylene as a function of temperature and salinity and its importance for nitrogen fixation assays. *Limnology and Oceanography: Methods*, 2: 282-288. DOI: [10.4319/lom.2004.2.282](https://doi.org/10.4319/lom.2004.2.282)
- CHEN, Y.B., ZEHR, J.P. & MELLON, M. (1996). Growth and nitrogen fixation of the diazotrophic filamentous nonheterocystous cyanobacterium *Trichodesmium* sp. IMS101 in defined media: Evidence for a circadian rhythm. *Journal of Phycology*, 32: 916-923. DOI: [10.1111/j.0022-3646.1996.00916.x](https://doi.org/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 Research*, 34: 1733-1743.
- 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  $\text{CO}_2$  on  $\text{CO}_2$ - and  $\text{N}_2$  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)
- LEWIS, E. & WALLACE, D.W.R. (1998). Program developed for  $\text{CO}_2$  System calculations. ORNL/CDIAC-105. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee. Available at: <http://cdiac.ornl.gov/oceans/co2rprt.html>
- MEHRBACH, Y., CULBERSON, C., HAWLEY, J. & PYTKOVICS, R. (1973). Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. *Limnology and Oceanography*, 18: 897-907.
- MONOD, J. (1949). The growth of bacterial cultures. *Annual Review of Microbiology*, 3: 371-394.
- 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.
- MULHOLLAND, M.R. (2007). The fate of nitrogen fixed by diazotrophs in the ocean. *Biogeosciences* 4: 37-51. DOI: [10.5194/bg-4-37-2007](https://doi.org/10.5194/bg-4-37-2007)
- MULHOLLAND, M.R. & BERNHARDT, P.W. (2005). The effect of growth rate, phosphorus concentration and temperature on  $\text{N}_2$ -fixation, carbon fixation, and nitrogen release in continuous cultures of *Trichodesmium* IMS101. *Limnology and Oceanography*, 50: 839-849. DOI: [10.4319/lo.2005.50.3.0839](https://doi.org/10.4319/lo.2005.50.3.0839)
- MULHOLLAND, M.R., BRONK, D.A. & CAPONE, D.G. (2004).  $\text{N}_2$  fixation and regeneration of  $\text{NH}_4^+$  and dissolved organic N by *Trichodesmium* IMS101. *Aquatic Microbial Ecology*, 37: 85-94. DOI: [10.3354/ame037085](https://doi.org/10.3354/ame037085)

## 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.

[ [table of contents](#) | [back to top](#) ]

---

## Data Files

File
<b>C_watsonii_CO2_expt.csv</b> (Comma Separated Values (.csv), 335 bytes) MD5:12300e3de68a121ea6d533de3359795c
Primary data file for dataset ID 3964

[ [table of contents](#) | [back to top](#) ]

---

## Parameters

Parameter	Description	Units
isolate	Name of Crocosphaera watsonii isolate.	text
pCO2	pCO2 level of the experiment (190, air, or 750 ppm).	ppm
gross_to_net_N2_fixation	Ratio of gross to net N2 fixation.	ratio
gross_to_net_N2_fixation_sd	Standard deviation of the ratio of gross to net N2 fixation.	ratio
cellular_growth_rate	Cellular growth rate per day.	per day
cellular_growth_rate_sd	Standard deviation of cellular growth rate per day.	per day

[ [table of contents](#) | [back to top](#) ]

---

## Instruments

<b>Dataset-specific Instrument Name</b>	Benchtop pH Meter
<b>Generic Instrument Name</b>	Benchtop pH Meter
<b>Dataset-specific Description</b>	Culture pH was measured using an Orion 5 Star (Thermo Scientific).
<b>Generic Instrument Description</b>	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.

<b>Dataset-specific Instrument Name</b>	CO2 Coulometer
<b>Generic Instrument Name</b>	CO2 Coulometer
<b>Dataset-specific Description</b>	Total CO2 was analyzed with a carbon coulomb meter (CM140, UIC, Joliet, IL). CM140 Instrument Brochure.
<b>Generic Instrument Description</b>	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.

<b>Dataset-specific Instrument Name</b>	Hemocytometer
<b>Generic Instrument Name</b>	Hemocytometer
<b>Dataset-specific Description</b>	Culture cell density was determined using a haemocytometer and an Olympus BX51 microscope.
<b>Generic Instrument Description</b>	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: <a href="http://hlsweb.dmu.ac.uk/ahs/elearning/RITA/Haem1/Haem1.html">http://hlsweb.dmu.ac.uk/ahs/elearning/RITA/Haem1/Haem1.html</a> .

<b>Dataset-specific Instrument Name</b>	Light Meter
<b>Generic Instrument Name</b>	Light Meter
<b>Dataset-specific Description</b>	During culturing, light was measured with a LI-250A light meter (LI-COR Biosciences, light sensor serial # SPQA 4020).
<b>Generic Instrument Description</b>	Light meters are instruments that measure light intensity. Common units of measure for light intensity are $\mu\text{mol}/\text{m}^2/\text{s}$ or $\text{uE}/\text{m}^2/\text{s}$ (micromoles per meter squared per second or microEinsteins per meter squared per second). (example: LI-COR 250A)

[ [table of contents](#) | [back to top](#) ]

## Deployments

### lab\_Hutchins\_07-12\_diazotrophs

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

## Project Information

### CO<sub>2</sub> control of oceanic nitrogen fixation and carbon flow through diazotrophs (Diaz N<sub>2</sub>-Fix in High CO<sub>2</sub>)

**Coverage:** Laboratory

*From NSF award abstract:*

The importance of marine N<sub>2</sub> fixation to present ocean productivity and global nutrient and carbon biogeochemistry is now universally recognized. Marine N<sub>2</sub> 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 N<sub>2</sub> fixers in the ocean such as *Trichodesmium* and unicellular N<sub>2</sub> fixing cyanobacteria to past, present and future global atmospheric CO<sub>2</sub> regimes. Our preliminary data demonstrate that N<sub>2</sub> and CO<sub>2</sub> fixation rates, growth rates, and elemental ratios of Atlantic and Pacific *Trichodesmium* isolates are controlled by the ambient CO<sub>2</sub> concentration at which they are grown. At projected year 2100 pCO<sub>2</sub> (750 ppm), N<sub>2</sub> 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 N<sub>2</sub> and C fixation due to elevated CO<sub>2</sub> were of similar relative magnitude regardless of the growth temperature or P availability. Thus, the influence of CO<sub>2</sub> appears to be independent of other common growth-limiting factors. Equally important, *Trichodesmium* growth and N<sub>2</sub> fixation were completely halted at low pCO<sub>2</sub> levels (150 ppm), suggesting that diazotrophy by this genus may have been marginal at best at last glacial maximum pCO<sub>2</sub> levels of ~190 ppm. Genetic evidence indicates that *Trichodesmium* diazotrophy is subject to CO<sub>2</sub> 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 N<sub>2</sub> 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-CO<sub>2</sub> ocean.

We propose an interdisciplinary project to examine the relationship between ocean N<sub>2</sub> fixing cyanobacteria and changing pCO<sub>2</sub>. A combined field and laboratory approach will incorporate in situ measurements with experimental manipulations using natural and cultured populations of *Trichodesmium* and unicellular N<sub>2</sub> fixers over range of pCO<sub>2</sub> spanning glacial era to future concentrations (150-1500 ppm). We will also examine how effects of pCO<sub>2</sub> on N<sub>2</sub> 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 pCO<sub>2</sub>, including growth rates, N<sub>2</sub> and CO<sub>2</sub> 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 N<sub>2</sub> fixation in the ocean. Many of our current ideas about the interactions between oceanic N<sub>2</sub> fixation, atmospheric CO<sub>2</sub>, 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 N<sub>2</sub> in the ocean, may respond to our rapidly changing world during the century to come.

## Funding

Funding Source	Award
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-0722337</a>

[ [table of contents](#) | [back to top](#) ]