

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

**Website:** <https://www.bco-dmo.org/dataset/3962>

**Data Type:** experimental

**Version:** 1

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## 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>)

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## Abstract

Results of laboratory experiment examining growth, CO<sub>2</sub>- and N<sub>2</sub>-fixation of *Crocospaera watsonii* isolates in differing light intensities; conducted in the Hutchins Laboratory, USC.

## 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, CO<sub>2</sub>-fixation and gross and net N<sub>2</sub>-fixation rate capacities of two isolates of *Crocospaera watsonii*, WH0401 and WH0402, in response to a range of light intensities. 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 1):

García, 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* CO<sub>2</sub> experiment

C *watsonii* CO<sub>2</sub>-light experiment

## Methods & Sampling

### Culturing and experimental conditions

Stock cultures of the two Atlantic *C. watsonii* isolates 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', 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 5.0-30 × 10<sup>3</sup> 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). 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 <sup>15</sup>N<sub>2</sub>-fixation rates, CO<sub>2</sub>-fixation rates, and particulate elemental composition.

### Light experiments

In order to quantify differences in growth and in the CO<sub>2</sub>- and N<sub>2</sub>-fixation rate capacities of these two isolates of *C. watsonii*, the investigators measured growth, CO<sub>2</sub>-fixation and gross and net N<sub>2</sub>-fixation rates, and particulate carbon and nitrogen composition in response to a range of light intensities.

### 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.

### 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). 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 <sup>15</sup>N<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 <sup>15</sup>N<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 degree C, 4 h) GF/F filters for the analysis of particulate <sup>15</sup>N, 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 the experiments were conducted, the investigators were not aware of the criticisms of the <sup>15</sup>N<sub>2</sub> uptake method that have been discussed by Mohr et al. (2010b). Thus, for another independent estimate of net N<sub>2</sub> fixation, the investigators calculated a particulate N (PN) accumulation rate in cultures over time ( $\Delta PN = PN_{final} - PN_{initial}$ ). Particulate N was measured in subsamples of experimental replicates that were incubated with <sup>15</sup>N<sub>2</sub> at the end of the dark period and used as the end-period PN measurement ( $PN_{final}$ ). Because only one sample of PN was collected, the investigators back-calculated an estimate of  $PN_{initial}$  based on their measurements of cellular growth rate using the equation:  $\text{growth rate} (d^{-1}) = [\ln(PN_{final}) - \ln(PN_{initial})] / (t_2 - t_1)$ , 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 N<sub>2</sub>-fixation rate:PN-accumulation rate ratio (hereafter the gross:PN accumulation ratio) was then calculated and compared to the ratio of gross N<sub>2</sub>-fixation rate:net <sup>15</sup>N<sub>2</sub>-fixation rate ratio (gross:net), which is a proxy for cellular N retention (Mulholland et al., 2004; Mulholland, 2007).

### CO<sub>2</sub> fixation

The rate of CO<sub>2</sub> fixation was determined as described in Garcia et al. (2011) using the H<sup>14</sup>CO<sub>3</sub><sup>-</sup> incorporation method. CO<sub>2</sub>-fixation rates were determined by first calculating the ratio of the radioactivity of <sup>14</sup>C incorporated into cells during 24 hours to the total radioactivity of H<sup>14</sup>CO<sub>3</sub><sup>-</sup>. This ratio was then multiplied by

the total CO<sub>2</sub> concentration (TCO<sub>2</sub>). TCO<sub>2</sub> concentrations were measured in the CO<sub>2</sub>-light experiments and were applied to all experiments to calculate CO<sub>2</sub>-fixation rates for corresponding CO<sub>2</sub> treatments. For the light experiments, the investigators used a TCO<sub>2</sub> value that was measured in the present-day pCO<sub>2</sub> treatments of the CO<sub>2</sub>-light experiments (2053 μM TCO<sub>2</sub>).

## References:

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- MULHOLLAND, M.R., BRONK, D.A. & CAPONE, D.G. (2004). N<sub>2</sub> fixation and regeneration of NH<sub>4</sub><sup>+</sup> 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](#) ]

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## Data Files

File
<b>C_watsonii_light_expt.csv</b> (Comma Separated Values (.csv), 1.55 KB) MD5:c42d89433ea91da45575c638a7f9b1e7
Primary data file for dataset ID 3962

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

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## Parameters

Parameter	Description	Units
isolate	Name of Crocosphaera watsonii isolate.	text
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 <sup>-2</sup> s <sup>-1</sup> )
growth_rate	Growth rate.	per day
growth_rate_sd	Standard deviation of growth rate.	per day
cell_diameter	Cell diameter in micrometers.	micrometers (um)
cell_diameter_sd	Standard deviation of cell diameter.	micrometers (um)
C_specific_CO2_fix	C-specific CO2 fixation.	per hour
C_specific_CO2_fix_sd	Standard deviation of C-specific CO2 fixation.	per hour
N_specific_gross_N2_fix	N-specific gross N2 fixation.	per hour
N_specific_gross_N2_fix_sd	Standard deviation of N-specific gross N2 fixation.	per hour
N_specific_net_15N2_fix	N-specific net 15N2 fixation.	per hour
N_specific_net_15N2_fix_sd	Standard deviation of N-specific net N2 fixation.	per hour
gross_to_net_N2fix	Ratio of gross N2 fixation to net 15N2 fixation.	ratio
gross_to_net_N2fix_sd	Standard deviation of the ratio of gross N2 fixation to net 15N2 fixation.	ratio

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

## Instruments

<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 $\mu\text{E}/\text{m}^2/\text{s}$ (micromoles per meter squared per second or microEinsteins per meter squared per second). (example: LI-COR 250A)

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

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## 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".

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

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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 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 revolutionize 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.

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

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## 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](#) ]