Results of laboratory experiment examining growth, CO2- and N2-fixation of Crocosphaera watsonii in differing pCO2 and light levels; conducted in the Hutchins Laboratory, USC

Website: https://www.bco-dmo.org/dataset/3965
Version: 13 June 2013
Version Date: 2013-06-13

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

Contributors	Affiliation	Role
Hutchins, David A.	University of Southern California (USC)	Principal Investigator, Contact
Fu, Feixue	University of Southern California (USC)	Contact
Rauch, Shannon	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 CO2-fixation rate of two isolates of *Crocosphaera watsonii*, WH0401 and WH0402, in response to different pCO2 levels and light intensities. Isolates of *C. watsonii*, a unicellular marine N2-fixing cyanobacterium, were obtained from the western tropical Atlantic Ocean and cultured in the laboratory. WH0402 was grown at two concentrations of CO2 (air and 750 ppm) at five light intensities (18-300 umol quanta m-2 s-1). WH0401 was grown at three concentrations of CO2 (190, air, and 750 ppm) under the same range of light intensities (however WH0401 could not be acclimated to 18 or 50 umol quanta m-2 s-1).

Detailed methods and results are described in the following publication (see Figures 3, 4, and 5):
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 CO2 and irradiance on the unicellular N2-fixing cyanobacterium Crocosphaera 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

Related datasets:
C watsonii light experiment
C watsonii CO2 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 6º 58.78' N, 49º 19.70' W and WH0402 from 11º 42.12S', 32º 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–1 = 50–500 × 103 for experiments with WH0401 and 5.0–30 × 103 for WH0402) to prevent light limitation of photosynthesis and deviation from the expected pH values for respective pCO2 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 CO2 experiments, media and cultures were bubbled with filtered air from the room (0.2 µm filtered, present-day pCO2 concentration of ~385 ppm) or premixed air prepared by Gilmore Liquid Air Company with certified values of 190 ppm pCO2 (last glacial maximum levels: Petit et al., 1999) and 750 or 761 ppm pCO2 (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 pCO2 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 15N2-fixation rates, CO2-fixation rates, particulate elemental composition, and carbonate system measurements (for CO2 experiments).

CO2–light experiments

To determine if light influences the effect of elevated pCO2 on growth, CO2-fixation and N2-fixation rates of *C. watsonii*, the investigators first grew WH0402 with two concentrations of CO2 (air and 750 ppm) at five light intensities (18-300 µmol quanta m–2 s–1). In this experiment, growth and N2-fixation rates were measured at the two CO2 concentrations. When examining responses of WH0401 with this experimental design, a low CO2 treatment (190 ppm) was added under the same range of light intensities. Despite several attempts, the investigators were not able to acclimate WH0401 to any of these CO2 concentrations at 18 or 50 µmol quanta m–2 s–1 for unknown reasons.

Growth rate and cell density estimates

Growth rate was determined as an increase in culture cell density over time with the equation $N_T=N_0e^{\mu T}$, 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 µ 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 CO2 experiments with a pH meter using the NBS seawater scale (Orion 5 star Thermo Scientific, Beverly, MA, USA). Samples for total CO2 (TCO2) measurements were preserved in unfiltered water collected from cultures (5–70 ml; stored at 4°C) with a 5% HgCl2 solution (0.5% final concentration) until later analysis with a carbon coulomb meter (CM 140, UIC, Joliet, IL, USA). TCO2 was measured by acidifying a 5 ml sample with phosphoric acid (1–2% final concentration) and quantifying the CO2 trapped in an acid sparging column as described in Garcia et al. (2011). TCO2 analyses were not available in the preliminary CO2 experiments. pCO2 was calculated with the CO2sys program (Lewis & Wallace, 1998) using the NBS pH scale and K1 and K2 constants from Mehrbach et al. (1973), refit by Dickson & Millero (1987).

N2 fixation

The acetylene reduction assay described by Capone et al. (1993) was used to estimate the gross N2-fixation rate. Rate measurements were initiated at the beginning of the 12-h dark period, when C. watsonii is known to fix N2 (Mohr et al., 2010a; Saito et al., 2011). For the CO2 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 CO2–light experiments) or 60 ml (CO2 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 N2-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 : N2-fixation ratio of 3:1.

Net N2-fixation rates were measured using the 15N2 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 15N2 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 15N2 uptake method that have been discussed by Mohr et al. (2010b). Thus, for another independent estimate of net N2 fixation, the investigators calculated a particulate N (PN) accumulation rate in cultures over time (deltaPN = PNfinal - PNinitial) by using estimates of particulate N. Particulate N was measured in subsamples of experimental replicates that were incubated with 15N2 at the end of the dark period and used as the end-period PN measurement (PNfinal). Because only one sample of PN was collected, the investigators back-calculated an estimate of PNinitial based on their measurements of cellular growth rate using the equation: growth rate (d–1) = [ln(PNfinal)–ln(PNinitial)]/(t2–t1), where t1 is the initial time and t2 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 N2-fixation rate:PN-accumulation rate ratio (hereafter the gross:PN accumulation ratio) was then calculated and compared with the ratio of gross N2-fixation rate:net 15N2-fixation rate ratio (gross:net), which is a proxy for cellular N retention (Mulholland et al., 2004; Mulholland, 2007).

CO2 fixation
The rate of CO2 fixation was determined as described in Garcia et al. (2011) using the H14CO3- incorporation method. CO2-fixation rates were determined by first calculating the ratio of the radioactivity of 14C incorporated into cells during 24 hours to the total radioactivity of H14CO3–. This ratio was then multiplied by the total CO2 concentration (TCO2). TCO2 concentrations were measured in the CO2-light experiments and were applied to all experiments to calculate CO2-fixation rates for corresponding CO2 treatments. For the light experiments, the investigators used a TCO2 value that was measured in the present-day pCO2 treatments of the CO2-light experiments (2053 µM TCO2).

Particulate C and N
Culture samples from each experimental replicate (100 ml) were filtered onto precombusted (450 degree C, 4 h) GF/F filters for the analysis of cellular N and C. Filters were then dried at 80–90 degrees C and compressed into pellets, and the amounts of C and N were determined using an elemental analyser (Costech Instruments, model 4010).

N-Specific and C-Specific Fixation Rates
C-specific CO2-fixation and N-specific N2-fixation rates were determined by normalizing CO2-fixation rates to particulate organic carbon measurements and by normalizng N2-fixation rates to particulate organic nitrogen measurements.

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

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

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 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

LEWIS, E. & WALLACE, D.W.R. (1998). Program developed for CO2 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

MULHOLLAND, M.R. & BERNHARDT, P.W. (2005). The effect of growth rate, phosphorus concentration and temperature on N2-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

MULHOLLAND, M.R., BRONK, D.A. & CAPONE, D.G. (2004). N2 fixation and regeneration of NH4+ and dissolved organic N by Trichodesmium IMS101. Aquatic Microbial Ecology, 37: 85–94. DOI: 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
C_watsonii_CO2_light_expt.csv (Comma Separated Values (.csv), 3.08 KB) MD5:f12d6c48e32da586e657729d9fe2ba94 Primary data file for dataset ID 3965

[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).	parts per million (ppm)
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)
specific_growth_rate	Specific growth rate.	per day
specific_growth_rate_sd	Standard deviation of specific growth rate.	per day
cellular_CO2_fix	Cellular CO2 fixation rate.	femtomole (fmol) C per cell per hour
cellular_CO2_fix_sd	Standard deviation of cellular CO2 fixation rate.	fmol C per cell per hour
gross_N2_fix	Gross N2 fixation, determined using the acetylene reduction assay described by Capone et al. (1993).	fmol N per cell per hour
gross_N2_fix_sd	Standard deviation of gross_N2_fix.	fmol N per cell per hour
net_15N2_fix	Net N2 fixation measured using the 15N2 isotope tracer method (Mulholland & Bernhardt, 2005; Mulholland et al., 2004).	fmol N per cell per hour
net_15N2_fix_sd	Standard deviation of net_15N2_fix.	fmol N per cell per hour
PN_accumulation	Particulate nitrogen (PN) accumulation.	fmol N per cell per hour
PN_accumulation_sd	Standard deviation of particulate nitrogen accumulation.	fmol N per cell 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 15N2 fixation	per hour
gross_to_net_15N2fix	Ratio of gross to net 15N2 fixation.	ratio
gross_to_net_15N2fix_sd	Standard deviation of ratio of gross to net 15N2 fixation.	ratio
gross_to_net_PN_accum	Ratio of gross to net particulate nitrogen accumulation.	ratio
gross_to_net_PN_accum_sd	Standard deviation of ratio of gross to net particulate nitrogen accumulation.	ratio

[table of contents | back to top]

Instruments

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

Dataset-specific Instrument Name	CO2 Coulometer
Generic Instrument Name	CO2 Coulometer
Dataset-specific Description	Total CO2 was analyzed with a carbon coulomb meter (CM140, UIC, Joliet, IL). CM140 Instrument Brochure.
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	Culture cell density was determined using a haemocytometer and an Olympus BX51 microscope.
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	Light Meter
Generic Instrument Name	Light Meter
Dataset-specific Description	During culturing, light was measured with a LI-250A light meter (LI-COR Biosciences, light sensor serial # SPQA 4020).
Generic Instrument Description	Light meters are instruments that measure light intensity. Common units of measure for light intensity are umol/m2/s or uE/m2/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

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".

[table of contents | back to top]

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.

[table of contents | back to top]

Funding

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

[table of contents | back to top]