Results of laboratory experiments examining Cyanobacteria (Trichodesmium and Crocosphaera watsonii) N2-fixation responses to pCO2; conducted in the Hutchins Laboratory, USC

Website: https://www.bco-dmo.org/dataset/3966
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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

Cyanobacteria N2 fixation responses to pCO2 using four *Trichodesmium* isolates representing three species and three *Crocosphaera watsonii* strains, isolated from locations across the North and South Pacific and Atlantic Oceans (Webb et al. 2009, Hynes et al. 2012) and spanning the taxonomic diversity within each genus.

Detailed methods and results will be available in the following publication (see Figures 1 and 2):
Hutchins, D.A., Fu, F.-X., Webb, E.A. and Tagliabue, A. (In press). Taxon-specific response of marine nitrogen fixers to elevated carbon dioxide concentrations. Nature Geoscience.

Methods & Sampling

The seven isolates were grown across a pCO2 range in triplicate steady-state semi-continuous cultures at 28 degrees C on a 12 h dark:12 h light cycle using cool white fluorescent bulbs at 120 µmol photons m-2 s-1, in 0.2 µm-filtered, microwave-sterilized artificial seawater enriched with 20 µM phosphate and Aquil trace metals and vitamins, but with no fixed nitrogen (Hutchins et al. 2007, Fu et al. 2008). Cultures were diluted every 2-3 days based on their growth rates, and were sampled when acclimation to experimental conditions was verified by statistically invariant growth rates over 10-15 generations. For *Trichodesmium* KO4-20, H9-4, and 2174 and *Crocosphaera* WH0401, WH0003 and WH8501 experimental CO2 concentrations were 100ppm, 190ppm, 280ppm, 750ppm, 1500ppm, and 2000ppm. The CO2 response curve for *Trichodesmium* GBR was obtained from data from a previous experiment (Hutchins et al. 2007) at concentrations of 150ppm, 370ppm, 750ppm, 1250ppm and 1500 ppm. Gentle bubbling with certified commercial air/CO2 mixtures (Praxair) was used to obtain these seawater concentrations, which were verified by dissolved inorganic carbon (DIC) and pH measurements (Hutchins et al. 2007, Fu et al. 2008). Triplicate preserved 25 mL DIC samples (200 µL 5% HgCl2) were stored in borosilicate flasks at 4 degrees C for <7 days until analysis. Total DIC was measured coulomb-metrically (model CM 140, UIC, Joliet, IL, USA) and pH was monitored daily using a microprocessor pH meter (NBS system) with three point buffer calibrations. Certified pCO2 values were verified using measured

DIC and pH values with CO2 SYS software (http://cdiac.ornl.gov/ftp/co2sys/). Since measured values never deviated >1-2% from commercial certified values, pCO2 is reported as the latter value.

Rates of N2 fixation were measured at the same time of day for each culture (during the dark period for *Crocosphaera* and light period for *Trichodesmium*) with the acetylene reduction method using a 3:1 ratio to convert ethylene production to N2 fixation, and were normalized to culture chlorophyll a levels. Microscope counts were used to calculate cell-specific exponential growth rates (μ)($N_T=N_0e^{uT}$, where N is the initial cell density, N_T is the cell density one day later, and T is one day) (Hutchins et al. 2007, Fu et al. 2008).

CO2 response curves for N2 fixation rates in each of the triplicate cultures for each isolate in each pCO2 treatment were fitted to Michaelis-Menten (1913) rectangular hyperbolic saturation equation curves (N2 fixation rate = Vmax * pCO2 / (K1/2 + pCO2)) using SigmaPlot software (SPSS), including determination of kinetic constants and curve correlation coefficients. Means and standard deviations of K1/2 (ppmv CO2) and Vmax (μmol N mg Chl a-1 h-1) values from the triplicate response curves are reported; significance of differences in K1/2 and Vmax values between isolates were tested using one-way ANOVA, followed by Fisher's Least Significant Difference to compare the mean of one group with the mean of another with SPSS statistics software (Hutchins et al. 2007, Fu et al. 2008). Multi-variate Principle Coordinate Analysis (PCoA) and Hierarchical Clustering were used to analyze the variance between all treatments and replicates in order to group them using their K1/2 and Vmax values as metrics of their responses to the CO2 treatments (Ramette 2007).

References

Fu, F.-X., Mulholland, M.R., Garcia, N., Beck, A., Bernhardt, P.W., Warner, M.E., Sañudo-Wilhelmy, S.A. and Hutchins, D.A. 2008. Interactions between changing pCO2, N2 fixation, and Fe limitation in the marine unicellular cyanobacterium Crocosphaera. Limnology and Oceanography 53 (6): 2472- 2484. DOI: 10.4319/lo.2008.53.6.2472

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Webb, E. A., Ehrenreich, I. M., Brown, S. L., Valois, F. W., and Waterbury, J. B. 2009. Phenotypic and genotypic characterization of multiple strains of the diazotrophic cyanobacterium, *Crocosphaera watsonii*, isolated from the open ocean. Environmental Microbiology 11:338-348. DOI: 10.1111/j.1462-2920.2008.01771.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
cyano_fixation.csv (Comma Separated Values (.csv), 2.94 KB) MD5:6f0b2b4d842a4c28a2aa7a669ecb7f71 Primary data file for dataset ID 3966

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Parameters

Parameter	Description	Units
isolate	Name of the Trichodesmium or Crocosphaera watsonii isolate.	text
s_to_v_ratio	Surface area (um^2) to volume (um^3) ratio.	ratio (square:cubic micrometers)
half_sat	Half-saturation value, K1/2	ppm pCO2
half_sat_sd	Standard deviation of K1/2.	ppm pCO2
vmax	Vmax	umol N mg Chla-1 h-1
vmax_sd	Standard deviation of Vmax.	umol N mg Chla-1 h-1
pCO2	pCO2 level of the experiment.	parts per million (ppm)
N2_fixation	Nitrogen fixation rate normalized to culture chlorophyll-a levels (pmol N ng Chla-1 h-1).	picomoles Nitrogen per nanogram chl-a per hour (pmol N ng Chla-1 h-1)
N2_fixation_sd	Standard deviation of N2 fixation rate normalized to culture chlorophyll-a levels.	picomoles Nitrogen per nanogram chl-a per hour (pmol N ng Chla-1 h-1)

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Instruments

Dataset-specific Instrument Name	Benchtop pH Meter
Generic Instrument Name	Benchtop pH Meter
Dataset-specific Description	pH was monitored daily using a microprocessor pH meter (NBS system).
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 DIC was measureed coulomb-metrically (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.

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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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