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

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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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Abstract
Results of laboratory experiment examining growth, CO2- and N2-fixation of Crocosphaera watsonii isolates in differing light intensities; conducted in the Hutchins Laboratory, USC.

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Dataset Description

Results of laboratory experiments examining growth, CO2-fixation and gross and net N2-fixation rate capacities of two isolates of *Crocosphaera watsonii*, WH0401 and WH0402, in response to a range of 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.

Detailed methods and results are described in the following publication (see Figure 1):
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 CO2 experiment
C watsonii CO2-light experiment

Acquisition Description

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 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). 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, and particulate elemental composition.

Light experiments

In order to quantify differences in growth and in the CO2- and N2-fixation rate capacities of these two isolates of *C. watsonii*, the investigators measured growth, CO2-fixation and gross and net N2-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 $N_T=N_0e^{\mu T}$, 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.

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). 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 degree 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 the 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). 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 to 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).

References:

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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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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-2 s-1)
growth_rate	Growth rate.	per day
growth_rate_sd	Standard deviation of growth rate.	per day
cell_diameter	Cell diamter 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

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Instruments

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)

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.

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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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