Experimental results from a study of CO2 and N2 fixation and trichome length of Trichodesmium erythraeum under varying pCO2 and light conditions (PhytoTM_in_HighCO2 project)

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Project
» Changing Phytoplankton Trace Metal Requirements in a High CO2 Ocean (PhytoTM_in_HighCO2)

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Table of Contents

Dataset Description

Experimental data on the effects of pCO2 and light on CO2 and N2 fixation in two *Trichodesmium erythraeum* strains (IMS101 and GBRTRLI101) and on trichome length in one strain (IMS101).

Data and methods are described in:
Garcia, N. S., Fu, F.-X., Breene, C. L., Bernhardt, P. W., Mulholland, M. R., Sohm, J. A. and Hutchins, D. A. (2011), INTERACTIVE EFFECTS OF IRRADIANCE AND CO2 ON CO2 FIXATION AND N2 FIXATION IN THE DIAZOTROPH TRICHODESMIUM ERYTHRAEUM (CYANOBACTERIA). Journal of Phycology, 47: 1292–1303. doi: 10.1111/j.1529-8817.2011.01078.x

Methods & Sampling

The methods below are described in Garcia et al. (2011).

Culture conditions
Stock and experimental cultures of *Trichodesmium erythraeum* GBRTRL101 (GBR; from the Great Barrier Reef, Pacific Ocean) and IMS101 (IMS; from coastal North Carolina, Atlantic Ocean) were cultured at 24 degrees C (unless otherwise stated) in an artificial seawater medium without fixed N using a modified version of the YBCII recipe of Chen et al. (1996). Phosphate and trace metal solutions were filtered (0.2 um) and added in concentrations equivalent to the AQUIL recipe (Morel et al. 1979) to microwave- (experiment with GBR) or autoclave-sterilized (experiments with IMS) seawater. Irradiance was supplied with cool white fluorescent bulbs

on a 12:12 light:dark cycle. For all experiments, cultures were grown in triplicate using a semi-continuous batch culturing method to achieve steady state exponential growth for approximately 7-10 generations prior to sampling, in order to fully acclimatize cells to treatment pCO2 and irradiance conditions. We monitored cell density every 2-3 days using microscopic cell counts. When the biomass reached approximately 100-200 trichomes per mL (~100-200 nmol C per mL), we diluted cultures with fresh medium to 50-100 trichomes per mL (~50-100 nmol C per mL). In this semi-continuous culturing method, the growth rate determines the dilution rate; this culturing technique does not attempt to control the growth rate with the dilution rate, as continuous culturing methods do.

Experimental Design: **CO2/light experiments**
Two separate experiments were conducted with two *T. erythraeum* strains: GBR and IMS. Cultures of Trichodesmium sp. (GBR) were grown in 12 1-L polycarbonate bottles at 35 and 220 umol quanta per square meter per second at two concentrations of CO2 (see below). In the experiment with IMS, cultures were grown in 18 1-L polycarbonate bottles at 38, 100 and 220 umol quanta per square meter per second at two concentrations of CO2. Both experiments were conducted on a 12:12 light:dark cycle at 24 degrees C.

Within each irradiance treatment for both experiments, cultures were bubbled with 0.2 um filtered lab air (for the experiment with IMS) or pre-mixed air (prepared by Gilmore Liquid Air Company, South El Monte, CA, USA) containing present-day (380 ppm certified value for the experiment with GBR) and elevated, 100-year predicted (750 ppm certified value for both CO2 experiments) atmospheric CO2 concentrations. The rate of bubbling was visually monitored daily to ensure that cultures were bubbled with sufficient positive gas flow to keep the pH of the cultures at an appropriate level for respective CO2 treatments. Based on rates of gas utilization from the supply cylinders, estimated gas flow rates were between 30-60 mL per min.

Due to incubator availability constraints in the laboratory, the light manipulation and the CO2/light manipulation experiments were conducted at slightly different temperatures. While we do not have growth and N2 fixation rates in response to temperature for GBR, rates of Trichodesmium isolate IMS are not significantly different from each other at 24 degrees C and 27 degrees C (Breitbarth et al. 2007).

N2 fixation
We estimated N2 fixation rates with two methods: the acetylene reduction method and the 15N2 isotope tracer method. For the acetylene reduction method, described in Capone (1993), two 10-mL samples from each experimental replicate were incubated under treatment-specific conditions of irradiance and temperature in air-tight vials for ~10 hours (starting from the beginning of the light period) with 2 mL acetylene in 16.75 mL of headspace. The amount of ethylene accumulation was then estimated in 200 uL headspace gas with a gas chromatograph (model: GC-8A, Shimadzu Scientific Instruments, Columbia, MD, USA) at the 2nd, 4th, 6th, and 8th hour of the light period yielding 3 two-hour rates of ethylene accumulation. The accumulation of ethylene over 8 hours was used to calculate total gross N2 fixation rates (see explanation below) using a conversion ratio of 3:1 for acetylene to N2 reduction. Maximum gross N2 fixation rates were determined by finding the maximum rate of ethylene accumulation over a ~2-hour period. To calculate the concentration of ethylene in seawater from the concentration in the vial headspace, we used the Bunsen coefficient (0.088; from Breitbarth et al. 2004) for ethylene in seawater at 24 degrees C and a salinity of 35. We also estimated N2 fixation rates with the 15N2 isotope tracer method (Mulholland and Bernhardt 2005) by injecting 160 uL of highly enriched (99%) 15N2 gas into combusted (4 hours, 450 degrees C) 159 mL gas tight bottles filled with culture (without headspace) from a treatment replicate (triplicate samples for each experimental treatment). Culture samples were then incubated for 12 hours under treatment-specific conditions of irradiance and temperature during the light period only and we terminated incubations by filtering samples onto pre-combusted (450 degrees C, 4 hours) Whatman GF/F filters. pH was not determined in culture subsamples that were used to estimate N2 fixation after the incubation period in our experiments. Samples were stored frozen and dried before analysis with a Europa 20/20 isotope ratio mass spectrometer equipped with an automated nitrogen and carbon analyzer (ANCA). Estimates of N2 fixation made with the isotope tracer (15N2) method should represent net N2 fixation rates, because it estimates fixed N that is retained within cells. We assumed that the acetylene reduction method estimates gross N2 fixation rates because this estimate includes N fixed regardless of fate (see Mulholland & Bernhardt 2005; Mulholland et al. 2004). We estimated net 15N2 fixation rates in our experiment with IMS, but not in our experiment with GBR.

Carbon fixation
We inoculated two 30 mL samples from each treatment replicate with 25 or 50 uL of 1 mCurie (mCi) stock solution of sodium bicarbonate (H14CO3-; 0.83-1.7 uCi per mL final concentration). Samples were incubated for 24 hours under treatment-specific conditions of irradiance and temperature and then filtered onto Whatman GF/F filters and rinsed 3 times with ~5 mL filtered seawater to remove extracellular H14CO3-. Non-photosynthetically driven 14C incorporation was determined by incubating replicate culture samples (30 mL) for 24 hours in opaque bottles at the experimental temperature with the same concentration of H14CO3-;

these values were subtracted from measured total 14C incorporation to estimate photosynthetic incorporation. The total radioactivity of H14CO3- was determined by stabilizing 25 or 50 uL of the 1 mCi H14CO3- with 100 uL of a basic solution of phenylethylamine (99%) before adding 4 mL of Ultima Gold® XR (PerkinElmer, Shelton CT, USA) Radioactivity of 14C was determined on a Multi-purpose Scintillation Counter (model: LS-6500, Beckman Coulter, Fullerton, CA, USA). Carbon fixation rates (moles of CO2 fixed per unit time) were estimated by calculating the ratio of the radioactivity of photosynthetically-driven 14C incorporation into cells over 24 hours to the total radioactivity of H14CO3- and multiplying that ratio by the total dissolved inorganic carbon concentration (DIC).

Trichome length
In our light/CO2 manipulation experiment with IMS, trichome length was measured in samples collected from treatment-specific acclimated cultures 2 days prior to and on the final sampling day. Length data from the two time points were averaged and the resulting mean value from each experimental replicate was used in our analyses.

Seawater carbonate system estimates
Total dissolved inorganic carbon (DIC) was preserved in whole water samples (5-70 mL; stored at 4 degrees C) with a 5% HgCl2 solution (final concentration diluted to 0.5% HgCl2) as described in Fu et al. (2007), and estimated by acidifying 5 mL and quantifying the CO2 trapped in an acid sparging column (model: CM 5230) with a carbon coulometer (model: CM 140, UIC inc., Joliet, IL, USA). Reference material for the DIC analysis was prepared by Andrew Dickson at Scripps Institute of Oceanography. pH was measured with a pH meter (model: Orion 5 star Thermo Scientific, Beverly, MA, USA) and was monitored to ensure that perturbations of the seawater with the either air or certified pre-mixed air (Gilmore Liquid Air Company 750 ppm) resulted in the desired target pH of either ~8.2 or ~7.95. For the CO2/light manipulation experiment with GBR, samples for total DIC were taken from cultures at the same time CO2 and N2 fixation rates were estimated. For the CO2/light manipulation experiment with IMS, measurements of pH were paired with total DIC samples 5-6 days prior to measuring rates of CO2- and N2 fixation and were used to calculate pCO2 at 24 degrees C using the CO2sys program provided by Lewis and Wallace (1998) with K1 and K2 constants from Mehrbach et al. (1973) refit by Dickson and Millero (1987).

Carbonate system parameters in the experiment:

Strain	pCO2 treatment	DIC (uM)	pH	Calculated pCO2 (ppm)
IMS	Present day	2018 ±27	8.23 ±0.01	435 ± 9
IMS	100-year projected	2116 ±15	8.00 ± 0	771 ± 8
GBR	Present-day	2037 ±9	n.d.	n.d.
GBR	100-year projected	2165 ±9	n.d.	n.d.

References:
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Mulholland, M. R. and 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. Limnol. Oceangr. 50:839-849.

Data Processing Description

BCO-DMO merged data submitted as 3 separate tables into one dataset. Parameter names were modified to conform with BCO-DMO conventions.

[table of contents | back to top]

Data Files

File
tricho_fixation.csv (Comma Separated Values (.csv), 2.01 KB) MD5:5fd9934f42d6c7687ae27d511ff222a9 Primary data file for dataset ID 3776

[table of contents | back to top]

Parameters

Parameter	Description	Units
strain	Name of the T. erythraeum strain. IMS101 = from Coastal North Carolina, Atlantic Ocean; GBRTRLI101 = from Great Barrier Reef, Pacific Ocean.	text
pCO2	Partial pressure of pCO2. Present-day levels = 380 ppm; 100-year predicted levels = 750 ppm.	ppm
irradiance	Light/irradiance level measured in umol quanta per square meter per second.	umol quanta m-2 s-1
C_spec_net_CO2	C-specific net CO2 fixation per hour.	moles CO2 fixed per hour
C_spec_net_CO2_sd	Standard deviation of C_spec_net_CO2 based on the means of triplicate samples.	moles CO2 fixed per hour
cell_net_CO2	Cell-specific net CO2 fixation.	fmol C per cell per hour
cell_net_CO2_sd	Standard deviation of cell_net_CO2 based on means of triplicate samples.	fmol C per cell per hour
tot_N_spec_gross_N2	Total N-specific gross N2 fixation.	moles N2 fixed per hour
tot_N_spec_gross_N2_sd	Standard deviation of tot_N_spec_gross_N2 based on means of triplicate samples.	moles N2 fixed per hour
tot_cell_gross_N2	Total cell-specific gross N2 fixation.	fmol N per cell per hour

tot_cell_gross_N2_sd	Standard deviation of tot_cell_gross_N2 based on means of triplicate samples.	fmol N per cell per hour
max_N_spec_gross_N2	Maximum N-specific gross N2 fixation.	moles N2 fixed per hour
max_N_spec_gross_N2_sd	Standard deviation of max_N_spec_gross_N2 based on means of triplicate samples.	moles N2 fixed per hour
max_cell_gross_N2	Maximum cell-specific gross N2 fixation.	fmol N per cell per hour
max_cell_gross_N2_sd	Standard deviation of max_cell_gross_N2 based on means of triplicate samples.	fmol N per cell per hour
N_spec_net_N2	Net N-specific N2 fixation rates.	15N uptake per hour
N_spec_net_N2_sd	Standard deviation of N_spec_net_N2 based on means of triplicate samples.	15N uptake per hour
cell_net_N2	Net cell-specific N2 fixation rate.	fmol 15N per cell per hour
cell_net_N2_sd	Standard deviation of cell_net_N2 based on means of replicate samples.	fmol 15N per cell per hour
N2_gross_to_net	Ratio of total gross (8 h rate) to net (12 h rate) N2 fixation.	unitless
N2_gross_to_net_sd	Standard deviation of N2_gross_to_net based on means of the triplicate samples.	unitless
trichome_length	Trichome length in micrometers of T. erythraeum strain IMS101.	um
trichome_length_sd	Standard deviation of trichome_length based on means of the triplicate samples.	um

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	Benchtop pH Meter
Generic Instrument Name	Benchtop pH Meter
Dataset-specific Description	pH was measured with an Orion 5 star Thermo Scientific (Beverly, MA, USA) pH meter.
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	Gas Chromatograph
Generic Instrument Name	Gas Chromatograph
Dataset-specific Description	Gas chromatograph model: GC-8A, Shimadzu Scientific Instruments, Columbia, MD, USA used in determination of N2 fixation.
Generic Instrument Description	Instrument separating gases, volatile substances, or substances dissolved in a volatile solvent by transporting an inert gas through a column packed with a sorbent to a detector for assay. (from SeaDataNet, BODC)

Dataset-specific Instrument Name	Isotope-ratio Mass Spectrometer
Generic Instrument Name	Isotope-ratio Mass Spectrometer
Dataset-specific Description	A Europa 20/20 isotope ratio mass spectrometer equipped with an automated nitrogen and carbon analyzer (ANCA) was used in determination of N2 fixation rates. The Europa was originally manufactured by Europa Scientific Inc., Cincinnati OH, USA and was refurbished by PDZ Europa Limited, Elworth, Sandbach, Cheshire, UK.
Generic Instrument Description	The Isotope-ratio Mass Spectrometer is a particular type of mass spectrometer used to measure the relative abundance of isotopes in a given sample (e.g. VG Prism II Isotope Ratio Mass-Spectrometer).

Dataset-specific Instrument Name	Liquid Scintillation Counter
Generic Instrument Name	Liquid Scintillation Counter
Dataset-specific Description	Radioactivity of 14C was determined on a Multi-purpose Scintillation Counter (model: LS-6500, Beckman Coulter, Fullerton, CA, USA).
Generic Instrument Description	Liquid scintillation counting is an analytical technique which is defined by the incorporation of the radiolabeled analyte into uniform distribution with a liquid chemical medium capable of converting the kinetic energy of nuclear emissions into light energy. Although the liquid scintillation counter is a sophisticated laboratory counting system used the quantify the activity of particulate emitting (ß and a) radioactive samples, it can also detect the auger electrons emitted from 51Cr and 125I samples.

[table of contents | back to top]

Deployments

lab_Fu

Website	https://www.bco-dmo.org/deployment/58877
Platform	USC
Start Date	2009-08-01
End Date	2012-07-01
Description	Laboratory experiments carried out by Feixue Fu et al. of the University of Southern California (USC) for the project "Changing Phytoplankton Trace Metal Requirements in a High CO2 Ocean".

[table of contents | back to top]

Project Information

Changing Phytoplankton Trace Metal Requirements in a High CO2 Ocean (PhytoTM_in_HighCO2)

Coverage: Laboratory

This award is funded under the American Recovery and Reinvestment Act of 2009 (Public Law 111-5). The award is also associated with the NSF Integrative Computing Education and Research (ICER) initiative.

Over the past two decades, the fundamental importance of iron and other bioactive trace metals in structuring marine food webs and biogeochemical cycles has been realized. Even more recently, over the past several years, the international ocean science community has begun to mobilize in an urgent effort to understand the ecosystem-level consequences of rising anthropogenic CO2 and acidification of the global ocean. This project examines the intersection of these two major research themes, by asking the question: **How will the trace element requirements of marine phytoplankton change in response to future increases in atmospheric pCO2?**

Preliminary data generated by the investigators suggests that changing pCO2 can indeed profoundly affect the cellular quotas of Fe, Mo, Zn, Cd, Co and Mn in both prokaryotic and eukaryotic phytoplankton. Trace metals play critical roles as enzymatic co-factors for processes that are closely linked to the availability of CO2 such as carbon and nitrogen fixation, photosynthetic electron transport, and nutrient acquisition. Therefore, it is important to develop methods to quantitatively predict how algal metal requirements will change in tomorrow's rapidly changing ocean.

The investigators will take a three-pronged approach to addressing this overarching question:
(1) Laboratory experiments will measure the trace metal quotas of steady-state cultures of key phytoplankton functional groups like diatoms, coccolithophores, Phaeocystis, and diazotrophic and pico-cyanobacteria while varying pCO2 both alone, and together with other limiting factors such as iron, temperature, and light.
(2) Field work in the Southern California bight will provide measurements in trace metal stoichiometry of natural phytoplankton communities over a seasonal cycle in relation to pCO2 and other environmental variables -- this region is already experiencing some of the largest increases in acidic upwelled water along the entire West Coast.
(3) This observational and correlative study will be coupled with manipulative experiments at the USC Catalina Island facility in which trace metal quotas of the same natural phytoplankton communities can be measured in relation to pCO2 shifts under controlled incubation conditions.

Together, these three complementary approaches will enable the investigators to determine over a variety of temporal and spatial scales how phytoplankton-driven trace element biogeochemistry is likely to change in a future high-CO2 ocean.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-0850730

[table of contents | back to top]