

Experimental results from a study of growth rates of two strains of *Trichodesmium erythraeum* under varying pCO₂ and light conditions (PhytoTM_in_HighCO₂ project)

Website: <https://www.bco-dmo.org/dataset/3778>

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Project

» [Changing Phytoplankton Trace Metal Requirements in a High CO₂ Ocean](#) (PhytoTM_in_HighCO₂)

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|--|---|---------------------------|
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Dataset Description

Experimental data on the growth rates of two strains of *Trichodesmium erythraeum* (IMS1010 and GBRTRL10) in response to irradiance and pCO₂. This experiment served as a reference for determining the range of irradiance levels used in the other CO₂ and light experiments on *T. erythraeum* during this project.

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](https://doi.org/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 µm) 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: IMS growth vs. light experiment

This experiment served as a reference for determining the range of irradiance levels used in our CO₂ and light experiments. Cultures of IMS were cultured at 27 degrees C in fifteen 800-mL culturing flasks on a 12:12 light:dark cycle at 25, 50, 100, 180 and 300 umol quanta per square meter per second irradiance. Once cells had achieved steady state, we determined cellular growth rates using microscopic cell counts between dilutions.

Analytical Methods: Growth rates

Growth rates were estimated by measuring relative increases in cell number per unit volume between dilutions (2-3 day periods) in steady-state cultures.

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 | pCO ₂ treatment | DIC (uM) | pH | Calculated pCO ₂ (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:

- Chen**, Y. B., Zehr, J. P. & Mellon, M. 1996. Growth and nitrogen fixation of the diazotrophic filamentous nonheterocystous cyanobacterium *Trichodesmium* sp IMS 101 in defined media: Evidence for a circadian rhythm. *J. Phycol.* 32:916-923. DOI: [10.1111/j.0022-3646.1996.00916.x](https://doi.org/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 Res.* 34:1733-1743. DOI: [10.1016/0198-0149\(87\)90021-5](https://doi.org/10.1016/0198-0149(87)90021-5)
- Lewis**, E. and D. W. R. Wallace (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. & Pytkovicz, R. 1973. Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. *Limnol. Oceanogr.* 18:897-907.
- Morel**, F. M. M., Rueter, J. G., Anderson, D. M. and Guillard, R. R. L. 1979. Aquil - chemically defined phytoplankton culture-medium for trace-metal studies. *J. Phycol.* 15:135-141. DOI: [10.1111/j.1529-8817.1979.tb02976.x](https://doi.org/10.1111/j.1529-8817.1979.tb02976.x)

Data Processing Description

Parameter names have been changed to conform with BCO-DMO conventions.

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Data Files

| File |
|---|
| tricho_growth.csv (Comma Separated Values (.csv), 355 bytes) MD5:938bfc4710ea865563e9c947d3da069b |
| Primary data file for dataset ID 3778 |

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Parameters

| Parameter | Description | Units |
|----------------|---|---|
| strain | Name of the <i>T. erythraeum</i> 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 |
| growth_rate | Cellular growth rate. Estimated from changes in cell number per unit volume over time. | cellular growth per day |
| growth_rate_SD | Standard deviation of growth_rate based on the means of the triplicate samples. | cellular growth per day |

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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. |

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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 CO ₂ Ocean". |

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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.

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Funding

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

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