Nitrogen and carbon fixation rates and POC and PON from thermal variation experiment of Trichodesmium GBR strain from 2016-2018

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Project
» How does intensity and frequency of environmental variability affect phytoplankton growth? (Enviro variability and phytoplankton growth)

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Coverage

Temporal Extent: 2016-12-02 - 2018-11-14

Dataset Description

This dataset includes nitrogen and carbon fixation rates as well as particulate organic carbon (POC) and nitrogen (PON) from subsamples taken from *Trichodesmium erythraeum* GBR strain incubated at different temperatures and phosphate concentrations to examine the interaction of intensity of thermal variability and phosphate limitation on growth rates, carbon fixation, and nitrogen fixation rates.

Methods & Sampling

The *Trichodesmium erythraeum* GBR strain used in this project was a tropical strain collected and isolated from the Great Barrier Reef (Fu and Bell 2003). In this study, the cultures were maintained with autoclaved artificial seawater by adding phosphate (10 µM), vitamins and trace metals as suggested by Aquil recipe (Garcia et al. 2011). Cool white fluorescent bulbs were used to provide a 12h dark: 12h light cycle at 150 µmol photons m-2s-1. Cultures were grown in acid-washed 120-ml plastic jars fitting into the thermal block that provides an even temperature gradient.

According to Fu et al. (2014), the temperature limit of this strain is 18-32°C, while the optimal growth range is

24-28°C. Within the optimal range, the growth of *Trichodesmium erythraeum* was at plateau stage, therefore, thermal variations that fall wholly within this range was expected to have negligible effects. 22°C and 30°C represents the “cold” and “warm” phases of the variation cycle. For each constant temperature, one or two variable treatments were used simultaneously, each with an average temperature equal to the corresponding constant temperature. There was an “intense” 18-26°C variable treatment and a “mild” 20-24°C variable treatment for 22°C. For 30°C, only one variable treatment “28-32°C” was used, because an “intense” one periodically exceeding the strain’s upper temperature limit was likely to kill the cultures.

For all the treatments, semi-continuous incubation methods were applied and dilution was conducted every four days. In each 4-day cycle, the first 48 hours of variable treatments were at a lower temperature (respectively 18, 20 and 28°C) and the second 48 hours were at a higher temperature (respectively 26, 24 and 32°C). In order to investigate the interactions between phosphate availability and thermal variation, there were triplicate bottles for phosphorus-replete (10 µmol/L) and phosphorus-limiting (0.2 µmol/L) conditions under the 5 constant and variable temperature treatments above.

Semi-continuous incubation was maintained until steady state was reached. Data on specific growth rates, nitrogen and carbon fixation rates were collected and analyzed. There were three sampling points in each cycle: the initial point (0 hour after the dilution and transfer to LT phase), the middle point (48 hours after the dilution, end of LT phase) and the final point (96 hours after the dilution, end of HT phase). For variable temperature treatments, nitrogen and carbon fixation data at the middle and final points and the average values of these two phases were compared to the corresponding data from the constant treatment.

Nitrogen fixation measurements. N2-fixation rate was determined using the Acetylene Reduction method (Capone 1993) by gas chromatography with a Shimadzu gas chromatograph GC-8a (Shimadzu Scientific Instruments). 10 ml of culture was added to a 27 ml serum vial. The vial was then air-tighten and 2 ml air was extracted. Then 2 ml of acetylene (C2H2) was added to the headspace of each vial. There is a theoretical 3:1 ratio (mol C2H2 to mol N2 reduced) to calculate the N2 fixation based on the rates of ethylene production (Montoya et al. 1996). Ethylene production was measured by injecting 200 µl of headspace to the GC device at 4-5 h intervals over the entire 12 h light period (Tuit et al. 2004). For each treatment, 3-6 replicates were incubated under the same light and temperature conditions. After total N2-fixation rates are measured, the cell count, PON and POC of each sample were measured and used to normalize N2-fixation rates.

C fixation rates. To measure uptake rates of carbon and iron, 0.2 µCi 14C-NaH14CO3 was added to 30 ml subsamples from each replicate (specific activity for final solutions was roughly 0.25 kBq/ml; PerkinElmer). The background dissolved inorganic carbon in the medium was determined by DIC measurements. Samples were then incubated for 24 h or select time under their respective experimental conditions, and filtered onto GF/F filters. To correct for filter adsorption, 30 ml of cultures from each treatment (10ml from each replicate bottle) was filtered immediately after adding equal amounts of NaH14CO3. All filters were rinsed with artificial seawater. Filters were then placed in 7 ml scintillation vials in the dark overnight after adding 4 ml scintillation fluid. To determine the total radioactivity (TA), 1 µCi 14C-NaH14CO3 together with 100 µl Phenylalanine was placed in identical scintillation vials with the addition of 4 ml scintillation solution. 14C radioactivity was measured using liquid scintillation counting (Perkin Elmer) for TA, blanks and samples (Xu et al. 2014).

Elemental stoichiometry. Elemental ratios were obtained by measuring particulate organic carbon and nitrogen (POC and PON). For particulate organic carbon and nitrogen (POC and PON), a large volume (determined by the biomass) was filtered onto a pre-combusted (500 °C, 2-3 h) GF/F filter, which was then wrapped in aluminum foil and dried at 55 °C. POC and PON were analyzed on a Costech Elemental Analyzer using methionine and atropine as references to calibrate the system at the beginning and between measurements of every twelve samples (Fu et al. 2007).

Methodology References:

Fu, F., & Bell, P. (2003). Factors affecting N2 fixation by the cyanobacterium trichodesmium sp. GBRTRLI101. FEMS Microbiology Ecology, 45(2), 203-209.

Fu, F., Warner, M. E., Zhang, Y., Feng, Y., & Hutchins, D. A. (2007). Effects of increased temperature and CO2 on photosynthesis, growth, and elemental ratios in marine Synechococcus and Prochlorococcus (cyanobacteria) 1. Journal of Phycology, 43(3), 485-496.

Fu, F., Yu, E., Garcia, N. S., Gale, J., Luo, Y., Webb, E. A., & Hutchins, D. A. (2014). Differing responses of marine N2 fixers to warming and consequences for future diazotroph community structure. Aquatic Microbial Ecology, 72(1), 33-46.

Garcia, N. S., Fu, F., Breene, C. L., Bernhardt, P. W., Mulholland, M. R., Sohm, J. A., & Hutchins, D. A. (2011).

Interactive effects of irradiance and CO2 on CO2 fixation and N2 fixation in the diazotroph Trichodesmium erythraeum (cyanobacteria) 1. Journal of Phycology, 47(6), 1292-1303.

Ihnken, S., Roberts, S., & Beardall, J. (2011). Differential responses of growth and photosynthesis in the marine diatom Chaetoceros muelleri to CO2 and light availability. Phycologia, 50(2), 182-193.

Tuit, C., Waterbury, J., & Ravizza, G. (2004). Diel variation of molybdenum and iron in marine diazotrophic cyanobacteria. Limnology and Oceanography, 49(4), 978-990.

Xu, K., Fu, F., & Hutchins, D. A. (2014). Comparative responses of two dominant Antarctic phytoplankton taxa to interactions between ocean acidification, warming, irradiance, and iron availability. Limnology and Oceanography, 59(6), 1919-1931.

Data Processing Description

Averages and standard deviations were calculated using Excel 14.4.2.

BCO-DMO Processing Notes:
- added conventional header with dataset name, PI name, version date
- modified parameter names to conform with BCO-DMO naming conventions
- removed degree symbols from temperature_treatment column
- replaced blank cells with '-' in statistical summary rows
- replaced 'n.a.' with 'nd' for 'no data'

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Data Files

File
CNfix_POCPON.csv (Comma Separated Values (.csv), 8.65 KB) MD5:b93edfdd4233ca5ede979cf522103885 Primary data file for dataset ID 722927

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Parameters

Parameter	Description	Units
temperature_treatment	temperature and treatment (constant or variable temperature)	degrees Celsius
sample_name	sample name	unitless
phosphate_conc	phosphate concentration	micromol/Liter (µmol/L)
date	date of measurement	unitless

sampling_point	sampling point: either final or middle of incubation	unitless
pmol_fixed_N_per_ml_per_hr	concentration of fixed Nitrogen in sample	picomol fixed N/milliliter/hour (pmol N/ml/hr)
mol_fixed_N_per_mol_PON_per_hr	rate of fixed Nitrogen to Particulate Organic Nitrogen (PON)	mol fixed Nitrogen/mol PON/hour (mol fixed N/mol PON/hr)
avg_mol_fixed_N_per_mol_PON_per_hr_each_treament	average rate of Nitrogen fixation to PON for each treatment	mol fixed Nitrogen/mol PON/hour (mol fixed N/mol PON/hr)
std_dev_mol_fixed_N_per_mol_PON_per_hr_each_treatment	standard deviation of rate of Nitrogen fixation to PON for each treatment	mol fixed Nitrogen/mol PON/hour (mol fixed N/mol PON/hr)
umol_fixed_C_per_h_per_L	Carbon fixation rate	umol fixed Carbon/hour/Liter (umol fixed C/h/L)
mol_fixed_C_per_mol_POC_per_hour	rate of Carbon fixation to Particulate Organic Nitrogen (POC)	mol fixed Carbon/mol POC/hour (mol fixed C/mol POC/hr)
avg_mol_fixed_C_per_mol_POC_per_hour_each_treament	average rate of Carbon fixation to POC for each treatment	mol fixed Carbon/mol POC/hour (mol fixed C/mol POC/hr)
std_dev_mol_fixed_C_per_mol_POC_per_hour_each_treatment	standard deviation of mol fixed C per mol POC per hour each treatment	mol fixed Carbon/mol POC/hour (mol fixed C/mol POC/hr)
umol_PON_per_L	concentration of Particulate Organic Nitrogen (PON)	umol PON/Liter
umol_POC_per_L	concentration of Particulate Organic Carbon (POC)	umol POC/Liter
POC_PON	POC:PON ratio	unitless
avg_POC_PON_each_treatment	average POC:PON of each treatment	unitless

std_dev_POC_PON_each_treatment	standard deviation of POC:PON of each treatment	unitless

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Instruments

Dataset-specific Instrument Name	Costech Elemental Analyzer ECS4010 CHNSO ANALYZER
Generic Instrument Name	Elemental Analyzer
Dataset-specific Description	Used to measure elemental ratios of POC and PON.
Generic Instrument Description	Instruments that quantify carbon, nitrogen and sometimes other elements by combusting the sample at very high temperature and assaying the resulting gaseous oxides. Usually used for samples including organic material.

Dataset-specific Instrument Name	Shimadzu gas chromatograph GC-8a (Shimadzu Scientific Instruments)
Generic Instrument Name	Gas Chromatograph
Dataset-specific Description	Used to measure Nitrogen fixation rates
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	Perkin Elmer liquid scintillation counter
Generic Instrument Name	Liquid Scintillation Counter
Dataset-specific Description	Used to measure C14 radioactivity for carbon fixation rates.
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. Liquid scintillation counters are instruments assaying alpha and beta radiation by quantitative detection of visible light produced by the passage of rays or particles through a suitable scintillant incorporated into the sample.

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Project Information

How does intensity and frequency of environmental variability affect phytoplankton growth? (Enviro variability and phytoplankton growth)

Coverage: laboratory experiment

NSF Award Abstract:
Microscopic plants called phytoplankton are key members of global oceanic ecosystems, since their photosynthesis supports the majority of the marine food chain and produces about as much oxygen as land plants. Because of this, oceanographers have often carried out experiments examining how factors such as temperature and carbon dioxide levels may affect phytoplankton growth. Most previous experiments have used constant levels of temperature and carbon dioxide, but it is clear from looking at measurements from real ocean ecosystems that these two factors often vary greatly over timescales of days to weeks. Using field and laboratory experiments along with computer modeling, this project will test how the growth of several major groups of phytoplankton differs under constant conditions of temperature and carbon dioxide, compared to conditions in which these factors fluctuate in intensity and frequency. This research will give marine scientists a better picture of how phytoplankton may respond to a varying natural environment today and in the future, and therefore help us to understand how ocean food webs function to support critical living resources such as fisheries. The project will train graduate and undergraduate students and a postdoctoral researcher, and the lead scientists will be involved in an ocean science education program for largely minority high school students from a downtown Los Angeles school district.

The goal of this project is to use laboratory culture and natural community experiments to understand how realistically fluctuating temperature and pCO2 conditions may affect globally important phytoplankton groups in ways that differ from the artificial constant exposures used in previous work. Culture experiments will test how the intensity and frequency of short-term thermal and carbonate fluctuations affects the growth responses of diazotrophic and picoplanktonic cyanobacteria, coccolithophores, and diatoms under both current and projected future environmental conditions. These lab results will be supported and extended by parallel experiments using mixed natural assemblages from the California upwelling regime, allowing us to test these same questions using phytoplankton communities that experience large seasonal shifts between highly dynamic thermal and carbonate system conditions during the spring upwelling season, and relatively much more static conditions during fall stratification events. These results will be synthesized using a new generation of numerical models that employ novel approaches to incorporating realistic environmental variations to allow more accurate predictions of phytoplankton responses to a dynamic environment in today's marine ecosystems, and in the future changing ocean.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1538525

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