

# Trichodesmium thermal curve from October to November 2018

**Website:** <https://www.bco-dmo.org/dataset/756942>

**Data Type:** Other Field Results

**Version:** 1

**Version Date:** 2019-02-26

## Project

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

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## Coverage

**Temporal Extent:** 2018-10-06 - 2018-11-12

## Dataset Description

This dataset includes specific growth rates 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* GBRTRLI101 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  $\mu$ M), 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  $\mu$ mol photons m<sup>-2</sup>s<sup>-1</sup>. 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.

*Growth rates.* During the semi-continuous incubation, real-time biomass was estimated with *in vivo* fluorescence before and after dilution, and subsequently validated by microscopy using preserved cell counts. Specific growth rates ( $\mu$ ) were calculated based on the *in vivo* fluorescence readings as:  $\mu = \ln(Nt2/Nt1)/(t2-t1)$ , where  $Nt1$ , and  $Nt2$  refer to biomass ( as *in vivo* fluorescence readings) at time 1 ( $t1$ ) and 2 ( $t2$ ) (in days) respectively (Ihnken et al. 2011).

*Nitrogen fixation measurements.* N<sub>2</sub>-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 (C<sub>2</sub>H<sub>2</sub>) was added to the headspace of each vial. There is a theoretical 3:1 ratio (mol C<sub>2</sub>H<sub>2</sub> to mol N<sub>2</sub> reduced) to calculate the N<sub>2</sub> 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 N<sub>2</sub>-fixation rates are measured, the cell count, PON and POC of each sample were measured and used to normalize N<sub>2</sub>-fixation rates.

*C fixation rates.* To measure uptake rates of carbon and iron, 0.2 µCi <sup>14</sup>C-NaH<sub>14</sub>CO<sub>3</sub> 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 NaH<sub>14</sub>CO<sub>3</sub>. 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 <sup>14</sup>C-NaH<sub>14</sub>CO<sub>3</sub> together with 100 µl Phenylalanine was placed in identical scintillation vials with the addition of 4 ml scintillation solution. <sup>14</sup>C radioactivity was measured using liquid scintillation counting (Perkin Elmer) for TA, blanks and samples (Xu et al. 2014).

## Data Processing Description

Microsoft 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
- appended start date and end date columns which were extracted from the date column.

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## Data Files

**File****thermal\_curve.csv**(Comma Separated Values (.csv), 4.30 KB)

MD5:59b26d76be1e65b508dd0b26a5627831

Primary data file for dataset ID 756942

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## Related Publications

Capone, D. G. 1993. Determination of nitrogenase activity in aquatic samples using the acetylene reduction procedure, p. 621-631. In P. F. Kemp, B. F. Sherr, E. B. Sherr, and J. J. Cole (ed.), Handbook of methods in aquatic microbial ecology. Lewis Publishers, Boca Raton, Fla.

*Methods*

Fu, F., Yu, E., Garcia, N., Gale, J., Luo, Y., Webb, E., & Hutchins, D. (2014). Differing responses of marine N<sub>2</sub> fixers to warming and consequences for future diazotroph community structure. *Aquatic Microbial Ecology*, 72(1), 33-46. doi:[10.3354/ame01683](https://doi.org/10.3354/ame01683)

*Methods*

Fu, F.-X., & Bell, P. R. . (2003). Factors affecting N<sub>2</sub> fixation by the cyanobacterium *Trichodesmium* sp. GBRTLI101. *FEMS Microbiology Ecology*, 45(2), 203-209. doi:10.1016/s0168-6496(03)00157-0

[https://doi.org/10.1016/S0168-6496\(03\)00157-0](https://doi.org/10.1016/S0168-6496(03)00157-0)

*Methods*

Fu, F.-X., Warner, M. E., Zhang, Y., Feng, Y., & Hutchins, D. A. (2007). Effects of Increased temperature and CO<sub>2</sub> on photosynthesis, growth, and elemental ratios in marine *Synechococcus* and *Prochlorococcus* (cyanobacteria). *Journal of Phycology*, 43(3), 485-496. doi:[10.1111/j.1529-8817.2007.00355.x](https://doi.org/10.1111/j.1529-8817.2007.00355.x)

*Methods*

Garcia, N. S., Fu, F.-X., Breene, C. L., Bernhardt, P. W., Mulholland, M. R., Sohm, J. A., & Hutchins, D. A. (2011). INTERACTIVE EFFECTS OF IRRADIANCE AND CO<sub>2</sub> ON CO<sub>2</sub> FIXATION AND N<sub>2</sub> FIXATION IN THE DIAZOTROPH TRICHODESMIUM ERYTHRAEUM (CYANOBACTERIA)1. *Journal of Phycology*, 47(6), 1292-1303.

doi:[10.1111/j.1529-8817.2011.01078.x](https://doi.org/10.1111/j.1529-8817.2011.01078.x)

*Methods*

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

*Methods*

Tuit, C., Waterbury, J., & Ravizza, G. (2004). Diel variation of molybdenum and iron in marine diazotrophic cyanobacteria. *Limnology and Oceanography*, 49(4), 978-990. doi:[10.4319/lo.2004.49.4.0978](https://doi.org/10.4319/lo.2004.49.4.0978)

*Methods*

Xu, K., Fu, F.-X., & 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. doi:[10.4319/lo.2014.59.6.1919](https://doi.org/10.4319/lo.2014.59.6.1919)

*Methods*

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## Parameters

Parameter	Description	Units
Sample_name	sample name	unitless
Temperature	temperature	degrees Celsius
Phosphate_concentration	concentration of phosphate	micromole per Liter (umol/L)
dates	date duration	unitless
specific_growth_rate	specific growth rate	per day
start_date	start date in yyyy-mm-dd format	unitless
end_date	end date in yyyy-mm-dd format	unitless

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## Instruments

<b>Dataset-specific Instrument Name</b>	ECS4010 CHNSO ANALYZER
<b>Generic Instrument Name</b>	CHN Elemental Analyzer
<b>Dataset-specific Description</b>	Costech Elemental Analyzer
<b>Generic Instrument Description</b>	A CHN Elemental Analyzer is used for the determination of carbon, hydrogen, and nitrogen content in organic and other types of materials, including solids, liquids, volatile, and viscous samples.

<b>Dataset-specific Instrument Name</b>	
<b>Generic Instrument Name</b>	Microscope - Optical
<b>Dataset-specific Description</b>	Used to validate growth rates calculated from fluorescence.
<b>Generic Instrument Description</b>	Instruments that generate enlarged images of samples using the phenomena of reflection and absorption of visible light. Includes conventional and inverted instruments. Also called a "light microscope".

<b>Dataset-specific Instrument Name</b>	
<b>Generic Instrument Name</b>	Turner Designs Fluorometer 10-AU
<b>Dataset-specific Description</b>	Used for in vivo fluorescence
<b>Generic Instrument Description</b>	The Turner Designs 10-AU Field Fluorometer is used to measure Chlorophyll fluorescence. The 10AU Fluorometer can be set up for continuous-flow monitoring or discrete sample analyses. A variety of compounds can be measured using application-specific optical filters available from the manufacturer. (read more from Turner Designs, turnerdesigns.com, Sunnyvale, CA, USA)

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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 pCO<sub>2</sub> 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
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-1538525</a>

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