# Fatty acid profiles by biovolume of replicate populations of Thalassiosira pseudonana, selected at 16 and 31C for $\sim$ 500 generations and assayed at 4 temperatures

Website: https://www.bco-dmo.org/dataset/780155

**Data Type**: experimental

Version: 1

Version Date: 2019-10-28

#### **Project**

» <u>Dimensions: Collaborative Research: Genetic, functional and phylogenetic diversity determines marine phytoplankton community responses to changing temperature and nutrients</u> (Phytoplankton Community Responses)

#### **Program**

» Dimensions of Biodiversity (Dimensions of Biodiversity)

Contributors	Affiliation	Role
<u>Litchman, Elena</u>	Michigan State University (MSU)	Principal Investigator
O'Donnell, Daniel R.	Michigan State University (MSU)	Co-Principal Investigator, Contact
Copley, Nancy	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

#### **Abstract**

Fatty acid profiles by biovolume of replicate populations of Thalassiosira pseudonana, selected at 16 and 31C for  $\sim$ 500 generations and assayed at 4 temperatures.

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### Coverage

**Spatial Extent**: Lat:42.4061 Lon:-85.4007 **Temporal Extent**: 2017-12-09 - 2018-01-05

## **Dataset Description**

Fatty acid profiles by biovolume of replicate populations of *Thalassiosira pseudonana*, selected at 16 and 31C for  $\sim$ 500 generations and assayed at 4 temperatures.

These data were published in O'Donnell and Litchman (2019) Evol. Appl. and can also be found in Supporting Information file eva12798-sup-0001-Supinfo.docx, Figures 1-5 of the main text, and on Dryad: <a href="https://datadryad.org/stash/dataset/doi:10.5061/dryad.pn04m84">https://datadryad.org/stash/dataset/doi:10.5061/dryad.pn04m84</a>.

#### Methods & Sampling

Details of the long-term selection experiment can be found in another dataset associated with this project, entitled "Temperature-growth rate curves for *Thalassiosira pseudonana*", and in O'Donnell et al. (2018), Global Change Biology. After  $\sim 500$  generations of experimental selection (5 replicate populations at  $16^{\circ}$ C and 5 at  $31^{\circ}$ C), we acclimated each replicate population for 10 generations at  $20^{\circ}$ C, then acclimated each at 10, 16, 26 and  $31^{\circ}$ C for an additional 10 generations. We then sub-cultured each acclimated population into triplicate batch cultures, starting with 100,000 cells in 40 ml of L1 marine culture medium in a 50 ml tissue culture flask. We grew all experimental cultures (2 selection temperatures x 5 replicate populations x 4 assay temperatures x 3 assay replicates = 120 total experimental units) to late log phase, estimating per-capita growth rates by placing each tissue culture flask in a spectrophotometer daily and measuring absorbance at 436 nm. We harvested 15 ml of culture for fatty acid analysis, retaining  $\sim 25 \, \mu l$  for enumeration on a CASY particle counter. The remaining volume was used for analyses not addressed here.

Cells were harvested by filtration through a 25mm Whatman GF/B filter. We extracted fatty acids from *T. pseudonana* cells using a solution of chloroform, methanol, and formic acid (10:20:1). We analyzed fatty acid profiles by performing fatty acid methyl ester (FAME) reactions on each GF/B filter sample (Wang and Benning 2011; adapted for algae by Boyle et al. 2012) and quantifying fatty acids using gas chromatography.

#### **Data Processing Description**

All calculations were done and data processed and analyzed using R statistical programming software, version 3.3.2. Cell volumes were estimated by measuring the length and width of *T. pseudonana* frustules under oil immersion at 1000x magnification, then using these measurements to calculate the volume of a cylinder ( $\pi$  x r^2 x h). We thusly measured 10 cells per sample (10 x 120 = 1200 total cells). Mean per-biovolume fatty acid contents were estimated using these volumes.

## **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 duplicate mol w3 by column

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#### **Data Files**

#### File

**fatty\_acids\_biovol.csv**(Comma Separated Values (.csv), 61.21 KB)
MD5:7fb151634b4b20380a1a479cdf639595

Primary data file for dataset ID 780155

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#### **Related Publications**

Boyle, N. R., Page, M. D., Liu, B., Blaby, I. K., Casero, D., Kropat, J., ... Merchant, S. S. (2012). Three Acyltransferases and Nitrogen-responsive Regulator Are Implicated in Nitrogen Starvation-induced Triacylglycerol Accumulation inChlamydomonas. Journal of Biological Chemistry, 287(19), 15811–15825. doi:10.1074/jbc.m111.334052 <a href="https://doi.org/10.1074/jbc.M111.334052">https://doi.org/10.1074/jbc.M111.334052</a> Methods

O'Donnell, D. R., Du, Z., & Litchman, E. (2019). Experimental evolution of phytoplankton fatty acid thermal reaction norms. Evolutionary Applications, 12(6), 1201–1211. doi:10.1111/eva.12798

Results

O'Donnell, D. R., Hamman, C. R., Johnson, E. C., Kremer, C. T., Klausmeier, C. A., & Litchman, E. (2018). Rapid thermal adaptation in a marine diatom reveals constraints and trade-offs. Global Change Biology, 24(10), 4554-4565. doi:10.1111/gcb.14360

# Methods

Wang, Z., & Benning, C. (2011). *Arabidopsis thaliana* Polar Glycerolipid Profiling by Thin Layer Chromatography (TLC) Coupled with Gas-Liquid Chromatography (GLC). Journal of Visualized Experiments, (49). doi: 10.3791/2518 *Methods* 

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# **Parameters**

Parameter	Description	Units
evol_temp	Growth chamber temperature long-term selection experiment	degrees Celsius
assay_temp	Growth chamber temperature during temperature-dependent growth assay	degrees Celsius
evol_rep	Replicate population ID during long-term selection experiment	unitless
assay_rep	Experimental replicate during temperature-dependent fatty acid production assay (3 replicates)	unitless
numID	A sequential identifier used post-sample processing	unitless
Sample	A sample ID used only to indicate a sample's position on the GC rack. Sequential; but does not correspond to "numID"	unitless
SampID	A sample ID that includes (in order) evol_temp; assay_temp; evol_rep and assay_rep	unitless
strain	A second replicate population ID; also indicating selection temperature; identifies only eval_temp and evol_rep	unitless
good	Quality flag: indicates whether a sample was flagged as unusable: yes = usable & used; no = unusable & not used	unitless
cells_ml	T pseudonana cells per ml of culture	cells/milliliter
mol_total_bv	Total mols of fatty acids per cubic micron of biovolume	mol/micron cubed
mol_SFA_bv	Mols of saturated fatty acid per cubic micron of biovolume	mol/micron cubed
mol_UFA_bv	Mols of unsaturated fatty acid per cubic micron of biovolume	mol/micron cubed

mol_MUFA_bv	Mols of monounsaturated fatty acid per cubic micron of biovolume	mol/micron cubed
mol_PUFA_bv	Mols of polyunsaturated fatty acid per cubic micron of biovolume	mol/micron cubed
mol_w3_bv	Mols of omega-3 fatty acid per cubic micron of biovolume	mol/micron cubed
mol_bv_14_0	mol per cubic micron of 14:0 fatty acid. 14 indicates the length of the fatty acid chain (carbon atoms); 0 indicates the number of double bonds.	mol fatty acid per cubic micron
mol_bv_16_0	mol per cubic micron of 16:0 fatty acid. 14 indicates the length of the fatty acid chain (carbon atoms); 0 indicates the number of double bonds.	mol fatty acid per cubic micron
mol_bv_16_1a	mol per cubic micron of 16:0 fatty acid. 14 indicates the length of the fatty acid chain (carbon atoms); 1 indicates the number of double bonds.	mol fatty acid per cubic micron
mol_bv_16_1b	mol per cubic micron of 16:0 fatty acid. 14 indicates the length of the fatty acid chain (carbon atoms); 1 indicates the number of double bonds.	mol fatty acid per cubic micron
mol_bv_16_2a	mol per cubic micron of 16:0 fatty acid. 14 indicates the length of the fatty acid chain (carbon atoms); 2 indicates the number of double bonds.	mol fatty acid per cubic micron
mol_bv_16_2b	mol per cubic micron of 16:0 fatty acid. 14 indicates the length of the fatty acid chain (carbon atoms); 2 indicates the number of double bonds.	mol fatty acid per cubic micron
mol_bv_16_3	mol per cubic micron of 16:0 fatty acid. 14 indicates the length of the fatty acid chain (carbon atoms); 3 indicates the number of double bonds.	mol fatty acid per cubic micron
mol_bv_16_4	mol per cubic micron of 16:0 fatty acid. 14 indicates the length of the fatty acid chain (carbon atoms); 4 indicates the number of double bonds.	mol fatty acid per cubic micron
mol_bv_18_0	mol per cubic micron of 18:0 fatty acid. 18 indicates the length of the fatty acid chain (carbon atoms); 0 indicates the number of double bonds.	mol fatty acid per cubic micron
mol_bv_18_1_9	mol per cubic micron of 18:0 fatty acid. 18 indicates the length of the fatty acid chain (carbon atoms); 1 indicates the number of double bonds.	mol fatty acid per cubic micron

mol per cubic micron of 18:0 fatty acid. 18 indicates the length of the fatty acid chain (carbon atoms); 1 indicates the number of double bonds.	mol fatty acid per cubic micron
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mol per cubic micron of 20:0 fatty acid. 20 indicates the length of the fatty acid chain (carbon atoms); 5 indicates the number of double bonds.	mol fatty acid per cubic micron
mol per cubic micron of 20:0 fatty acid. 20 indicates the length of the fatty acid chain (carbon atoms); 6 indicates the number of double bonds.	mol fatty acid per cubic micron
Total mols of fatty acids in a sample	mol fatty acid
Mean fatty acid chain length	C atoms per fatty acid molecule.
Mean degree of fatty acid unsaturation	Double bonds per fatty acid molecule.
	fatty acid chain (carbon atoms); 1 indicates the number of double bonds.  mol per cubic micron of 18:0 fatty acid. 18 indicates the length of the fatty acid chain (carbon atoms); 2 indicates the number of double bonds.  mol per cubic micron of 18:0 fatty acid. 18 indicates the length of the fatty acid chain (carbon atoms); 3 indicates the number of double bonds.  mol per cubic micron of 20:0 fatty acid. 20 indicates the length of the fatty acid chain (carbon atoms); 0 indicates the number of double bonds.  mol per cubic micron of 20:0 fatty acid. 20 indicates the length of the fatty acid chain (carbon atoms); 5 indicates the number of double bonds.  mol per cubic micron of 20:0 fatty acid. 20 indicates the length of the fatty acid chain (carbon atoms); 6 indicates the number of double bonds.  Total mols of fatty acids in a sample  Mean fatty acid chain length

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# Instruments

Dataset-specific Instrument Name	CASY particle counter, Schärfe System GmbH, Reutlingen, Germany
Generic Instrument Name	Automated Cell Counter
	An instrument that determines the numbers, types or viability of cells present in a sample.

Dataset- specific Instrument Name	Agilent Technologies 7890A Gas Chromatography system (Agilent Technologies)
Generic Instrument Name	Gas Chromatograph
Dataset- specific Description	Gas chromatograph using the Agilent capillary DB-23 column for FAME analysis. Temperature settings: initial temperature 140°C, increased by 30°C/min to 160°C, then by 8°C/min to 240°C, and held at 240°C for 2 min.
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)

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# **Project Information**

Dimensions: Collaborative Research: Genetic, functional and phylogenetic diversity determines marine phytoplankton community responses to changing temperature and nutrients (Phytoplankton Community Responses)

Coverage: Narragansett Bay, RI and Bermuda, Bermuda Atlantic Time-series Study (BATS)

#### NSF Award Abstract:

Photosynthetic marine microbes, phytoplankton, contribute half of global primary production, form the base of most aquatic food webs and are major players in global biogeochemical cycles. Understanding their community composition is important because it affects higher trophic levels, the cycling of energy and elements and is sensitive to global environmental change. This project will investigate how phytoplankton communities respond to two major global change stressors in aquatic systems: warming and changes in nutrient availability. The researchers will work in two marine systems with a long history of environmental monitoring, the temperate Narragansett Bay estuary in Rhode Island and a subtropical North Atlantic site near Bermuda. They will use field sampling and laboratory experiments with multiple species and varieties of phytoplankton to assess the diversity in their responses to different temperatures under high and low nutrient concentrations. If the diversity of responses is high within species, then that species may have a better chance to adapt to rising temperatures and persist in the future. Some species may already be able to grow at high temperatures; consequently, they may become more abundant as the ocean warms. The researchers will incorporate this response information in mathematical models to predict how phytoplankton assemblages would reorganize under future climate scenarios. Graduate students and postdoctoral associates will be trained in diverse scientific approaches and techniques such as shipboard sampling, laboratory experiments, genomic analyses and mathematical modeling. The results of the project will be incorporated into K-12 teaching, including an advanced placement environmental science class for underrepresented minorities in Los Angeles, data exercises for rural schools in Michigan and disseminated to the public through an environmental journalism institute based in Rhode Island.

Predicting how ecological communities will respond to a changing environment requires knowledge of genetic, phylogenetic and functional diversity within and across species. This project will investigate how the interaction of phylogenetic, genetic and functional diversity in thermal traits within and across a broad range of species determines the responses of marine phytoplankton communities to rising temperature and changing nutrient regimes. High genetic and functional diversity within a species may allow evolutionary adaptation of that species to warming. If the phylogenetic and functional diversity is higher across species, species sorting and ecological community reorganization is likely. Different marine sites may have a different balance of genetic and functional diversity within and across species and, thus, different contribution of evolutionary and ecological responses to changing climate. The research will be conducted at two long-term time series sites in the Atlantic Ocean, the Narragansett Bay Long-Term Plankton Time Series and the Bermuda Atlantic Time Series (BATS) station. The goal is to assess intra- and inter-specific genetic and functional diversity in thermal responses at contrasting nutrient concentrations for a representative range of species in communities at the two sites in

different seasons, and use this information to parameterize eco-evolutionary models embedded into biogeochemical ocean models to predict responses of phytoplankton communities to projected rising temperatures under realistic nutrient conditions. Model predictions will be informed by and tested with field data, including the long-term data series available for both sites and in community temperature manipulation experiments. This project will provide novel information on existing intraspecific genetic and functional thermal diversity for many ecologically and biogeochemically important phytoplankton species, estimate generation of new genetic and functional diversity in evolution experiments, and develop and parameterize novel eco-evolutionary models interfaced with ocean biogeochemical models to predict future phytoplankton community structure. The project will also characterize the interaction of two major global change stressors, warming and changing nutrient concentrations, as they affect phytoplankton diversity at functional, genetic, and phylogenetic levels. In addition, the project will develop novel modeling methodology that will be broadly applicable to understanding how other types of complex ecological communities may adapt to a rapidly warming world.

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# **Program Information**

Dimensions of Biodiversity (Dimensions of Biodiversity)

Website: <a href="http://www.nsf.gov/funding/pgm\_summ.jsp?pims\_id=503446">http://www.nsf.gov/funding/pgm\_summ.jsp?pims\_id=503446</a>

Coverage: global

(adapted from the NSF Synopsis of Program)

Dimensions of Biodiversity is a program solicitation from the NSF Directorate for Biological Sciences. FY 2010 was year one of the program. [MORE from NSF]

The NSF Dimensions of Biodiversity program seeks to characterize biodiversity on Earth by using integrative, innovative approaches to fill rapidly the most substantial gaps in our understanding. The program will take a broad view of biodiversity, and in its initial phase will focus on the integration of genetic, taxonomic, and functional dimensions of biodiversity. Project investigators are encouraged to integrate these three dimensions to understand the interactions and feedbacks among them. While this focus complements several core NSF programs, it differs by requiring that multiple dimensions of biodiversity be addressed simultaneously, to understand the roles of biodiversity in critical ecological and evolutionary processes.

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# **Funding**

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1638958
NSF Division of Ocean Sciences (NSF OCE)	OCE-1638804
NSF Division of Ocean Sciences (NSF OCE)	OCE-1638834

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