Diatom growth rates from samples collected on the Gould cruise LMG1411 in the Western Antarctica Peninsula from 2014 (Polar Transcriptomes project)

Website: https://www.bco-dmo.org/dataset/666201
Data Type: experimental
Version: 1
Version Date: 2016-11-28

Project
» Iron and Light Limitation in Ecologically Important Polar Diatoms: Comparative Transcriptomics and Development of Molecular Indicators (Polar_Transcriptomes)

Contributors	Affiliation	Role
Marchetti, Adrian	University of North Carolina at Chapel Hill (UNC-Chapel Hill)	Principal Investigator, Contact
Ake, Hannah	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
Diatom growth rates from samples collected on the Gould cruise LMG1411 in the Western Antarctica Peninsula from 2014 (Polar Transcriptomes project)

Table of Contents

- Coverage
- Dataset Description
  - Methods & Sampling
  - Data Processing Description
- Data Files
- Parameters
- Instruments
- Deployments
- Project Information
- Funding

Coverage

Temporal Extent: 2014 - 2014

Dataset Description

Diatom growth rates from samples obtained on LMG1411.

Diatom isolates were obtained from the Western Antarctic Peninsula surface waters.

Methods & Sampling

Nine species of diatoms were isolated from the Western Antarctic Peninsula along the PalmerLTER sampling grid in 2013 and 2014. Isolations were performed using an Olympus CKX41 inverted microscope by single cell isolation with a micropipette (Anderson 2005). Diatom species were identified by morphological characterization and 18S rRNA gene (rDNA) sequencing. DNA was extracted with the DNeasy Plant Mini Kit according to the manufacturer's protocols (Qiagen). Amplification of the nuclear 18S rDNA region was achieved with standard PCR protocols using eukaryotic-specific, universal 18S forward and reverse primers. Primer sequences were obtained from Medlin et al. (1982). The length of the region amplified is approximately 1800 base pairs (bp). Pseudo-nitzschia species are often difficult to identify by their 18S rDNA sequence, therefore, additional support of the taxonomic identification of P. subcurvata was provided through

sequencing of the 18S-ITS1-5.8S regions. Amplification of this region was performed with the 18SF-euk and 5.8SR_euk primers of Hubbard et al. (2008). PCR products were purified using either QIAquick PCR Purification Kit (Qiagen) or ExoSAP-IT (Affymetrix) and sequenced by Sanger DNA sequencing (Genewiz). Sequences were edited using Geneious Pro software (http://www.geneious.com, Kearse et al., 2012) and BLASTn sequence homology searches were performed against the NCBI nucleotide non-redundant (nr) database to determine species with a cutoff identity of 98%.

Diatom phylogenetic analysis was performed with Geneious Pro and included 71 additional diatom 18S rDNA sequences from publically available genomes and transcriptomes, including those in the MMETSP database. Diatom sequences were trimmed to the same length and aligned with MUSCLE (Edgar 2004). A phylogenetic tree was created in Mega with the Maximum-likelihood method of tree reconstruction, the Jukes-Cantor genetic distance model (Jukes and Cantor 1969), and 100 bootstrap replicates.

Isolates were maintained at 4 deg C in constant irradiance at intensities of either 10 umol photons m-2 s-1 (low light) or 90 umol photons m-2 s-1 (growth saturating light) and with media containing high and low iron concentrations. Cultures were grown in the synthetic seawater medium, AQUIL, enriched with filter sterilized vitamin and trace metal ion buffer containing 100 umol L-1 EDTA. The growth media also contained 300 µmol L-1 nitrate, 200 umol L-1 silicic acid and 20 umol L-1 phosphate. Premixed Fe-EDTA (1:1) was added separately for total iron concentrations of either 1370 nmol L-1 or 3.1 nmol L-1. Cultures were grown in acid-washed 28 mL polycarbonate centrifuge tubes (Nalgene) and maintained in exponential phase by dilution. Specific growth rates of successive transfers were calculated from the linear regression of the natural log of in vivo chlorophyll a fluorescence using a Turner 10-AU fluorometer (Brand et al. 1981).

Statistical analyses of growth rates and photophysiological data were performed with SigmaPlot 12.5 (SysStat Software Inc.). To test for significant differences between treatments, Two-Way Analysis of Variance (ANOVA) was performed with a significance level set to $p<0.05$. ANOVA also tests for normality using Shapiro-Wilks and Equal Variance tests. Because ANOVA does not test all interactions, an unpaired t-test was performed between –FeLL and +FeSL for u, Fv:Fm, and oPSII. All tests passed the Shapiro-Wilks Normality tests unless otherwise stated, in which case p-values are representative of the Mann-Whitney Rank Sum test. Post-hoc Tukey tests were also performed in order to determine which treatments differed significantly ($p < 0.05$).

Cultures for high throughput sequencing of mRNA were grown in acid-washed 2L polycarbonate bottles in iron-replete conditions under growth-saturating light (90 umol photons m-2 s-1). After reaching late exponential/early stationary phase, cultures were harvested onto polycarbonate filters (3.0 um pore size, 25 mm) and stored at -80 deg C. Total RNA was extracted using the RNAqueous 4PCR Kit (Ambion) according to the manufacturer's protocols. Residual genomic DNA was eliminated by DNAseI digestion at 37 deg C for 45 min. An Agilent Bioanalyzer 2100 was used to determine RNA integrity. mRNA libraries were generated with ~2 ug of total RNA and prepared with the Illumina TruSeq Stranded mRNA Library Preparation Kit. Samples were individually barcoded and pooled prior to sequencing on the Illumina MiSeq platform at the High Throughput Sequencing Facility (HTSF) at UNC-Chapel Hill. Sequencing resulted in approximately 0.7-2 million paired-end reads of 2x300bp per sample.

Data Processing Description

BCO-DMO Data Processing Notes:

-reformatted column names to comply with BCO-DMO standards
-replaced spaces with underscores
-replaced blank cells with "nd"
-added a species column
-filled in blank column name

[table of contents | back to top]

Data Files

File
growth_rates.csv (Comma Separated Values (.csv), 3.72 KB) MD5:b2429ce298ac39ad935aabe5812041a5 Primary data file for dataset ID 666201

[table of contents | back to top]

Parameters

Parameter	Description	Units
species	Species analyzed	unitless
treatment	Treatment condition	unitless
mean_specific_u	Average growth rate in a specific treatment	d -1
relative_u	Relative growth rate	unitless
std_error_u	Standard error of relative growth rate	unitless
propogation_error_u	Relative growth rate error	unitless
sample_size_u	Number of samples recorded	unitless
mean_FvFm	Average photosynthetic efficiency	unitless
relative_FvFm	Relative photosynthetic efficiency	unitless
std_error_FvFm	Relative photosynthetic efficiency standard error	unitless
propogation_error_FvFm	Relative photosynthetic efficiency error	unitless
sample_size_FvFm	Relative photosynthetic efficiency number of samples recorded	unitless
mean_sigma	Average functional absorption cross-section of PSII	A2 quanta -1
relative_sigma	Relative function absorption cross-section of PSII	unitless
std_error_sigma	Functional absorption cross-section of PSII standard error	unitless
propogation_error_sigma	Functional absorption cross-section of PSII error	unitless
sample_size_sigma	Functional absorption cross-section of PSII number of samples recorded	unitless

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	Agilent Bioanalyzer 2100
Generic Instrument Name	Bioanalyzer
Dataset-specific Description	Used to determine RNA integrity
Generic Instrument Description	A Bioanalyzer is a laboratory instrument that provides the sizing and quantification of DNA, RNA, and proteins. One example is the Agilent Bioanalyzer 2100.

Dataset-specific Instrument Name	Turner 10-AU
Generic Instrument Name	Fluorometer
Dataset-specific Description	Used to determine cell growth rates
Generic Instrument Description	A fluorometer or fluorimeter is a device used to measure parameters of fluorescence: its intensity and wavelength distribution of emission spectrum after excitation by a certain spectrum of light. The instrument is designed to measure the amount of stimulated electromagnetic radiation produced by pulses of electromagnetic radiation emitted into a water sample or in situ.

Dataset-specific Instrument Name	Olympus CKX41
Generic Instrument Name	Inverted Microscope
Dataset-specific Description	Used to perform isolations
Generic Instrument Description	An inverted microscope is a microscope with its light source and condenser on the top, above the stage pointing down, while the objectives and turret are below the stage pointing up. It was invented in 1850 by J. Lawrence Smith, a faculty member of Tulane University (then named the Medical College of Louisiana). Inverted microscopes are useful for observing living cells or organisms at the bottom of a large container (e.g. a tissue culture flask) under more natural conditions than on a glass slide, as is the case with a conventional microscope. Inverted microscopes are also used in micromanipulation applications where space above the specimen is required for manipulator mechanisms and the microtools they hold, and in metallurgical applications where polished samples can be placed on top of the stage and viewed from underneath using reflecting objectives. The stage on an inverted microscope is usually fixed, and focus is adjusted by moving the objective lens along a vertical axis to bring it closer to or further from the specimen. The focus mechanism typically has a dual concentric knob for coarse and fine adjustment. Depending on the size of the microscope, four to six objective lenses of different magnifications may be fitted to a rotating turret known as a nosepiece. These microscopes may also be fitted with accessories for fitting still and video cameras, fluorescence illumination, confocal scanning and many other applications.

[table of contents | back to top]

Deployments

LMG1401

Website	https://www.bco-dmo.org/deployment/675566
Platform	ARSV Laurence M. Gould
Start Date	2014-11-27
End Date	2014-12-21

[table of contents | back to top]

Project Information

Iron and Light Limitation in Ecologically Important Polar Diatoms: Comparative Transcriptomics and Development of Molecular Indicators (Polar_Transcriptomes)

Website: http://www.nsf.gov/awardsearch/showAward?AWD_ID=1341479

Coverage: Antarctica

The Southern Ocean surrounding Antarctica is changing rapidly in response to Earth's warming climate. These changes will undoubtedly influence communities of primary producers (the organisms at the base of the food chain, particularly plant-like organisms using sunlight for energy) by altering conditions that influence their growth and composition. Because primary producers such as phytoplankton play an important role in global biogeochemical cycling, it is essential to understand how they will respond to changes in their environment. The growth of phytoplankton in certain regions of the Southern Ocean is constrained by steep gradients in chemical and physical properties that vary in both space and time. Light and iron have been identified as key variables influencing phytoplankton abundance and distribution within Antarctic waters. Microscopic algae known as diatoms are dominant members of the phytoplankton and sea ice communities, accounting for significant proportions of primary production. The overall objective of this project is to identify the molecular bases for the physiological responses of polar diatoms to varying light and iron conditions. The project should provide a means of evaluating the extent these factors regulate diatom growth and influence net community productivity in Antarctic waters. The project will also further the NSF goals of making scientific discoveries available to the general public and of training new generations of scientists. It will facilitate the teaching and learning of polar-related topics by translating the research objectives into readily accessible educational materials for middle-school students. This project will also provide funding to enable a graduate student and several undergraduate students to be trained in the techniques and perspectives of modern biology.

Although numerous studies have investigated how polar diatoms are affected by varying light and iron, the cellular mechanisms leading to their distinct physiological responses remain unknown. Using comparative transcriptomics, the expression patterns of key genes and metabolic pathways in several ecologically important polar diatoms recently isolated from Antarctic waters and grown under varying iron and irradiance conditions will be examined. In addition, molecular indicators for iron and light limitation will be developed within these polar diatoms through the identification of iron- and light-responsive genes -- the expression patterns of which can be used to determine their physiological status. Upon verification in laboratory cultures, these indicators will be utilized by way of metatranscriptomic sequencing to examine iron and light limitation in natural diatom assemblages collected along environmental gradients in Western Antarctic Peninsula waters. In order to fully understand the role phytoplankton play in Southern Ocean biogeochemical cycles, dependable methods that provide a means of elucidating the physiological status of phytoplankton at any given time and location are essential.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Office of Polar Programs (formerly NSF PLR) (NSF OPP)	PLR-1341479

[table of contents | back to top]