

Presence and absence of iron and light-related functional genes collected on the Gould (LMG1411) cruise in the Western Antarctica Peninsula during 2014 (Polar Transcriptomes project)

Website: <https://www.bco-dmo.org/dataset/665407>

Data Type: experimental

Version: 1

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Project

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

Contributors	Affiliation	Role
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Abstract

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Coverage

Temporal Extent: 2014 - 2014

Dataset Description

Presence absence of iron and light-related functional genes. Protein information obtained from samples collected on LMG1411.

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

Acquisition Description

Nine species of diatoms were isolated from the Western Antarctic Peninsula along the Palmer LTER 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 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (low light) or 90 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (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 $\mu\text{mol L}^{-1}$ EDTA. The growth media also contained 300 $\mu\text{mol L}^{-1}$ nitrate, 200 $\mu\text{mol L}^{-1}$ silicic acid and 20 $\mu\text{mol L}^{-1}$ 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).

Photophysiological parameters were measured with a Fluorescence Induction Relaxation System (FIRe) (Satatlantic). Samples were dark acclimated for at least 10 minutes and measurements were taken of each culture for photosynthetic efficiency (Fv:Fm), and functional absorption cross-section of PSII (oPSII [A2 quanta⁻¹]). FIRe parameters were set to measure single turnover flash of PSII reaction centers (single closure event) with a sample delay of 100, and a total of 50 samples (Gorbunov and Falkowski 2004).

Processing Description

BCO-DMO Data Processing Notes:

- reformatted column names to comply with BCO-DMO standards
- replaced spaces with underscores
- added names to columns that were unnamed
- pivoted table so that species became its own column
- added accession links

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Parameters

Parameter	Description	Units
species	Species analyzed	unitless
description	Category of genes of interest	unitless
protein	Gene name	unitless
RPKM	Presence of a gene is denoted with semi-qualitative RPKM (Reads Per Kilobase of transcript per Million) values	unitless
evaluate	E-values	unitless
KO_num	Listed for genes that had a homolog in the KEGG database	unitless
accession_link	Accession link for K0 number	unitless

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Instruments

Dataset-specific Instrument Name	Olympus CKX41
Generic Instrument Name	Inverted Microscope
Dataset-specific Description	Used to perform isolations
Generic Instrument Description	<p>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.</p>

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.

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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

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.

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	PLR-1341479