Results from trace metal controlled diel microarray experiments with diatoms carried out in the Kustka and Allen labs at Rutgers in Newark, NJ from 2007-2011

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Data Type: experimental
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Project
» Expression profiling and functional genomics of a pennate diatom: Mechanisms of iron acquisition, stress acclimation, and recovery (Pennate Diatom Genomics)

Contributors	Affiliation	Role
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Abstract
Results from trace metal controlled diel microarray experiments with diatoms carried out in the Kustka and Allen labs at Rutgers in Newark, NJ from 2007-2011. Ten diel microarray experiments were completed (seven for P. tricornutum encompassing three Fe concentrations, and three for T. pseudonana encompassing two Fe concentrations).

Table of Contents

Dataset Description

Ten diel microarray experiments were completed (seven for *P. tricornutum* encompassing three Fe concentrations, and three for *T. pseudonana* encompassing two Fe concentrations).

Methods & Sampling

The following description is from a final report for an NSF OCE award provided by the dataset contact.

As biomass increases, steady state Fe prime invariably changes; this was minimized to less than 5% under all Fe conditions by making modifications to the Aquil recipe. In addition, excursions in media pH (which also change Fe' prime) were minimized by acclimating cells to pH buffer (EPPS) treated with chelex-100 resin.

A maximum of 750 pmol/L Fe prime is assumed because of Fe hydroxide precipitation, but for simplicity, the investigators report the results as Fe prime even at concentrations exceeding this maximum. Steady-state uptake rates for several diatoms continue to increase with increasing total Fe within the region of Fe hydroxide precipitation (Sunda and Huntsman 1995; Mar Chem 50:189).

In *T. pseudonana* experiment E, addition of Fe to remaining low Fe cultures after diel sampling resulted in an increase in growth rate after 24 h, confirming growth rate limitation by Fe. The similar Fv/Fm values under low

and high Fe are consistent with the data of Price 2005 (Limnol. Oceanogr. 50:1159).

In *P. tricornutum* experiment C, the pH buffer stock solution was not properly buffered before use in cultures. While these particular experiments will need to be repeated, these samples may be useful for comparing the diatom transcriptomes during holocene and anthropocene conditions, as these pH values are consistent with predicted values for 2100 (Gruber et al. 1996 Global Biogeochemical Cycles).

Data Processing Description

PI-provided parameter names were modified to conform with BCO-DMO conventions.

[table of contents | back to top]

Data Files

File	
trace_metal_microarray.csv	(Comma Separated Values (.csv), 593 bytes) MD5:6d0c6b64ba46d655815f8e154ca94b47
Primary data file for dataset ID 3667	

[table of contents | back to top]

Parameters

Parameter	Description	Units
species	Name of the diatom species.	dimensionless
exp_id	Identifier for the experiment; originally named 'cluster'.	dimensionless
fe_prime	Fe prime is the sum of all concentrations of Fe not bound to EDTA species. It is calculated according to thermodynamic equilibrium constants and photochemical reduction rate constants for FeEDTA species.	pMol
growth_rate	Cell specific growth rate (per day), calculated as the slope of the linear regression between ln (cell density) versus time. Originally named 'mu'.	ln(cell density)/day
growth_rate_se	Standard error of growth_rate.	dimensionless
Fv_to_Fm	Also referred to as (delta F)/Fm; Fv_to_Fm is the variable to maximum chlorophyll fluorescence, explicitly calculated as [F(knot)-F(maximum)]/F(maximum) and measured using the DCMU method.	dimensionless
Fv_to_Fm_sd	Standard deviation of Fv_to_Fm.	dimensionless
pH	pH of buffer solution.	pH scale
pH_sd	Standard deviation of the pH.	dimensionless

[table of contents | back to top]

Deployments

lab_Kustka_Allen

Website	https://www.bco-dmo.org/deployment/58828
Platform	Rutgers_Newark
Start Date	2007-09-01
End Date	2011-08-01
Description	Research for the project 'Expression profiling and functional genomics of a pennate diatom: Mechanisms of iron acquisition, stress acclimation, and recovery' was conducted at Dr. Kustka's lab at the Rutgers-Newark campus: 101 Warren Street, Smith Hall Room 140 Newark, New Jersey, 07102

[table of contents | back to top]

Project Information

Expression profiling and functional genomics of a pennate diatom: Mechanisms of iron acquisition, stress acclimation, and recovery (Pennate Diatom Genomics)

Abstract:
Iron (Fe) availability plays an increasingly well known role regulating the fate of upwelled nitrate and determining the size structure and community composition of phytoplankton assemblages in the ocean. All Fe enrichment experiments conducted to date have reported increases in the biomass and photosynthetic capacity of diatoms. Mounting evidence from field experiments, detailed physiological investigation, and genomic sequence data suggest fundamental differences in Fe bioavailability and uptake mechanisms, storage capacity, and stress recovery between pennate and centric diatoms. Pennate diatoms often dominate the phytoplankton assemblage after mesoscale Fe addition experiments because, in part, they are able to maintain cell viability during long periods of chronic Fe stress. The underlying molecular bases for these adaptations are virtually unknown. Preliminary primary metabolite data of Fe-limited *P. tricornutum* suggest that metabolic reconfigurations are necessary to meet increased demand for Fe-stress metabolites such as those involved in defense from reactive oxygen species (ROS) and intracellular metal chelation. Cellular nitrogen (N) status, and the accumulation of glutamate in particular, appears likely to play a primary role in recovery from Fe stress. This project capitalizes on the extremely well annotated *Phaeodactylum tricornutum* genome sequence to characterize global patterns of gene expression in response to shifts into and out of Fe and N stress and over the course of the diel cycle. The primary goal is to determine the molecular and physiological processes that constrain and define different phases and levels of Fe-stress acclimation. Oceanic physiological regimes have recently been defined according to different combinations of Fe and N availability and physiological indicators of the resident phytoplankton. This research will provide molecular-level insights into defense, acclimation, and regulatory mechanisms and pathways that govern survival strategies in situations of oceanographically-relevant stress and thus are of major ecological and biogeochemical consequence. Preliminary EST and partial genome microarray data, for example, indicate that chaperones and proteases play a significant role in monitoring cellular health and balancing the difference between investment in defense or activation of programmed cell death (PCD).

The proposed research will provide insights into the regulation of this fascinating and delicate balance. Such basic cellular processes play an important biogeochemical role in controlling bloom dynamics and regulating particle flux. Analysis of global gene expression will be compared with state of the art monitoring of intracellular metal levels and primary metabolite profiles using ICP-MS and gas chromatograph-mass spectroscopy (GC-MS) to determine the factors that determine cell survivability. The combination of global gene expression profiling and analysis of intracellular metal and metabolite pools will supply, for the first time, a holistic picture of the global cellular response of a marine pennate diatom to Fe-stress. *P. tricornutum* transcriptome profiles resulting from exposure to Fe - hydroxamate siderophores and heme-bound Fe (two classes of Fe binding ligands that are believed to comprise two major components of Fe in seawater) will be evaluated to understand the network of genes involved in recognizing and assimilating these compounds. An advanced reverse-genetics system for manipulating levels of gene expression in *P. tricornutum* will be used to evaluate the specific role of particular genes and pathways in facilitating Fe stress acclimation.

Broader Impacts: This research integrates important current themes in biogeochemistry, microbial ecology, marine sciences, and genome biology and will provide insight into factors that control the distribution and nutrient biogeochemistry of diatoms. By partnering with Affymetrix, through their Microbiology Program, a diatom microarray resource will be made available for the first time for open purchase and use. As part of the proposed research, a high school teacher from one of the local school systems with large underrepresented student populations will be recruited to work on a related topic. Upon completion of his/her paid internship, the teacher will design a classroom activity for use the following school year. As a further point of dissemination, the activity will be incorporated into a curriculum installment focused on marine and phytoplankton genomics for an existing mobile laboratory program called DISCOVER GENOMICS!, which interacts with middle school students in the Washington, D.C. Metropolitan area.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-0727997

[table of contents | back to top]