Experimental results: Fe limited diatoms were supplied with excess 57Fe and the new Fe was tracked to better understand storage mechanisms; conducted in the Kustka lab at Rutgers

Website: https://www.bco-dmo.org/dataset/516730
Version: 13 June 2014
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Project
» Iron storage in diatoms and N2 fixing cyanobacteria: mechanisms, regulation and biogeochemical significance (Mechanisms of Fe storage in phytoplankton)

Contributors	Affiliation	Role
Kustka, Adam	Rutgers University	Lead Principal Investigator
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Dataset Description

Status (June 2014): Data and metadata have been contributed to BCO-DMO. Data are restricted until April 2015. Manuscript submission is expected during the fall 2014.

Data contributed include 3 Excel files of ion intensities of 12C, 28Si, 31P, 56Fe, 40Ca and 57Fe within each region of interest as well as the Mathematica script written by A. Kustka used to conduct the Monte Carlo permutation.

Methods & Sampling

Experiments were conducted to probe the intracellular iron (Fe) distribution in *Thalassiosira pseudonana*. These experiments can be summarized as follows:

Three cells of *T. pseudonana* were analyzed for relative elemental contents at nanometer scale resolution using NanoSIMS mass spectrometry. These diatoms were first grown under low Fe reflecting natural abundance iron isotopic ratios and then spiked with excessive concentrations of Fe heavily enriched in 57Fe, in order to track the fate of newly incorporated 57Fe relative to pre-existing pools of 56Fe. The spatial distribution of this newly incorporated iron was evaluated using Geary's C statistic of spatial correlation, and significant deviations from random were established by a Monte Carlo permutation experiment.

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Parameters

Parameter	Description	Units
diatom	Number identifying the diatom studied.	integer
ROI_num	Identification number for the region of interest (ROI).	integer
C12	Ion intensity of Carbon-12 (12C).	dimensionless
Si28	Ion intensity of Silicon-28 (28Si).	dimensionless
P31	Ion intensity of Phosphorus-31 (31P).	dimensionless
Fe56	Ion intensity of Iron-56 (56Fe).	dimensionless
Ca40	Ion intensity of Calcium-40 (40Ca).	dimensionless
Fe57	Ion intensity of Iron-57 (57Fe).	dimensionless
ROI_area	Area of the region of interest (ROI).	microns^2
ROI_diam	Diamter of the region of interest (ROI).	microns
ROI_X	?	?
ROI_Y	?	?
ROI_XSTG	X stage coordinate on the nanoSIMS microscope.	dimensionless
ROI_YSTG	Y stage coordinate on the nanoSIMS microscope.	dimensionless

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Instruments

Dataset-specific Instrument Name	NanoSIMS mass spectrometry
Generic Instrument Name	Mass Spectrometer
Dataset-specific Description	Three cells of T. pseudonana were analyzed for relative elemental contents at nanometer scale resolution using Nanoscale Secondary Ion Mass Spectrometry (NanoSIMS). Secondary Ion Mass Spectrometry (SIMS) is a surface analysis technique that provides information about the lateral distribution of any element and its isotopes, and quantitative information about the isotopic composition of a sample. NanoSIMS allows for elemental and isotopic analysis down to the 50-nanomenter scale.
Generic Instrument Description	General term for instruments used to measure the mass-to-charge ratio of ions; generally used to find the composition of a sample by generating a mass spectrum representing the masses of sample components.

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Deployments

lab_Kustka

Website	https://www.bco-dmo.org/deployment/474414
Platform	Rutgers_Newark
Start Date	2007-09-01
End Date	2013-08-31
Description	Laboratory-based research for the projects "A Matter of Life or Death? Assessing the physiological roles of PCD-related genes to stress adaptation in diatoms" and "Iron storage in diatoms and N2 fixing cyanobacteria: mechanisms, regulation and biogeochemical significance" were conducted at Dr. Kustka's lab at the Rutgers-Newark campus: 101 Warren Street, Smith Hall Room 140 Newark, New Jersey, 07102

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Project Information

Iron storage in diatoms and N2 fixing cyanobacteria: mechanisms, regulation and biogeochemical significance (Mechanisms of Fe storage in phytoplankton)

From NSF Award Abstract:
Most studies on the Fe physiology of phytoplankton have focused on the induction of high affinity uptake pathways or the rearrangement of photosynthetic machinery to decrease cellular demand. By contrast, little attention has been given to the mechanisms of intracellular Fe storage. Proper handling and storage of Fe on timescales of generations can ensure adequate Fe nutrition in episodic environments. Furthermore short term storage of Fe is essential to "buffer" the intracellular redox-labile Fe concentration and prevent Fenton production of reactive oxygen species. Even though sufficient Fe can be stored for at least 4 cell divisions, much more than in the cases of P, N and (especially) C, our understanding of Fe storage lags far behind what is known for those elements. Since the biogeochemical cycles of Fe and C, N and P are linked via the Fe quotas of phytoplankton, it is critical that we understand the environmental and physiological controls of Fe storage. Fe can be stored in proteins such as those of the ferritin superfamily or sequestered into intracellular vacuoles. Some marine diatoms, such as Phaeodactylum tricornutum have ferritin genes. However ferritin has not been detected bioinformatically or by evolutionary PCR methods in other diatoms such as Thalassiosira pseudonana.

The investigators have measured the Fe-dependent regulation of transcript and protein abundance of NRAMP, a protein likely involved in vacuolar Fe metabolism, an alternative method of Fe storage found in Arabidopsis thaliana and yeast. It is proposed that the regulation and biogeochemical significance of ferritin and vacuole-mediated Fe storage may differ for different diatom groups. The filamentous N2 fixing cyanobacterium, Trichodesmium erythraeum, possesses three ferritin/ bacterioferritin genes, suggesting specialization of these proteins. Both Fe storage and Fe buffering are likely critical functions in Trichodesmium, yet nothing is known of either aspect of Fe homeostasis. This project aims to elucidate intracellular cycling and storage of Fe in marine diatoms and N2 fixing cyanobacteria and the relationship between Fe storage and cell quota. Specific objectives are to:

1) Determine the factors that regulate ferritin transcription, apo-protein synthesis and ferritin iron content in P. tricornutum lab cultures. The underlying hypothesis is that ferritins serve as Fe storage reservoirs over long generational time scales. Because they are targeted to chloroplasts, ferritins may also buffer Fe to prevent oxidative stress during degradation and synthesis of photosynthetic components.

2) Determine the role of storage vacuoles and NRAMP in Fe storage and mobilization in lab cultures of T. pseudonana, based on the hypothesis that vacuoles store Fe and NRAMP helps mobilize Fe in T. pseudonana, T. oceanica, and possibly other centric diatoms.

3) Evaluate the relationships between Fe storage proteins and cellular quota in culture and field populations of Trichodesmium; it is proposed that one or more of these proteins serve as Fe reservoir over long generational, time scales, in which case they may indicate nutritional Fe status. It is hypothesized that one or more of these proteins are co-localized in cells specifically responsible for N2 fixation in Trichodesmium colonies as a mechanism to buffer the Fe released through the diel degradation of the Fe-rich nitrogenase proteins.

The above objectives will be addressed using genetic, immunological, and synchrotron-based approaches applied to laboratory cultures of P. tricornutum, T. pseudonana,and Trichodesmium. Trichodesmium trichomes collected from the Sargasso Sea will also be analyzed to determine the biogeochemical importance of (bacterio)ferritins as a storage mechanism in this group.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-0727889

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