Q incubation data from the R/V Melville (MV1405) cruise along the California coast during July 2014

Website: https://www.bco-dmo.org/dataset/663689
Data Type: Cruise Results
Version: 1
Version Date: 2016-10-28

Project
» Collaborative Research: Investigating the Ecological Importance of Iron Storage in Diatoms (Diatom Iron Storage)

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

Abstract
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Coverage

Spatial Extent: **N**:42.4 **E**:-121.44 **S**:35.56 **W**:-125
Temporal Extent: 2014 - 2014

Dataset Description

Water was collected from 4 different locations and incubation experiments were performed, with 22 incubations total per site. The data details physiological parameters associated with each incubation sample (Q ID).

Methods & Sampling

Methodology from the IRNBRU cruise report

During the IRNBRU (MV1405) cruise, multiple incubation experiments were performed at different locations in relation to varying iron environments, including regions of high iron, upwelled waters as well as relatively low iron waters. Incubated phytoplankton community response was assessed using physiological and molecular methods in order to examine the effects of varying biogeochemical environments on metabolism and gene expression. Each experiment contained several treatments and time points consisting of the following: a 5 nmol L-1 FeCl3 addition, a 200 nmol L-1 desferroxamine B (DFB) addition, an initial 5 nmol L-1 FeCl3 addition followed by a 200 nmol L-1 DFB addition during the midpoint of the experiment, and an unamended control.

For three of the experiments (sites 1, 2, and 4), seawater was collected from the near surface (2-5m) using a trace-metal clean sampling system which consisted of a towed GeoFish sampler attached to KevlarTM line, PFA Teflon tubing and a Teflon dual-diaphragm pump that pumped seawater directly into a positive pressure trace-metal clean bubble constructed in the main laboratory of the ship. The seawater was placed into a large 55 gallon acid-cleaned HDPE drum for homogenization before being distributed into10L flexible acid-cleaned polyethylene cubitainers and placed in on-deck plexiglass incubators with flow-through seawater to maintain near-ambient surface temperatures. For the experiment at site 3, seawater was obtained from the depth corresponding to the 10 deg C isotherm (91 m) in order to simulate an upwelling event. Cleaning protocols for all cubitainers included soaking the inside walls in 1.2 mol L-1 hydrochloric acid (reagent grade) for 3 d followed by three rinses with Milli-Q H20, soaking in 1.2 mol L-1 hydrochloric acid (trace metal grade) for 1 week followed by three rinses with Milli-Q H20, and soaking in 0.1 mol L-1 acetic acid (trace-metal grade). Prior to filling the cubitainers with seawater, the dilute acetic acid was removed and the cubitainers were rinsed thoroughly three times with ambient, low-iron seawater. Incubators were covered with neutral density screening to reduce irradiance to ca. 30% of the incident. For sample collection from initial conditions, triplicate cubitainers were immediately filtered. All incubations were initiated and terminated just prior to dawn. Following 96 hours of incubation, the seawater was removed from the incubators and stored in a dark, cool room until filtration. Subsamples for dissolved nutrients, size-fractionated chlorophyll a ,Fv/Fm, particulate nutrients, nutrient uptake, biogenic silica, domoic acid, dissolved iron, and RNA were collected from each cubitainer. Methods performed on the ship for each measurement are briefly explained below.

Dissolved nitrate + nitrite (NO3- + NO2-), phosphate (PO43-), and silicic acid (H4SiO4) concentrations were measured shipboard using a Lachat Quick Chem 8000 Flow Injection Analysis system. Particles were removed by filtering the sample through a GF/F filter using a syringe prior to analysis. Reference materials for nutrients in seawater (Lots BY and CA, KANSO Technos, Osaka, Japan) were run alongside samples for quality control.

For biomass determination, 400 mL of seawater was gravity-filtered through a 5 um polycarbonate filter (47 mm) followed by a GF/F filter (25 mm) under gentle vacuum pressure (<100 mm Hg) using a series filter cascade for size fractionation. Filters were rinsed with 0.45 um filtered seawater and immediately frozen at -80 deg C until analysis. Chlorophyll a extraction was performed on the ship using 90% acetone at -20 deg C for 24 hours and measured via in vitro fluorometry using a Turner Designs 10-AU fluorometer.

In order to assess changes in photophysiology among treatments, the maximum photochemical yield of Photosystem II (Fv:Fm) was measured shipboard using a Satlantic FIRe. Before each measurement, a subsample (5 mL) of each culture was placed in the dark for 20 minutes. The resulting Fv:Fm was derived from the induction profile using a saturating pulse (20,000 umol photons m2 s-1) for a duration of 100-200 us.

Particulate nitrate (PN), carbon (PC) and nitrate (NO3-) uptake was obtained by spiking 500 mL of seawater with 15N-NH4Cl at no more than 10% of ambient nitrate concentration and incubated for 8 hours in the flow-through plexiglass incubator. Following incubation, seawater filtration commenced immediately and was performed by gravity through a 5 um polycarbonate filter (47 mm), and with an in-line vacuum (<100 mm Hg) onto a precombusted (450 deg C for 5 hours) GF/F filter (25 mm). Cells on the 5 um polycarbonate filter were then rinsed onto an additional precombusted GF/F filter (25 mm) using an artificial saline solution. Filters were then stored at -20 deg C. In the laboratory, filters were heated at 50 deg C for 24 hours, wrapped in tin capsules, and pelletized in preparation for analysis of the atom % 15N, PN, and PC using an elemental analyzer paired with an isotope ratio mass spectrometer (EA-IRMS).

For inorganic carbon uptake, or measurements of primary productivity, 60mL samples from each cubitainer were distributed into a light and dark bottles cleaned with 10% HCl. For each bottle, 1.2 uCi of NaH14CO3 was added. A 1 mL subsample was taken and added to vials containing NaOH. The light and dark bottles were incubated on-deck for 6.5-8 hours and stacked polycarbonate filters (5 um and 1 um) with a mesh spacer between them. The blanks were filtered onto a GF/F filter after 5 minutes. Filters were vacuumed dried, placed in scintillation vials with 0.5 mL of 6M HCl, permitted to degas for 24h, and counted using a Beckman Coulter LS 6500 scintillation counter.

Biogenic silica was determined shipboard via filtration of 335 mL onto 1.2 um polycarbonate filters (45 mm). Concentrations were measuring using a NaOH digestion in teflon tubes3 and a colorimetric ammonium molybdate method.

Samples for iron (Fe) analysis were acidified at sea with the equivalent of 4 mL 6N quartz-distilled HCl per liter of seawater (to pH 1.7-1.8) and stored in LDPE bottles that had been cleaned as per the GEOTRACES cookbook (http://www.geotraces.org/science/intercalibration/222-sampling-and-sample-handling-protocols-for-geotraces-cruises). Samples were analyzed by preconcentrating the Fe on 2 cm columns of Nobias chelate PA1 resin, and analyzing the eluent on the Thermo-Element high-resolution XR ICP-MS at UC Santa Cruz. The resin columns are rinsed and conditioned with 0.05 M NH4Ac buffer at pH 6.0, and the samples are buffered to

pH 6.0 with NH4Ac immediately before loading. The samples are eluted from the columns with ~1 mL of 1N quartz distilled HNO3. One to three days before preconcentration, samples were irradiated with UV light for 2 hours to ensure full recovery of other metals of interest.

Seawater for RNA analysis was filtered directly onto 0.8 um Pall Supor filters (142 mm) using a peristaltic pump. Filters were placed in cyrovials and immediately flash frozen in liquid nitrogen. Samples were later transferred to storage in -80 deg C freezers until RNA extractions were performed. Filters were briefly thawed on ice before being extracted individually using the ToTALLY RNA Total RNA Isolation Kit (Ambion). RNA-Seq library prep was conducted with the Illumina TruSeq Stranded mRNA Library Preparation Kit and HiSeq v4 reagents. Bioinformatic pipeline consisted of the following: trimming of reads for quality and removal of adapters, contig assemblies, read mapping for quantitative analysis, taxonomic and functional assignment, and differential gene expression determination.

Data Processing Description

BCO-DMO Data Processing Notes:

-reformatted column names to comply with BCO-DMO standards
-filled in blank cells with "nd"

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Data Files

File
Q_incubations.csv (Comma Separated Values (.csv), 9.50 KB) MD5:50f571a9710c63e88c9fa80685453def Primary data file for dataset ID 663689

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Parameters

Parameter	Description	Units
site	Numbered experiment site ID	unitless
lat	Latitude; N is positive	decimal degrees
lon	Longitude; E is positive	decimal degrees
Q_ID	Incubation sample ID	unitless
timepoint	Sampling timepoint in hours	hours
treatment	Incubation treatment; C Fe DFB or FeDFB	unitless
replicate	Replicate code; A B or C	unitless
NO3	Nitrate concentration	uM
PO4	Phosphate concentration	uM
SiO4	Silicate concentration	uM
Chl_greaterThan5um	Concentration of chloride over 5 um	micrograms per liter (ug/L)
Chl_GFF	Concentration of chloride over 0.7 um	micrograms per liter (ug/L)
FvFm	Maximum quantum yield	dimensionless
relative_sigma	Sigma t density	(kilograms/meters cubed) - 1000
biogenic_silica	Concentration of biogenic silica	uM
bSi_new	32Si silica production rate; bSi new	micromoles of silica per liter (umol Si/L)
rho	32Si silica production rate; rho	micromoles of silica per liter (umol Si/L)
Vb	32Si silica production rate; Vb	d -1
primaryProductivity_02um	Primary productivity greater than 0.2 um	moles of carbon per liter per hour (mol C/L/h)
primaryProductivity_1um	Primary productivity greater than 1 um	moles of carbon per liter per hour (mol C/L/h)
primaryProductivity_5um	Primary productivity greater than 5 um	moles of carbon per liter per hour (mol C/L/h)

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Instruments

Dataset-specific Instrument Name	LDPE bottles
Generic Instrument Name	Bottle
Dataset-specific Description	Contained seawater samples
Generic Instrument Description	A container, typically made of glass or plastic and with a narrow neck, used for storing drinks or other liquids.

Dataset-specific Instrument Name	Lachat Quick Chem 8000 Flow Injection Analysis system
Generic Instrument Name	Flow Injection Analyzer
Dataset-specific Description	Dissolved nitrate + nitrite (NO3- + NO2-), phosphate (PO43-), and silicic acid (H4SiO4) concentrations were measured shipboard.
Generic Instrument Description	An instrument that performs flow injection analysis. Flow injection analysis (FIA) is an approach to chemical analysis that is accomplished by injecting a plug of sample into a flowing carrier stream. FIA is an automated method in which a sample is injected into a continuous flow of a carrier solution that mixes with other continuously flowing solutions before reaching a detector. Precision is dramatically increased when FIA is used instead of manual injections and as a result very specific FIA systems have been developed for a wide array of analytical techniques.

Dataset-specific Instrument Name	Turner Designs 10-AU fluorometer
Generic Instrument Name	Fluorometer
Dataset-specific Description	Chlorophyll a extraction was performed on the ship using 90% acetone at -20 deg C for 24 hours and measured via in vitro fluorometry.
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	GeoFish Sampler
Generic Instrument Name	GeoFish Towed near-Surface Sampler
Dataset-specific Description	Used to collect seawater
Generic Instrument Description	The GeoFish towed sampler is a custom designed near surface (

Dataset-specific Instrument Name	EA-IRMS
Generic Instrument Name	Isotope-ratio Mass Spectrometer
Dataset-specific Description	Analysis of the atom % 15N, PN, and PC using an elemental analyzer paired with an isotope ratio mass spectrometer.
Generic Instrument Description	The Isotope-ratio Mass Spectrometer is a particular type of mass spectrometer used to measure the relative abundance of isotopes in a given sample (e.g. VG Prism II Isotope Ratio Mass-Spectrometer).

Dataset-specific Instrument Name	Beckman Coulter LS 6500 scintillation counter
Generic Instrument Name	Liquid Scintillation Counter
Dataset-specific Description	Scintillation vials with 0.5 mL of 6M HCl, permitted to degas for 24h were counted.
Generic Instrument Description	Liquid scintillation counting is an analytical technique which is defined by the incorporation of the radiolabeled analyte into uniform distribution with a liquid chemical medium capable of converting the kinetic energy of nuclear emissions into light energy. Although the liquid scintillation counter is a sophisticated laboratory counting system used the quantify the activity of particulate emitting (ß and a) radioactive samples, it can also detect the auger electrons emitted from 51Cr and 125I samples.

Dataset-specific Instrument Name	Teflon dual-diaphragm pump
Generic Instrument Name	Pump
Dataset-specific Description	Pumped seawater directly into a positive pressure trace-metal clean bubble constructed in the main laboratory of the ship.
Generic Instrument Description	A pump is a device that moves fluids (liquids or gases), or sometimes slurries, by mechanical action. Pumps can be classified into three major groups according to the method they use to move the fluid: direct lift, displacement, and gravity pumps

Dataset-specific Instrument Name	Flow-through plexiglass incubator
Generic Instrument Name	Shipboard Incubator
Dataset-specific Description	Particulate nitrate (PN), carbon (PC) and nitrate (NO3-) uptake was obtained by spiking 500 mL of seawater with 15N-NH4Cl at no more than 10% of ambient nitrate concentration and incubated for 8 hours.
Generic Instrument Description	A device mounted on a ship that holds water samples under conditions of controlled temperature or controlled temperature and illumination.

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Deployments

MV1405

Website	https://www.bco-dmo.org/deployment/559966
Platform	R/V Melville
Start Date	2014-07-03
End Date	2014-07-26
Description	Deployment MV1405 on R/V Melville. Cruise took place during July 2014.

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Project Information

Collaborative Research: Investigating the Ecological Importance of Iron Storage in Diatoms (Diatom Iron Storage)

Coverage: North Pacific, California coast and subarctic gyre

NSF Award Abstract:
Diatoms are responsible for a significant fraction of primary production in the ocean. They are associated with enhanced carbon export and usually dominate the response of phytoplankton to additions of the micronutrient iron in high-nutrient, low-chlorophyll (HNLC) regions. Diatoms, particularly those isolated from the open ocean, appear to have a significant capacity to store iron for later use, and in some groups of diatoms this ability is enabled by the iron storage protein ferritin. Such luxury uptake of iron has long been observed in laboratory cultures and hypothesized to provide diatoms with an ecological benefit in the low-iron waters that cover 40% of the global ocean. However iron storage has been difficult to observe in natural systems due to the methodological challenges of working with mixed plankton assemblages, and a physiological understanding of the impacts of iron on ocean diatoms is lacking. This project combines state-of-the-art high-throughput transcriptomic sequencing and single-cell element analysis with novel laboratory and field incubation experiments to quantify iron storage abilities of cultured and natural diatoms that either contain or lack ferritin and determine the ecological impacts of this process. The overall objective of this project is to examine the ecological importance of iron storage as a selective mechanism controlling the distributions of diatoms along iron gradients in marine ecosystems. The proposed research includes three specific objectives:

A. Determine if there is a consistent physiological difference in the ability of pennate versus centric diatoms to store iron.

B. Examine whether iron storage capacities across diverse diatom taxa consistently provide a mechanistic explanation for continued growth in the absence of iron.

C. Determine whether enhanced iron storage provides diatoms with a competitive within natural phytoplankton assemblages in both coastal and oceanic regions.

Transcriptomic sequencing on a variety of ecologically important pennate and centric diatoms will be used to survey for the presence of ferritin-like genes in order to establish biogeographical and/or phylogenetic patterns of occurrence of diatom ferritin. Laboratory culture experiments will be used to quantify the iron storage abilities of these diatoms, as well as the number of cell divisions that can be supported by the stored iron, providing valuable physiological data to inform the understanding of plankton ecology in iron-limited coastal and HNLC systems. The laboratory experiments will be complemented by measurements of ferritin expression and iron storage in coastal and ocean diatoms sampled across gradients of iron availability on two cruises-of-opportunity to the northeast Pacific Ocean.

The NCBI bioproject page can be found here.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1334935
NSF Division of Ocean Sciences (NSF OCE)	OCE-1334632

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