Data on phytoplankton physiology and iron and temperature interactions collected in the Ross Sea during 2013 (Ross Sea Microb Ecophys project)

Website: https://www.bco-dmo.org/dataset/628230
Data Type: experimental
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Project
» Synergistic Effects of Iron, Carbon Dioxide and Temperature on the Fate of Nitrate: Implications for Future Changes in Export Production in the Southern Ocean (Ross_Sea_Microb_Ecophys)

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Dataset Description

There are five species/strains of phytoplankton: *Pseudo-nitzschia subcurvata* LN, *Pseudo-nitzschia subcurvata* HN, *Chaetoceros* sp. LN, *Fragilariopsis cylindrus* HN, and *Phaeocystis antarctica* HN. The physiological response of these five species/strains to four treatments was investigated: 0°C-Fe limited (1nM), 0°C-Fe replete (500nM), 4°C-Fe limited (1nM), 4°C-Fe replete (500nM).

We report: carbon fixation rates, Fe uptake rates, Fe uptake rates to carbon fixation rates ratio; carbon quota, nitrogen quota, phosphorus quota, and silicate quota; cell volume, cell surface area to cell volume ratios; carbon quota to Chl a ratio, Chl a per cell; and Fv/Fm of *Pseudo-nitzschia subcurvata* LN and *Chaetoceros* sp. LN .

Methods & Sampling

Methodology:

Strains and growth conditions: Unialgal cultures of Pseudo-nitzschia subcurvata, Chaetoceros sp., Fragilariopsis cylindrus, and Phaeocystis antarctica were isolated from the ice edge in McMurdo Sound (77.62°S, 165.47°E) in the Ross Sea, Antarctica during January and February 2013. All stock cultures were maintained in 0.2 µM-filtered seawater that was collected using trace metal clean techniques from the same locale as the culture isolates (Hare et al. 2007, King et al. 2012). Cultures were grown at 0°C in a walk-in incubator under 24 hour (h) cold white fluorescence light (80 µmol photons m-2 s-1).

Experiments examined interactions between temperature and iron availability under four conditions: 0°C-Fe limited (+1nM Fe, abbreviated as 0C-Fe), 0°C-Fe replete (+500nM Fe, or 0C+Fe), 4°C-Fe limited (+1nM Fe, or 4C-Fe), and 4°C-Fe replete (+500nM Fe, or 4C+Fe). Fe concentration was amended by adding EDTA chelated FeCl3 (100: 1) into 0.2 µM-filtered trace metal-clean Ross Sea seawater. The seawater was collected late in the Antarctic summer, and so the concentrations of NO_3^-, PO_4^{3-} were relatively low for this region at 6.95 µmol L-1 and 0.66 µmol L-1 respectively. $Si(OH)_4$ and dissolved Fe concentrations were 52.91 µmol L-1, and 0.2 nmol L-1 respectively (Feng et al. 2010). Chaetoceros sp. and one strain of Pseudo-nitzschia subcurvata (P. subcurvata) were grown in this seawater medium without any added nutrients. Another isolate of P. subcurvata, Fragilariopsis cylindrus, and Phaeocystis antarctica were maintained in the same four conditions, but the seawater was enriched with chelexed nutrient stocks to 50 µmol L-1 NO_3^- and 10 µmol L-1 PO_4^{3-} to examine growth effects of these two variables at higher nutrient levels. Cultures grown at high and low major nutrient levels will be identified as HN and LN treatments, respectively.

Experimental cultures were grown in triplicate 500 ml acid washed polycarbonate bottles under 24 h irradiance (80 µmol photons m-2 s-1). Semi-continuous culturing methods were used, whereby the cultures were diluted every 2 days with medium pre-acclimated to their respective temperatures. Dilution rates were based on the individually calculated growth rate of each replicate bottle (see 'Growth rates' section below), allowing each bottle to reach its own steady state exponential growth rate. All of the cultures were acclimated to their respective environmental conditions for 8 weeks before the commencement of the experiment. After the growth rates remained stable for at least three to five consecutive transfers, indicating steady state growth had been attained, the cultures were sampled 48 h after dilution.

Growth rates: Ten ml aliquot of culture samples were taken for visual cell counts directly before and after each treatment was diluted. Cell count samples were preserved with 0.5% glutaraldehyde (final concentration) and stored at 4°C for subsequent counting on a hemacytometer using an Olympus BX51 microscope (Olympus, Japan). Due to poor preservation, cell count samples of P. antarctica at 4°C for phosphorus cell quotas and Chl a per cell calculations were lost. Specific growth rates, expressed as h-1, were calculated as: $\mu = (\ln N1 - \ln N0)/t$, where N0 and N1 are the cell density at the beginning and end of a dilution period, respectively, and t is the duration of the dilution period.

Elemental analysis: 50 ml and 20 ml culture samples of each treatment were filtered onto pre148 combusted GF/F filters (500°C for 2h) and dried in a 60°C oven overnight for particulate organic carbon/nitrogen (POC/PON) and particulate organic phosphorus (POP) analyses, respectively. POC/PON samples were analyzed using a 440 Elemental Analyzer (Costech Inc, Valencia, CA) following Fu et al. (2007) and Garcia et al. (2014). POP was analyzed using a molybdate colorimetric method according to Fu et al. (2007). A 20 ml aliquot of Chaetoceros sp. and P. subcurvata LN sample from each treatment was filtered onto 2 µm polycarbonate filters and dried in a 60°C oven overnight for biogenic silicate (BSi) analysis (Passche et al. 1973). Chlorophyll a analysis: 20 to 50 ml culture samples were filtered onto GF/F filters (Whatman) and extracted with 90% aqueous acetone for 24 h at -20°C, and measured using the non157 acidification method on a 10-AUTM fluorometer (Turner Designs, CA) (Fu et al. 2007). Cell volume and surface area: A minimum of 50 cells from each treatment were measured using an Olympus BX51 microscope (Olympus, Japan) with a coupled Excelis HD camera (ACCU8 SCOPE, NY). The length, height, or diameter of all cells were measured using ImageJ (NIH), and the volume and surface area of each cell was calculated following Hillebrand et al. (1999). Active fluorescence characteristics: A 6 ml aliquot of culture sample of P. subcurvata LN and Chaetoceros sp. LN from each treatment was dark-adapted for 5 min, and minimum fluorescence (F0) was measured using a 10-AUTM fluorometer (Turner Designs, CA). Next, maximum fluorescence (Fm) was recorded by adding 6 µl DCMU (dichloromethylurea) to each sample followed by shaking for 30 seconds. The quantum efficiency of photosystem II Fv/Fm was calculated according to the equation $Fv/Fm = (Fm – F0)/Fm$ (Schreiber 2004).

C fixation and Fe uptake rates: To measure carbon fixation rates and iron uptake rates, a 30 ml aliquot of culture sample from each treatment was incubated with 37 kBq 14C -bicarbonate (MP Biomedicals), or ~2 kBq 55FeCl3 (PerkinElmer, 0.33 nM 55FeCl3 complexed to 120 µmol L-1 EDTA) under their respective treatment conditions. Samples were filtered onto GF/F filters after 24 h incubation. For Fe uptake rate samples, the filters were washed in oxalate reagent for 5 min to remove surface-adsorbed Fe (Tovar-Sanchez et al. 2003). To correct for filter absorption of both radiotracers, the same amount of stock solution was added to a 30 ml aliquot of sample and filtered immediately; these filter absorption count values were subtracted from reported activities. The radioactivities of 14C and 55Fe in each sample were counted in a Tri-Carb 2500TR (Packard, now Perkin Elmer). Carbon fixation rates and Fe uptake rates were calculated using the initial dissolved inorganic carbon (DIC) concentrations and initial total Fe concentrations of each bottle (1nM Fe and 500 nM for iron limited and iron replete cultures, respectively), and were normalized to cell density (Garcia et al. 2014). Because 55Fe additions were a large fraction of the total Fe present in the Fe-limited samples, these uptake values represent an upper rate estimate for this treatment.

Statistical analysis: All statistical analyses, including student t-tests, ANOVA, Tukey's HSD test, and two-way ANOVA were conducted using the open source statistical software R version 3.1.2 (Systat Software, CA).

References:

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Garcia NS, Fu F, Sedwick PN, Hutchins DA (2014) Iron deficiency increases growth and nitrogen-fixation rates of phosphorus-deficient marine cyanobacteria. ISME J 2015(9): 238–245

Hare CE, DiTullio GR, Riseman SF, Crossley AC, Popels LC, Sedwick PN, Hutchins DA (2007) Effects of changing continuous iron input rates on a Southern Ocean algal assemblage. Deep-Sea Res I 54: 732-746

Hillebrand H, Dürselen CD, Kirschtel D, Pollingher U, Zohary T (1999) Biovolume calculation for pelagic and benthic microalgae. J Phycol 35(2): 403-424

King AL, Sañudo-Wilhelmy SA, Boyd PW, Twining BS, Wilhelm SW, Breene C, Ellwood MJ, Hutchins DA (2012) A comparison of biogenic iron quotas during a diatom spring bloom using multiple approaches. Biogeosciences 9: 667–687

Paasche E (1973) Silicon and the ecology of marine plankton diatoms. I. Thalassiosira pseudonana (Cyclotella nana) grown in a chemostat with silicate as limiting nutrient. Mar Bio 19(2): 117-126

Schreiber U (2004) Pulse-amplitude-modulation (PAM) fluorometry and saturation pulse method: an overview. In: Chlorophyll a Fluorescence, Springer Netherlands, p279-319

Tovar-Sanchez A, Sañudo-Wilhelmy SA, Garcia-Vargas M, Weaver RS, Popels LC, and others (2003) A trace metal clean reagent to remove surface-bound iron from marine phytoplankton. Marine Chemistry 82(1): 91-99.

Data Processing Description

BCO-DMO Processing:

- added conventional header with dataset name, PI name, version date, reference information
- renamed parameters to BCO-DMO standard
- reformatted data to flat file
- replaced spaces with underscores
- replaced special characters (+)

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Data Files

File
phyto_phys.csv (Comma Separated Values (.csv), 3.53 KB)   MD5:715d8209f97db356174a875fff2e32fe   Primary data file for dataset ID 628230

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Parameters

Parameter	Description	Units
species_strain	species and strain; whether grown in high (HN) or low nutrients (LN)	unitless
treatment	temperature and iron exposure	unitless
growth_rate	Specific growth rate	growth/day
growth_rate_sd	standard deviation of specific growth rate	growth/day
Cfix	Carbon fixation rate	pmol/cell/h
Cfix_sd	standard deviation of Carbon fixation rate	pmol/cell/h
Fe_uptake	Iron uptake rate	10-18mol/cell/h
Fe_uptake_sd	standard deviation of Iron uptake rate	10-18mol/cell/h
Fe_C	Iron to Carbon ratio	umol: mol
Fe_C_sd	standard deviation of Iron to Carbon ratio	umol: mol
POC	Particulate Organic Carbon	pmol/cell
POC_sd	standard deviation of POC	pmol/cell
PON	Particulate Organic Nitrogen	pmol/cell
PON_sd	standard deviation of PON	pmol/cell
POP	Particulate Organic Phosphate	pmol/cell
POP_sd	standard deviation of POP	pmol/cell
BSI	Biogenic silicate	pmol/cell
BSi_sd	standard deviation of Bsi	pmol/cell
Vol	cell volume	um^3
Vol_sd	standard deviation of cell volume	um^3
surf_area_vol	cell surface area/vVolume ratio	unitless
surf_area_vol_sd	standard deviation of cell surface area/volume ratio	unitless
POC_chla	POC: Chl-a ratio (g/g)	unitless
POC_chla_sd	standard deviation of POC: Chl-a ratio	unitless
chla_cell	Chl-a per cell	pg/cell
chla_cell_sd	standard deviation of Chl-a per cell	pg/cell
Fv_Fm	plant stress measured as Fv/Fm	unitless
Fv_Fm_sd	standard deviation of Fv/Fm	unitless

Instruments

Dataset-specific Instrument Name	
Generic Instrument Name	Camera
Dataset-specific Description	Excelis HD camera (ACCU8 SCOPE, NY)
Generic Instrument Description	All types of photographic equipment including stills, video, film and digital systems.

Dataset-specific Instrument Name	
Generic Instrument Name	Elemental Analyzer
Dataset-specific Description	440 Elemental Analyzer (Costech Inc, Valencia, CA)
Generic Instrument Description	Instruments that quantify carbon, nitrogen and sometimes other elements by combusting the sample at very high temperature and assaying the resulting gaseous oxides. Usually used for samples including organic material.

Dataset-specific Instrument Name	
Generic Instrument Name	Fluorometer
Dataset-specific Description	10AU fluorometer (Turner Designs, CA)
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	
Generic Instrument Name	Liquid Scintillation Counter
Dataset-specific Description	Tri-Carb 2500TR (Packard, now Perkin Elmer
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	
Generic Instrument Name	Microscope - Optical
Dataset-specific Description	Olympus BX51 microscope (Olympus, Japan)
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of reflection and absorption of visible light. Includes conventional and inverted instruments. Also called a "light microscope".

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Deployments

helicopter_Allen

Website	https://www.bco-dmo.org/deployment/628276
Platform	McMurdo Station
Start Date	2013-01-16
End Date	2015-01-23
Description	Water sample collections in the Ross Sea

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Project Information

Synergistic Effects of Iron, Carbon Dioxide and Temperature on the Fate of Nitrate: Implications for Future Changes in Export Production in the Southern Ocean (Ross_Sea_Microb_Ecophys)

Coverage: McMurdo Sound of the Ross Sea

Description from NSF award abstract:
This award provides support for "Collaborative Research: Synergistic Effects of Iron, Carbon Dioxide, and Temperature on the Fate of Nitrate: Implications for Future Changes in Export Production in the Southern Ocean" from the Antarctic Organisms and Ecosystems program in the Office of Polar Programs at NSF. The project will use a novel combination of research approaches to evaluate the effects of temperature, carbon dioxide, and iron on three ecologically- and biogeochemically-critical Southern Ocean phytoplankton functional groups: Large centric diatoms, small pennate diatoms, and Phaeocystis antarctica.

The Southern Ocean around Antarctic is undergoing several changes including increased temperature and carbon dioxide content, as well as changing levels of biologically available iron. The project goals are to understand how the individual and combined influences of these three variables affect Southern Ocean phytoplankton community structure, and to determine how these assemblage-level responses are linked to fundamental cellular responses at the levels of nutrient physiology and gene expression. The research team will focus on three different types of marine algae: large and small diatoms, and the prymnesiophyte, Phaeocystis antarctica. Shifts between these three major algal groups have very different consequences for nutrient and carbon biogeochemistry in the rapidly changing Antarctic marine environments. However, the mechanistic underpinnings of these environmentally-driven community shifts are not known. The project includes a US-based laboratory component with Antarctic isolates, field study at McMurdo Station, and then a cruise of

opportunity in the upwelling areas directly south of the Antarctic Circumpolar Current. The study also includes collection and analysis of environmental gene expression data, or meta-transcriptomics, both from the field and experimental settings. The transcriptomes will be generated under environmentally relevant conditions and will thus contain information critical for decoding the genomes of several newly sequenced polar phytoplankton species in addition to the three groups highlighted above.

Related publications:
Bertrand, E.M., McCrow, J.P., Moustafa, A., Zheng, H., McQuaid, J., Delmont, T., Post, A.F., Sipler, R., Spackeen, J.L., Xu, K., Bronk, D.A., Hutchins, D.A., Allen, A.E. 2015. Phytoplankton-bacterial interactions mediate micronutrient colimitation at the coastal Antarctic sea ice edge. Proceedings of the National Academy of Sciences. 10.1073/pnas.1501615112

Funding

Funding Source	Award
NSF Office of Polar Programs (formerly NSF PLR) (NSF OPP)	PLR-1043748