Response of Bathycoccus sp. to iron-limitation sampled aboard R/V Roger Revelle RR1813 in the Subarctic North Pacific near Station PAPA (50 N, 144.8 W) from August to September 2018

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Project
» Collaborative Research: Evaluating the contribution of small eukaryotes to nitrate-based new production in the North Pacific Subtropical Gyre (Small Euks)

Contributors	Affiliation	Role
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Abstract
Small eukaryotic phytoplankton can account for considerable amounts of biomass and primary production in high nutrient, low chlorophyll (HNLC) regions of the ocean where iron-limitation is pronounced. However, the strategies these cells invoke to cope under low iron conditions and the extent to which they are responsible for new production (i.e., the fraction of primary production supported by nutrients from outside of the euphotic zone) is unclear. Here, we examine how a representative picoeukaryote, the chlorophyte Bathycoccus sp., recently isolated from the iron-limited subarctic North Pacific Ocean, responds to iron-limitation and quantify its potential contribution to new production. Iron-limited Bathycoccus exhibits reductions in cellular growth rate, volume, and carbon and nitrogen quotas along with a restructuring of cellular metabolism as inferred through gene expression. Gene expression and pathway analyses show evidence of cellular strategies to mitigate iron limitation but conservation of genes related to nitrogen uptake and utilization. Additionally, when grown on nitrate, cellular carbon and nitrogen quotas ranged from 0.02 – 0.17 pmol C cell-1 and 3.3 x 10-3 – 2.2 x 10-2 pmol N cell-1 as a function of iron status.

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Coverage

Location: Ocean Station Papa, Subarctic North Pacific Ocean, approximate location lat:50, lon:-145
Spatial Extent: **Lat**:50 **Lon**:-145
Temporal Extent: 2018-08-16 - 2018-09-07

Methods & Sampling

Samples were taken at Ocean Station Papa, Subarctic North Pacific Ocean. Sampling depth during cruise RR1813 was 3 m. The culture was isolated from a sample taken at approximate location (lat:50, lon:-145).

Bathycoccus cells were isolated using a 10 mL pipette after whole seawater sampling from a trace metal clean rosette aboard the R/V Revelle. Cultures were transported back to the University of North Carolina at Chapel Hill and stored in Aquil media under constant 120-150 µmol photons m-2 d-1 light in a 12ºC incubator.

Cultures were grown with either 1.37x10-6 M total Fe ("Fe+") or 3.10x10-9 M total Fe ("Fe-") media. Upon reaching exponential phase growth (based on trends in relative fluorescence units as measured by a Turner fluorometer), cultures were filtered for chlorophyll a concentration, particulate carbon/particulate nitrogen, and RNA. Additional samples for cell count were collected and fixed with a 10% addition of paraformaldehyde. Measurements of the maximum quantum yield of photochemistry in photosystem II (Fv/Fm) and the functional absorption cross section of photosystem II (sigma) were collected. Filters used in chlorophyll and carbon/nitrogen sampling were uncombusted and combusted Whatman 0.7µm glass fiber filters, respectively.

For RNA sampling, Isopore 0.4 µm 47 mm polycarbonate filters were used. A RNAqueous-4PCR kit and RNeasy MinElute Clean-up kit were used for RNA extraction and clean-up.

Data Processing Description

Chlorophyll was analyzed at the University of North Carolina on a Turner 10AU fluorometer following the protocol of Graff and Rynearson (2011). Particulate carbon/particulate nitrogen was analyzed on an elemental analyzer at the University of Maryland Center for Environmental Science Application Laboratory. Cell counts were measured at North Carolina State University on a Guava easyCyte flow cytometer equipped with a red laser. RNA was analyzed at the Azenta Genewiz Sequencing Facility via Illumina high throughput sequencing with 350M raw paired-end reads per lane and single index, 2 x 150 bp per lane.

RNA transcripts were processed according to the pipeline outlined in https://github.com/omtorano/Exports-Metatranscriptomics (Author: Olivia Torano). Programs for processing include Trim_galore (v.0.6.2), FastQC (v.0.11.9), Trinity (v.2.8.6), cdhit (v.4.8.1), Salmon (v.10.9.1), tximport, and the DESeq2 package in RStudio. Sequences were blasted to the Kyoto Encyclopedia of Genes and Genomes (Release 88.2).

BCO-DMO Processing Description

* Renamed parameters to comply with database requirements
* Added cruise ID and apporximate sampling location to data

Problem Description

Sample NO3- Fe+ A had low Fv/Fm and high sigma, suggesting it was no longer in exponential phase and thus scientifically different from NO3- Fe+ B and C. Therefore, NO3- Fe+ A was removed from average analyses.

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Parameters

Parameter	Description	Units
Cruise	Cruise ID	unitless
Latitude	Approximate sampling latitude	unitless
Longitude	Approximat sampling longitude	unitless
Treatment	Treatment (NO3- Fe-, NO3- Fe+) - iron treatment, Fe- refers to Aquil media concentrations of 1.37 x 10-6 M Fe and Fe+ refers to Aquil media concentrations of 3.10 x 10-6 M Fe	unitless
Replicate	Replicate (A, B, C) - culture replicate	unitless
Volume	Measured volume	liter (L)
Cell_Count	Cell Count	cell per liter (cell L-1)
Carbon	Particulate carbon concentration	grams (g)
Nitrogen	Particulate nitrogen concentration	grams (g)
Fv_Fm	Estimates of maximum photochemical yield of photosystem II	unitless
Sigma	Cross-sectional absorption area of photosystem II	unitless
Growth_Rates	Growth rates (d-1)	per day (d-1)

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Instruments

Dataset-specific Instrument Name	
Generic Instrument Name	Elemental Analyzer
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	Guava easyCyte flow cytometer
Generic Instrument Name	Flow Cytometer
Generic Instrument Description	Flow cytometers (FC or FCM) are automated instruments that quantitate properties of single cells, one cell at a time. They can measure cell size, cell granularity, the amounts of cell components such as total DNA, newly synthesized DNA, gene expression as the amount messenger RNA for a particular gene, amounts of specific surface receptors, amounts of intracellular proteins, or transient signalling events in living cells. (from: http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm)

Dataset-specific Instrument Name	Turner 10AU fluorometer
Generic Instrument Name	Turner Designs Fluorometer 10-AU
Generic Instrument Description	The Turner Designs 10-AU Field Fluorometer is used to measure Chlorophyll fluorescence. The 10AU Fluorometer can be set up for continuous-flow monitoring or discrete sample analyses. A variety of compounds can be measured using application-specific optical filters available from the manufacturer. (read more from Turner Designs, turnerdesigns.com, Sunnyvale, CA, USA)

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Deployments

RR1813

Website	https://www.bco-dmo.org/deployment/772777
Platform	R/V Roger Revelle
Report	https://datadocs.bco-dmo.org/docs/EXPORTS/data_docs/RR1813_Cruise_Report.pdf
Start Date	2018-08-10
End Date	2018-09-12
Description	Additional cruise information is available from the Rolling Deck to Repository (R2R): https://www.rvdata.us/search/cruise/RR1813

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Project Information

Collaborative Research: Evaluating the contribution of small eukaryotes to nitrate-based new production in the North Pacific Subtropical Gyre (Small Euks)

Coverage: Station ALOHA - North Pacific Subtropical Gyre

NSF abstract:
The subtropical oceans in the middle latitudes are “ocean deserts,” where there are relatively little nutrients in the surface waters. Nitrogen is an important nutrient that is particularly scarce. Nitrogen in the ocean occurs

as nitrate or ammonium. As a result of limited nutrients in the subtropical gyres, marine algae or phytoplankton there grow slowly. In deeper waters below the surface, there are more nutrients. However, it is not well understood how phytoplankton living in surface waters in summer can access the nutrients in the deeper waters. This award will investigate how phytoplankton access subsurface nitrogen at Station ALOHA in the North Pacific Subtropical Gyre (NPSG). Phytoplankton will be sorted by size and pigment composition. The researchers will make measurements of the stable nitrogen isotope ratios of sorted populations and determine where phytoplankton are getting the needed nutrients, either nitrate from the subsurface or ammonium from the surface waters. The researchers will use metatranscriptomic analysis to investigate the physiological pathways that different phytoplankton populations use to take up the needed nitrogen. The metatranscriptomic analysis involves analyzing the RNA sequences transcribed by plankton, comparing the sequence to documented gene sequences and mapped to documented physiological pathways. The researchers will assess which taxonomic groups are using nitrate versus recycled sources of nitrogen. They will test the hypothesis that eukaryotic phytoplankton primarily use nitrate whereas prokaryotic phytoplankton mostly use recycled sources of nitrogen. With these measurements, the researchers will help the society anticipate climate impacts on the productivity of subtropical gyres. This project will support two graduate students and a post-doctoral researcher in laboratory and ship-based research. Principal Investigator (PI) Granger in Connecticut will engage high school students and sponsor graduate students from underrepresented groups via AGU's Bridge Partner Program. PI Marchetti in North Carolina will sponsor the internship of a high school student from a local minority county. PI White in Hawaii will engage with Hawaiian high school students though the Indigenous Partnership for Ocean Monitoring program to mentor and teach essential research skills.

The researchers of this project will investigate the drivers of productivity at station ALOHA in NPSG. They will exploit a high-sensitivity method for natural abundance N isotope analysis (the 'persulphate-denitrifier' method) to evaluate the extent to which taxonomically distinct components of the plankton sorted by flow cytometry rely on nitrate vs. reduced N sources in surface waters. We will also query physiological nitrogen pathways of the plankton with metatranscriptomic analysis to infer which clades are reliant on nitrate. We will quantify the fraction of primary production fueled by nitrate to characterize seasonal trends and explore potential mechanisms of nitrate supply to the euphotic zone and its mixed layer. The work proposed here will resolve the relative contribution of nitrate mixed from the ocean interior to surface production at station ALOHA, to better define seasonal dynamics of the biological pump in the NPSG. We will identify plankton groups that contribute dominantly to nitrate-based production and resolve eco-physiological strategies that confer fitness in a severely N-limited environment. Seasonal trends in the contributions of nitrate to total nitrogen production will be evaluated considering incident hydrography to infer potential mechanisms of nitrate supply.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-2219973

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