# Data from nutrient manipulation experiments (conducted on EXPORTS cruise DY131) aimed at relieving or inducing nutrient stress in phytoplankton and quantifying these responses using metatranscriptomic sequencing

Website: https://www.bco-dmo.org/dataset/948590 Data Type: Cruise Results, experimental Version: 1 Version Date: 2025-01-21

#### Project

» <u>Collaborative Research: Diatoms, Food Webs and Carbon Export - Leveraging NASA EXPORTS to Test the</u> <u>Role of Diatom Physiology in the Biological Carbon Pump</u> (Diatoms and carbon export)

#### Program

» EXport Processes in the Ocean from Remote Sensing (EXPORTS)

Contributors	Affiliation	Role
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#### Abstract

This dataset includes data from nutrient manipulation experiments aimed at relieving or inducing nutrient stress in phytoplankton and quantifying these responses using metatranscriptomic sequencing. Experiments were conducted by adding key macronutrients (N, P, Si) and Fe in different combinations over different growth periods, simulating potential alleviation of in situ nutrient stress or the induction of nutrient stress. Experiments were conducted on the EXports Processes in the Oceans from RemoTe Sensing (EXPORTS) cruise DY131 in the North Atlantic during May of 2021.

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# Coverage

Location: North Atlantic, near Porcupine Abyssal Plain Spatial Extent: N:49.079293 E:-14.782629 S:48.934083 W:-15.017959

#### Methods & Sampling

Seawater for five shipboard incubation experiments was collected using a surface towfish (Mellett and Buck 2020) at ~2 meters (m) depth on the RSS Discovery between 5 May 2021 and 26 May 2021. All experimental setup and sampling was conducted in a trace metal clean (TMC) lab using TMC methods. Water was prefiltered through a 150-micrometer (µm) mesh to remove large grazers. For all incubations, 20-liter (L) acid-cleaned carboys were filled in sets based on the replicate number (e.g. replicate 1 carboys for all treatments were filled in succession). For LT1, individual carboys were completely filled prior to filling the next. For all other experiments carboys were filled "round robin", filling each 1/3 at a time until all replicates in the set were full. Triplicate control and treatment carboys were amended for each experiment as indicated in Table 1 (see Supplemental File "EXPORTS\_2021\_dy131\_table1.pdf"). In addition, triplicate T0 carboys were also collected. T0 carboys were sampled immediately after the experimental start. Control and treatment carboys were sampled at the end of each incubation. T0 carboys are shared between LT2 and ST2 experiments.

Short-term incubations were carried out for 24 (ST1A and ST2) and 42 hours (ST1B). Long-term incubations were carried out for 6 (LT1) and 5 (LT2) days (Table 1). Short-term nutrient amendment treatments were selected to alleviate potential in situ nutrient stress. The +Si and +N treatments were amended to final concentrations of 20 micromolar ( $\mu$ M) silicate and nitrate respectively, in addition to in situ nutrient concentrations. The +Fe treatment was amended to a final concentration of 0.005  $\mu$ M <sup>57</sup>Fe. The long-term experiments were amended with all key macro and trace nutrients (N, P, Si, Fe) except one, with the aim of inducing a specified nutrient stress. For LT1 and LT2, we refer to the +20  $\mu$ M nitrate +1.25  $\mu$ M phosphate +0.005  $\mu$ M <sup>57</sup>Fe treatment as "AllButFe" and the +20  $\mu$ M nitrate +1.25  $\mu$ M phosphate +0.005  $\mu$ M <sup>57</sup>Fe treatment as "AllButSi".

Nutrient samples were filtered through 0.2 µm polycarbonate filters and frozen at -20 degrees Celsius (°C). Samples for biogenic silica concentrations were size fractionated by serial filtration through 5 µm and 0.6 µm polycarbonate filters Filters were stored frozen at -20°C. Particulate organic carbon and nitrogen were measured on samples from experiments examining the effect of added Fe and Si on carbon fixation. These samples were filtered through precombusted GFF filters placed in glass scintillation vials and frozen at -20°C.

Biogenic silica concentrations were determined by NaOH digestion followed by colorimetric analysis of the resulting dissolved Si. Particulate organic carbon and nitrogen samples were analyzed by Dumas combustion. Nutrient concentrations were determined by flow injection using a Lachat Instruments QuikChem 8500 Series 2 analyzer.

Metatranscriptomes were sequenced for experiments LT1 and ST1B. Metatranscriptomic samples were collected on 5 µm 47-millimeter (mm) polyester (PETE) membrane filters using a peristaltic pump. Filters were then preserved in 2 milliliters (mL) of RLT buffer (RNeasy Mini Kit, Qiagen, Germantown, MD, USA), flash frozen in liquid nitrogen, and stored at -80°C until RNA extraction. PolyA selection was performed on sample libraries to enrich for eukaryotic sequences and libraries were sequenced on an Illumina NovaSeq 6000 platform.

For more information on nutrient, particulate, and chlorophyll measurements see the Protocol documents attached as Supplemental Files and <u>https://msi.ucsb.edu/facilities-services/analytical-lab/services</u>.

#### **Data Processing Description**

Values below detection limits are left blank and flagged in the quality columns, detection limits are as follows:

Nitrate (NO3) + Nitrite (NO2): 0.1 µM

Nitrite (NO2): 0.1 µM

Phosphate (PO4): 0.1 µM

Silicic Acid (Si(OH)4): 0.1 µM

- Imported original file "EXPORTS\_dy131\_experiments.xlsx" into the BCO-DMO system.
- Renamed fields to comply with  $\overline{BCO}$ -D $\overline{MO}$  naming conventions.
- Saved the final file as "948590\_v1\_exports\_dy131\_experiments.csv".

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### **Data Files**

#### File

948590\_v1\_exports\_dy131\_experiments.csv(Comma Separated Values (.csv), 9.73 KB) MD5:c27c9d974b7521a913ddb900fb53d0d4

Primary data file for dataset ID 948590, version 1

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### Supplemental Files

File

Brzezinski\_Lab\_Chl\_filtration\_protocols.pdf

Supplemental file for dataset ID 948590, version 1

Brzezinski\_Lab\_Chl\_reading\_protocols.pdf

Supplemental file for dataset ID 948590, version 1

EXPORTS\_2021\_dy131\_table1.pdf

(Portable Document Format (.pdf), 264.08 KB) MD5:273e64b3c96f3b0afb4345f9844116ec

(Portable Document Format (.pdf), 207.84 KB) MD5:ca197e2c2cdb6c2d616db53b5d187a26

(Portable Document Format (.pdf), 21.83 KB) MD5:0efa728b8a3fb8afc0539186814f1c5a

Table 1. Supplemental file for dataset ID 948590, version 1. Contains a table of the experiment's duration and treatment information.

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### **Related Publications**

Mellett, T., & Buck, K. N. (2020). Spatial and temporal variability of trace metals (Fe, Cu, Mn, Zn, Co, Ni, Cd, Pb), iron and copper speciation, and electroactive Fe-binding humic substances in surface waters of the eastern Gulf of Mexico. Marine Chemistry, 227: 103891. doi:<u>10.1016/j.marchem.2020.103891</u> *Methods* 

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#### **Parameters**

Parameter	Description	Units
Cruise	cruise during which sample was collected	unitless
ISO_DateTime_UTC	date and time (UTC) of sample collection in ISO 8601 format	unitless
Date_UTC	date (UTC) of sample collection	unitless
	-	

Time_UTC	time (UTC) of sample collection	unitless
Event	event number from R2R event log	unitless
Activity	instrument used for sample collection	unitless
Station	station identifier	unitless
Latitude	latitude (positive values = North; negative values = South)	decimal degrees
Longitude	longitude (positive values = East; negative values = West)	decimal degrees
exp_id	Experiment Id	unitless
Incubation	Incubation number	unitless
Treatment	Experimental conditions applied to experimental units. In comparative experiments, members of the complementary group, the control group, receive either no treatment or a standard treatment.	unitless
replicate	an identifier used to distinguish between one or more replicate samples	unitless
BioProject	The National Center for Biotechnology Information (NCBI) BioProject identifier. A BioProject is a collection of biological data related to a single initiative, originating from a single organization or from a consortium. A BioProject record provides users a single place to find links to the diverse data types generated for that project.	unitless
BioSample	The National Center for Biotechnology Information (NCBI) BioSample accession number in the BioSample database. The BioSample database stores descriptive information about the physical biological materials used to generate data submitted to NCBI's primary data archives. Typical examples of a BioSample include a cell line, a tissue biopsy or an environmental isolate. The BioSample database promotes the use of structured and consistent attribute names and values to describe sample properties and provenance. BioSample records are reciprocally linked to data derived from that sample and to the BioProjects in which they participate.	unitless
SRA_sample	The National Center for Biotechnology Information (NCBI) Sample accession number in the Sequence Read Archive (SRA). A Sample in the SRA is an object that contains the metadata describing the physical sample upon which a sequencing experiment was performed. Imported from BioSample. An experiment targets one or more samples. Results are expressed in terms of individual samples or bundles of samples as defined by the experiment.	unitless

Phosphate	phosphate concentration (PO4) in micromoles	millimoles per cubic meter (mmol m^3)
Phosphate_flag	data quality flag: $1 = \text{good}$ ; $3 = \text{below detection limit}$	unitless
Silicate	silicic acid concentration (SiO4) in micromoles (also known as dissolved silicon concentration or dSi)	millimoles per cubic meter (mmol m^3)
Silicate_flag	data quality flag: $1 = \text{good}$ ; $3 = \text{below detection limit}$	unitless
Nitrite	dissolved nitrite (NO2) concentration in micromoles; analyzed in UCSB MSI Analytical lab	millimoles per cubic meter (mmol m^3)
Nitrite_flag	data quality flag: $1 = \text{good}$ ; $3 = \text{below detection limit}$	unitless
Nitrate	dissolved nitrate (NO3) concentration in micromoles; analyzed in UCSB MSI Analytical lab	millimoles per cubic meter (mmol m^3)
Nitrate_flag	data quality flag: $1 = \text{good}$ ; $3 = \text{below detection limit}$	unitless
chl_a_0_6um_to_5um	chlorophyll a in micrograms per liter for the 0.6 to 5 micrometer (um) size fraction	micrograms per cubic meter (ug m^3)
chl_a_gt_5um	chlorophyll a in micrograms per litre for the size fraction of 5 micrometers (um) and greater	micrograms per cubic meter (ug m^3)
Blank_Corrected_POC	particulate organic carbon (POC) in micromoles; analyzed in UCSB MSI Analytical lab	milligrams per cubic meter (mg m^3)
Blank_Corrected_PON	particulate organic nitrogen (PON) in micromoles; analyzed in UCSB MSI Analytical lab	milligrams per cubic meter (mg m^3)
bSi_0_6um_to_5um	particulate biogenic silica in nanomoles Si per litre for the 0.6 to 5 micrometer (um) size fraction	nanomoles Si per liter (nmol Si L^1)
bSi_gt_5um	particulate biogenic silica in nanomoles Si per litre for the size fraction of 5 micrometers (um) and greater	nanomoles Si per liter (nmol Si L^1)

### Instruments

Dataset-specific Instrument Name	Illumina NovaSeq 6000 platform
Generic Instrument Name	Automated DNA Sequencer
	A DNA sequencer is an instrument that determines the order of deoxynucleotides in deoxyribonucleic acid sequences.

Dataset- specific Instrument Name	Lachat Instruments QuikChem 8500 Series 2 analyzer
Generic Instrument Name	Lachat QuikChem 8500 flow injection analysis system
Generic Instrument	The Lachat QuikChem 8500 Series 2 Flow Injection Analysis System features high sample throughput and simple, but rapid, method changeover. The QuikChem 8500 Series 2 system maximises productivity in determining ionic species in a variety of sample types, from sub-ppb to percent concentrations. Analysis takes 20 to 60 seconds, with a sample throughput of 60 to 120 samples per hour.

Dataset-specific Instrument Name	surface towfish
Generic Instrument Name	towed unmanned submersible
	A vehicle towed by rigid cable through the water column at fixed or varying depth with no propulsion and no human operator (e.g. Towfish, Scanfish, UOR, SeaSoar).

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### Deployments

### DY131

Website	https://www.bco-dmo.org/deployment/893299
Platform	RRS Discovery
Report	https://www.bodc.ac.uk/resources/inventories/cruise_inventory/report/17779/
Start Date	2021-05-01
End Date	2021-06-01
Description	See additional information from the British Oceanographic Data Centre (BODC): https://www.bodc.ac.uk/resources/inventories/cruise_inventory/report/17779/

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# **Project Information**

Collaborative Research: Diatoms, Food Webs and Carbon Export - Leveraging NASA EXPORTS to

#### Test the Role of Diatom Physiology in the Biological Carbon Pump (Diatoms and carbon export)

Coverage: Sub-Arctic Pacific, Ocean Station Papa

#### NSF Award Abstract:

This project focuses on a group of microscopic single-celled photosynthetic organisms in the ocean called diatoms. Diatoms float in the surface ocean as part of a group of organisms collectively called phytoplankton. There are thousands of different species of diatoms distributed across the global ocean. A famous oceanographer Henry Bigelow once said "All fish is diatoms" reflecting the importance of diatoms as the base of the food chain that supports the world's largest fisheries. Despite their small size, diatom photosynthesis produces 20% of the oxygen on earth each year. That's more than all of the tropical rain forests on land. The major objective of the research is to understand how the metabolic differences among diatom species affects the amount of diatom organic carbon that is carried, or exported, from the surface ocean to the deep ocean. As diatoms are photo-synthesizers like green plants, their biological carbon comes from converting carbon dioxide dissolved in seawater from the atmosphere into organic forms. Diatoms also require a series of other nurtrients supplied by the ocean such as nitrogen and phosphorous and, uniquely for diatoms, the silicon used to construct their glass shells. This research will investigate how genetic and physiological differences among diatoms influence how each species react to changes in nutrient levels in the ocean and how those shifts affect the export of diatom carbon to the deep sea. The link between diatoms' physiological response and their carbon export comes about because shifts in physiology affect diatom attributes like how fast they sink and how tasty they are to predators. So if we can relate the physiological condition of different diatoms to the food-web pathways followed by different species, we can ultimately use knowledge of diatom physiological status and food web structure to predict how much diatom carbon gets to the deep sea. The research involves investigators with expertise in the physiology and genomics of diatoms and in the ocean's chemistry. The work will initially take place in the subarctic North Pacific in conjunction with the NASA Export Processes in the Ocean from RemoTe Sensing (EXPORTS) field program. The EXPORTS program is using a wide variety of methods to quantify the export and fate of photo-synthetically fixed carbon in the upper ocean. The research supports the training of undergraduate students, graduate students and a postdoctoral scholar. The research will also serve as the basis for activities aimed at K-12 and junior high school students.

The research will broadly impact our understanding of the biology of the biological pump (the transport of photo-synthetically fixed organic carbon to the deep sea) by forming a mechanistic basis for predicting the export of diatom carbon. It is hypothesized that the type and degree of diatom physiological stress are vital aspects of ecosystem state that drive export. To test this hypothesis, the genetic composition, rates of nutrient use and growth response of diatom communities will be evaluated and supported with measurements of silicon and iron stress to evaluate stress as a predictor of the path of diatom carbon export. The subarctic N. Pacific ecosystem is characterized as high nutrient low chlorophyll (HNLC) due to low iron (Fe) levels that are primary controllers constraining phytoplankton utilization of other nutrients. It has been a paradigm in low Fe, HNLC systems that diatoms grow at elevated Si:C and Si:N ratios and should be efficiently exported as particles significantly enriched in Si relative to C. However, Fe limitation also alters diatoms species composition and the high Si demand imposed by low Fe can drive HNLC regions to Si limitation or Si/Fe co-limitation. Thus, the degree of Si and/or Fe stress in HNLC waters can all alter diatom taxonomic composition, the elemental composition of diatom cells, and the path cells follow through the food web ultimately altering diatom carbon export.

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

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#### **Program Information**

#### EXport Processes in the Ocean from Remote Sensing (EXPORTS)

Website: http://oceanexports.org/

EXport Processes in the Ocean from Remote Sensing (EXPORTS) is a large-scale NASA-led field campaign that will provide critical information for quantifying the export and fate of upper ocean net primary production (NPP) using satellite observations and state of the art ocean technologies.

Ocean ecosystems play a critical role in the Earth's carbon cycle and the quantification of their impacts for both present conditions and for predictions into the future remains one of the greatest challenges in oceanography. The goal of the EXport Processes in the Ocean from Remote Sensing (EXPORTS) Science Plan is to develop a predictive understanding of the export and fate of global ocean net primary production (NPP) and its implications for present and future climates. The achievement of this goal requires a quantification of the mechanisms that control the export of carbon from the euphotic zone as well as its fate in the underlying "twilight zone" where some fraction of exported carbon will be sequestered in the ocean's interior on time scales of months to millennia. In particular, EXPORTS will advance satellite diagnostic and numerical prognostic models by comparing relationships among the ecological, biogeochemical and physical oceanographic processes that control carbon cycling across a range of ecosystem and carbon cycling states. EXPORTS will achieve this through a combination of ship and robotic field sampling, satellite remote sensing and numerical modeling. Through a coordinated, process-oriented approach, EXPORTS will foster new insights on ocean carbon cycling that maximizes its societal relevance through the achievement of U.S. and International research agency goals and will be a key step towards our understanding of the Earth as an integrated system.

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### Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	<u>OCE-1756816</u>
NSF Division of Ocean Sciences (NSF OCE)	<u>OCE-1756433</u>
NSF Division of Ocean Sciences (NSF OCE)	<u>OCE-1756442</u>

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