Diatom (Thalassiosira pseudonana) physiological data from experiments designed to study single-cell transcriptional profiling of nutrient acquisition heterogeneity in diatoms conducted in December of 2022

Website: https://www.bco-dmo.org/dataset/918841
Data Type: experimental
Version: 1
Version Date: 2024-01-30

Project
» EAGER: Diatom Programmed Cell Death at Single-Cell Resolution (Diatom Death)

Program
» Ocean Carbon and Biogeochemistry (OCB)

Contributors	Affiliation	Role
Orellana, Monica V.	University of Washington Friday Harbor Laboratories (FHL)	Principal Investigator
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Abstract
This dataset includes physiological data for diatom Thalassiosira pseudonana grown during experiments conducted as part of a study of "Single-Cell transcriptional profiling of nutrient acquisition heterogeneity in diatoms." See "Related Datasets" section for T. pseudonana gene and cell information collected as part of the same study and experiments. Study description: Diatoms (Bacillariophyceae) are unicellular photosynthetic algae, accounting for about 40% of total marine primary production (equivalent to terrestrial rainforests) and critical ecological players in the contemporary ocean. Diatoms can form enormous blooms in the ocean that can be seen from space and are the base of food webs in coastal and upwelling systems, support essential fisheries, and are central to the biogeochemical cycling of important nutrients such as carbon and silicon. Over geological time, diatoms have influenced the world's climate by changing the carbon flux into the oceans. Diatoms have traditionally been studied on a population level. Growth is often measured by the total increase in biomass, and gene expression is analyzed by isolating mRNA from thousands or millions of cells. These methods generate a valuable analysis on the population’s average functioning; however, they fail to show how each individual diatom cell contributes to the population phenotype. Bulk transcriptomes confound different stages and variability of cell states in heterogeneous populations. By contrast, single-cell transcriptomics measures gene expression in thousands of individual diatoms providing a quantitative and ultrahigh-resolution picture of transient cell states in population fractions enabling the reconstruction of the various phenotypic trajectories. Thus, the single-cell physiological and molecular parameters analysis allows an unsupervised assessment of cell heterogeneity within a population—a new dimension in diatoms and phytoplankton in general. In this dataset, we examine the model diatom Thalassiosira pseudonana clonal cells grown in different nitrogen conditions, at the single cell level when grown in a light: dark cycle (12:12 h). Nitrogen is the major limiting nutrient for primary production and growth in the ocean’s surface, specifically for diatoms and the food webs they support. We investigate nutrient limitation, starvation and recovery. We used droplet-based, single-cell transcriptomics to analyze ten samples in two stages. In the first stage ("starvation"), six samples were collected over four days of culture as nutrient levels decreased. In the second stage ("recovery"), four samples were collected over twelve hours after nutrients were replenished.

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Coverage

Location: Baliga Laboratory Institute for Systems Biology
Temporal Extent: 2022-12-04 - 2022-12-09

Methods & Sampling

Cultures: Axenic *Thalassiosira pseudonana* (CCMP 1335, National Center for Marine Algae and Microbiota, Maine, USA; LSID urn:lsid:marinespecies.org:taxname:148934) batch cultures were grown in enriched artificial seawater (ESAW) medium modified with reduced levels of nitrate (170 µM) to characterize starvation. Before the experiment, the diatoms were acclimated to a constant 20 °C temperature and under a 12: 12 h dark: light diurnal cycle at 300 µmol photons·m_{-2}·s_{-1} using cool fluorescent lights. The cultures were continuously equilibrated at ambient (420 ppm CO_2 by bubbling mixed gasses (air and CO_2 at 0.4 L/min) regulated using mass-flow controllers (GFC17, Aalborg) and monitored with a CO_2 analyzer (model Q-S151; Qubit Systems) into a 1.5-L glass bioreactor system. The bioreactors were inoculated with 150,000 cells/ml of acclimated, axenic *T. pseudonana* and grown for 5 days on a 12: 12 h dark: light cycle. The pH was monitored spectrophotometrically (Dickson et al. 2007); the photochemical yield of photosystem II (variable fluorescence/maximum fluorescence Fv/Fm) was measured with an AquaPen AP100; and total dissolved nitrogen was measure using the Nitrite/Nitrate, Colorimetric Test Roche # 11746081001) kit after syringe-filtering (0.2µm) the samples. Samples were taken twice a day, in the middle of dark-time, and the middle of the light-time representing different growth conditions regulating the metabolism based on light and nitrogen on days one and two (Ashworth et al. 2013). On days three and four, samples were only taken during the light time for experiment b. This experiment was repeated to evaluate the recovery of the cells from starvation by amending NO_3 (170 uM+/- 5uM) on day 5, after 70 hrs of starvation for *T. pseudonana* and sampled at T1: 0h, T2: 1h and T3: 3 hr, and T4: 6 hr after adding the supplemental nitrogen. Samples T1, T2, T3, were set on ice in the dark before analysis at when T4 was sampled.

Experiment Metadata (physiology, gene and cell information):

Experiment
Start_Date 12-4
Light:Dark 12:12
Lights_On 1:00 PM
Lights_(umols/m.s) 1000 umoles m-2s-1
CO2_ppm ~420
scRNAseq_Timepoints 18 hours, 30 hours, 42 hours, 54 hours, 68 hours, 80 hours, 92 hours
scRNAseq_Timepoint_Labels
Notes scRNA, Full ESAW nitrate limited (170uM). Starting innoculate = 150k/m

BCO-DMO Processing Description

* Sheet 1 of file "EAGER 10x Physiological metadata and measurements.xlsx" was imported into the BCO-DMO data system up to Excel row 15 (following rows containing metadata describing the columns was not imported as part of the data table, it was added to the "Parameters" section of the dataset metadata.

* DateTime (UTC) added in ISO 8601 format using provided dates and times (PST/PSD) .

* Column names adjusted to conform to BCO-DMO naming conventions designed to support broad re-use by a variety of research tools and scripting languages. [Only numbers, letters, and underscores. Can not start with a number]

* Three columns for replicates of F/cell flow were removed from table at submitter's request.

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Data Files

File
918841_v1_physiological-data.csv (Comma Separated Values (.csv), 2.70 KB) MD5:0dcc34596f03a7c84762c09bfe228ea8 Primary data file for dataset ID 918841, version 1

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Related Publications

Dickson, A.G.; Sabine, C.L. and Christian, J.R. (eds) (2007) Guide to best practices for ocean CO2 measurement. Sidney, British Columbia, North Pacific Marine Science Organization, 191pp. (PICES Special Publication 3; IOCCP Report 8). DOI: https://doi.org/10.25607/OBP-1342
Methods

Wolf, F. A., Angerer, P., & Theis, F. J. (2018). SCANPY: large-scale single-cell gene expression data analysis. Genome Biology, 19(1). https://doi.org/10.1186/s13059-017-1382-0
Methods

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Related Datasets

IsRelatedTo

Orellana, M. V., Lausted, C., Huang, S. (2024) **Diatom (Thalassiosira pseudonana) cell information from experiments designed to study single-cell transcriptional profiling of nutrient acquisition heterogeneity in diatoms conducted in December of 2022.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-01-30 doi:10.26008/1912/bco-dmo.918860.1 [view at BCO-DMO]
Relationship Description: Datasets were part of the same experiments conducted as part of a study of "Single-Cell transcriptional profiling of nutrient acquisition heterogeneity in diatoms."

Orellana, M. V., Lausted, C., Huang, S. (2024) **Diatom (Thalassiosira pseudonana) gene information from experiments designed to study single-cell transcriptional profiling of nutrient acquisition heterogeneity in diatoms conducted in December of 2022.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-01-30 doi:10.26008/1912/bco-dmo.918852.1 [view at BCO-DMO]
Relationship Description: Datasets were part of the same experiments conducted as part of a study of "Single-Cell transcriptional profiling of nutrient acquisition heterogeneity in diatoms."

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Parameters

Parameter	Description	Units
Species	Thaps = Thalassiosira pseudonana (LSID urn:lsid:marinespecies.org:taxname:148934)	unitless
Identification	identification "1"	unitless

Date	Date sampled (mm-dd-yy)	unitless
Time	Time sampled (HH:MM), 24hr	unitless
ISO_DateTime_UTC	DateTime with timezone sampled, ISO 8601 format	unitless
Hours	Hours since innoculation	unitless
Diel	Diel Cycle (Day/ Night)	unitless
PPM_CO2_analyzer	CO2 (CO2 Analyzer)	parts per million (ppm)
cell_counts_q1	Cell counts q1 (quadrant 1)	cells
cell_counts_q2	Cell counts q2 (quadrant 2)	cells
cell_counts_q3	Cell counts q3 (quadrant 3)	cells
cell_counts_q4	Cell counts q4 (quadrant 4)	cells
dF	Dilution factor	unitless
cells_mL_avgerage	cell concentration	cells per ml (cells/ml)
Quantum_yield_1	Quantum Yield replicate 1	unitless
Quantum_yield_2	Quantum Yield replicate 2	unitless
Quantum_Yield_3	Quantum Yield replicate 3	unitless
QY_AVG	Quantum Yield Average	unitless
FT1	Fluorescence replicate 1	unitless
FT2	Fluorescence replicate 2	unitless
FT3	Fluorescence replicate 3	unitless

FT_AVG	Fluorescence Average	unitless
pH	pH (potential for Hydrogen)	unitless
Sample_for_RNA	Sample for RNA (Yes/No)	unitless
Cell_Pellet	Cell pellet (Yes/No)	unitless
CF_Media	Cell free media for nutrient profiling	unitless
uM_Nitrate1	Nitrate replicate 1	micromolar (uM)
uM_Nitrate2	Nitrate replicate 2	micromolar (uM)
uM_Nitrate3	Nitrate replicate 3	micromolar (uM)
uM_Nitrate_AVG	Nitrate average	micromolar (uM)

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Instruments

Dataset-specific Instrument Name	CO2 analyzer (model Q-S151; Qubit Systems)
Generic Instrument Name	CO2 Analyzer
Generic Instrument Description	Measures atmospheric carbon dioxide (CO2) concentration.

Dataset-specific Instrument Name	AquaPen AP100, Photon Systems Instruments (Drásov, Czechia)
Generic Instrument Name	Fluorometer
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.

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Project Information

EAGER: Diatom Programmed Cell Death at Single-Cell Resolution (Diatom Death)

Coverage: Laboratory study

NSF Award Abstract:
Diatoms are important primary producers in sunlit oceans and lakes across the globe. They are key players in spring phytoplankton blooms, which in turn support food webs that include productive fisheries. In addition, these photosynthetic diatom cells are known to capture enormous amounts of carbon, which are exported to depth as blooms die off. In oceanic systems, this process effectively removes carbon from the atmosphere for thousands of years. However, the biological mechanisms that lead to carbon sequestration, the transfer of carbon from the atmosphere into the deep ocean, are not well understood. As diatom populations reach the end of the bloom cycle, individual cells start to deteriorate and undergo cell death. The collapse of diatom populations is regarded as a critical point (or tipping point), which can be predicted by understanding the genetic activity of the population. This project seeks to elucidate how environmental change influences diatom cell-death processes. A mechanistic understanding of these cellular processes will elucidate how climate change could alter carbon-removal from the atmosphere and affect ocean productivity. The knowledge and methods developed during this study are applicable across organisms from all domains of life. The broader impacts of this project are focused on high school education and new ideas and approaches for three-dimensional learning opportunities in support of Next Generation Science Standards (NGSS). Specifically, researchers are working with high school educators and partners to teach concepts of systems approaches, tipping points, and carbon sequestration in the marine environment through educational modules.

The goal is to determine the structure of diatom populations during the transition of actively growing cells towards population collapse by identifying the point of commitment (tipping point) at the level of individual cells. The model system is the diatom, Thalassiosira pseudonana, which is widespread and a common bloom-forming species. The random decision process of individual diatom cells is being characterized by establishing a genome-wide gene expression space through the physical and biochemical characterization of the cells. Implementation of the project is focused on measuring the phenotypic responses in the diatom to environmental factors including severe stress. Transcriptomic analyses during the transition of cell proliferation towards culture collapse include bulk (RNA-Seq) and single-cell (scRNA-Seq) high-throughput sequencing. Using a systems biology approach, the genetic information obtained from the samples is incorporated into predictive models to identify genetic transitions that occur prior to population collapse. The systems approach can detect changes in transcriptomic state that precede a critical point in the cell death process leading to predictions of how diatoms respond to environmental change. This work opens new vistas for the elucidation of mechanistic pathways of diatom cell populations.

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

Ocean Carbon and Biogeochemistry (OCB)

Website: http://us-ocb.org/

Coverage: Global

The Ocean Carbon and Biogeochemistry (OCB) program focuses on the ocean's role as a component of the global Earth system, bringing together research in geochemistry, ocean physics, and ecology that inform on and advance our understanding of ocean biogeochemistry. The overall program goals are to promote, plan, and coordinate collaborative, multidisciplinary research opportunities within the U.S. research community and with international partners. Important OCB-related activities currently include: the Ocean Carbon and Climate Change (OCCC) and the North American Carbon Program (NACP); U.S. contributions to IMBER, SOLAS, CARBOOCEAN; and numerous U.S. single-investigator and medium-size research projects funded by U.S. federal agencies including NASA, NOAA, and NSF.

The scientific mission of OCB is to study the evolving role of the ocean in the global carbon cycle, in the face of

environmental variability and change through studies of marine biogeochemical cycles and associated ecosystems.

The overarching OCB science themes include improved understanding and prediction of: 1) oceanic uptake and release of atmospheric CO2 and other greenhouse gases and 2) environmental sensitivities of biogeochemical cycles, marine ecosystems, and interactions between the two.

The OCB Research Priorities (updated January 2012) include: ocean acidification; terrestrial/coastal carbon fluxes and exchanges; climate sensitivities of and change in ecosystem structure and associated impacts on biogeochemical cycles; mesopelagic ecological and biogeochemical interactions; benthic-pelagic feedbacks on biogeochemical cycles; ocean carbon uptake and storage; and expanding low-oxygen conditions in the coastal and open oceans.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-2029738

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