

N:P ratio experiment physiology and carbonate chemistry laboratory experiments with *Pseudo-nitzschia australis* conducted from 2021 to 2022

Website: <https://www.bco-dmo.org/dataset/906858>

Data Type: experimental

Version: 1

Version Date: 2023-08-28

Project

» [MCA: Developing transcriptomics as a tool to investigate toxic diatom responses to ocean heatwave and upwelling events](#) (Toxic diatoms and heatwaves)

Contributors	Affiliation	Role
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Abstract

These raw data contain physiological data collected from laboratory experiments with *Pseudo-nitzschia australis*. This dataset includes replicate data for N:P ratio experiment physiology and carbonate chemistry. In this 3x2 matrix experiment, we compared each N:P ratio (5, 10, and 50), temperature (13 and 19°C), and total nutrient concentration (high and low) at every combination. See "Related Datasets" for other physiological measurements published as part of these experiments. See the results publication Kelley et al. (2003) for more detail. The following description provides details for all related physiological measurement datasets. These physiological measurements include: growth rates, domoic acid quotas, domoic acid production rates, net primary productivity, and nitrogen use efficiencies. Also included are pH and DIC measurement used to characterize the carbonate system. These data revealed novel insights into *P. australis* bloom dynamics and may be useful to harmful algal bloom modelers and were collected and analyzed by Kyla Kelly, Amjad Mansour, Chen Liang, Andrew Kim, Lily Mancini, Dr. Matthew Bertin, Dr. Bethany Jenkins, Dr. David Hutchins, and Dr. Fei-Xue Fu.

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Coverage

Spatial Extent: Lat:46.495103 Lon:-124.060591

Temporal Extent: 2020-11 - 2022-07

Methods & Sampling

These experiments were conducted with a strain of (strain NWFSC 731) isolated from Long Beach, Washington State, USA on November 3, 2020. The temperature and salinity were 14°C and 27 ppt, respectively at the time of collection. The data was collected in laboratory experiments at the University of Southern California. The experiments began in September 2021 and finished in July of 2022.

The following section provides a methodology summary for this dataset and references related datasets collected as part of the same experiment (see "Related Datasets" section for data access). A full methodology was published in "Simulated upwelling and marine heatwave events promote similar growth rates but differential domoic acid toxicity in *Pseudo-nitzschia australis*" in *Harmful Algae* (Kelly et al., 2023).

Pseudo-nitzschia australis was grown under upwelling heatwave, and extreme heatwave conditions (e.g., combined temperature, nutrient, and carbon dioxide levels specific to each condition) and in single-factor response curves for carbon dioxide, temperature, and varying nitrogen:phosphorus (N:P) ratios/total nutrient concentrations.

Samples for chlorophyll a (used to calculate growth rates) were filtered on GF/F filters, extracted in 6 mL of 90 % acetone at -20°C for 24 h, then analyzed using a Turner 10AU field fluorometer (Welschmeyer 1994; Fu et al. 2007).

For elemental analysis (particulate organic carbon and nitrogen, POC and PON), cells were filtered onto pre-combusted GF/F filters, dried, and analyzed on a Costech 4010 Elemental Analyzer (Fu et al. 2007).

Samples for particulate domoic acid were filtered onto Supor 0.2 µm 47 mm PES filters. Samples were analyzed using LC-MS/MS on a Prominence UFLC system (Shimadzu, Kyoto, Japan) coupled to a SCIEX 4500 QTRAP mass spectrometer (AB Sciex, Framingham, MA, USA). Methods described in Wang et al. 2012.

Primary production was determined by measuring the uptake of radiolabeled bicarbonate (Fu et al. 2008). ¹⁴C-bicarbonate was added to 45 mL sub-cultures at T24 h and incubated for 24 h (approximating net carbon fixation) under the respective experimental conditions. After the incubation period, cells were collected on GF/F filters and placed in a scintillation vial containing scintillation cocktail. Samples were stored for 24 h before being read on a Wallac System 1400 liquid scintillation counter.

pH measurements were made on a Mettler Toledo SevenCompact pH meter using a three-point calibration curve and total pH scale (Cooley and Yager 2006). Samples for total DIC analysis were collected at Tfinal. Seawater from undisturbed culture bottles was removed with a sterile syringe, ejected into pre-evacuated borosilicate Exetainers, and poisoned with 5% MgCl₂. Total DIC was then measured using a Picarro cavity ring-down spectrophotometer according to Subhas et al. (2015).

For cell count samples (for normalizing cellular domoic acid), 1 mL of the final experimental culture was preserved with 40 µl glutaraldehyde and stored at 4°C in the dark. Cells were counted on a Olympus BX51 microscope using a Sedgewick Rafter Chamber.

Organism:

Pseudo-nitzschia australis, LSID (urn:lsid:marinespecies.org:taxname:246604)

Data Processing Description

Data was processed in using excel, which was used to calculate rates, averages, and standard deviations.

BCO-DMO Processing Description

* File NP_ratio_experiments.csv was loaded into the BCO-DMO data system with missing identifier indicated by

"NA".

** Missing data values are displayed differently based on the file format you download. They are blank in csv files, "NaN" in MatLab files, etc.

* 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]

* degree symbol within treatment column values removed for broader interoperability (e.g. "13°C" -> "13C")

* particulate Domoic acid column was a mix of scientific notation and floats without scientific notation. Dataset at BCO-DMO was set to display all as floats no scientific notation.

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Data Files

File
N:P ratio experiments filename: 906858_v1_p-australis-n-to-p.csv(Comma Separated Values (.csv), 1.78 KB) MD5:4e16d0e6ea2b5a266fbb1339f35a1b32 This is the primary data table for dataset 906858 version 1. Replicate data for N:P ratio experiment physiology and carbonate chemistry

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Related Publications

Cooley, S. R., & Yager, P. L. (2006). Physical and biological contributions to the western tropical North Atlantic Ocean carbon sink formed by the Amazon River plume. *Journal of Geophysical Research*, 111(C8).

doi:10.1029/2005jc002954 <https://doi.org/10.1029/2005JC002954>

Methods

Fu, F.-X., Mulholland, M. R., Garcia, N. S., Beck, A., Bernhardt, P. W., Warner, M. E., Sañudo-Wilhelmy, S. A., & Hutchins, D. A. (2008). Interactions between changing pCO₂, N₂ fixation, and Fe limitation in the marine unicellular cyanobacterium *Crocospaera*. *Limnology and Oceanography*, 53(6), 2472–2484. Portico.

<https://doi.org/10.4319/lo.2008.53.6.2472>

Methods

Fu, F.-X., Zhang, Y., Feng, Y., & Hutchins, D. A. (2006). Phosphate and ATP uptake and growth kinetics in axenic cultures of the cyanobacterium *Synechococcus* CCMP 1334. *European Journal of Phycology*, 41(1), 15–28. <https://doi.org/10.1080/09670260500505037>

Methods

Kelly, K. J., Mansour, A., Liang, C., Kim, A. M., Mancini, L. A., Bertin, M. J., Jenkins, B. D., Hutchins, D. A., & Fu, F.-X. (2023). Simulated upwelling and marine heatwave events promote similar growth rates but differential domoic acid toxicity in *Pseudo-nitzschia australis*. *Harmful Algae*, 127, 102467.

<https://doi.org/10.1016/j.hal.2023.102467>

Results

Lewis, E., Wallace, D., & Allison, L. J. (1998). Program developed for CO₂ system calculations (No. ORNL/CDIAC-105). Brookhaven National Lab., Dept. of Applied Science, Upton, NY (United States); Oak Ridge National Lab., Carbon Dioxide Information Analysis Center, TN (United States). doi: [10.2172/639712](https://doi.org/10.2172/639712)

Methods

Subhas, A. V., Rollins, N. E., Berelson, W. M., Dong, S., Erez, J., & Adkins, J. F. (2015). A novel determination of calcite dissolution kinetics in seawater. *Geochimica et Cosmochimica Acta*, 170, 51–68.

<https://doi.org/10.1016/j.gca.2015.08.011>

Methods

Wang, Z., Maucher-Fuquay, J., Fire, S. E., Mikulski, C. M., Haynes, B., Doucette, G. J., & Ramsdell, J. S. (2012). Optimization of solid-phase extraction and liquid chromatography–tandem mass spectrometry for the determination of domoic acid in seawater, phytoplankton, and mammalian fluids and tissues. *Analytica Chimica Acta*, 715, 71–79. doi:[10.1016/j.aca.2011.12.013](https://doi.org/10.1016/j.aca.2011.12.013)

Methods

Welschmeyer, N. A. (1994). Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and pheopigments. *Limnology and Oceanography*, 39(8), 1985–1992. doi:[10.4319/lo.1994.39.8.1985](https://doi.org/10.4319/lo.1994.39.8.1985)
Methods

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Related Datasets

IsRelatedTo

Kelly, K. J., Fu, F., Hutchins, D. A., Bertin, M., Mansour, A., Mancini, L. A., Jenkins, B. D., Chen, L., Kim, A. (2023) **CO2 experiment physiology and carbonate chemistry from laboratory experiments with *Pseudo-nitzschia australis* conducted from 2021 to 2022**. Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2023-08-28 doi:[10.26008/1912/bco-dmo.906938.1](https://doi.org/10.26008/1912/bco-dmo.906938.1) [[view at BCO-DMO](#)]

Relationship Description: Related dataset published in the same results publication (Kelly et al., 2023).

Kelly, K. J., Fu, F., Hutchins, D. A., Bertin, M., Mansour, A., Mancini, L. A., Jenkins, B. D., Chen, L., Kim, A. (2023) **Cluster (combined temperature, nutrient concentration, and CO2) results from laboratory experiments with *Pseudo-nitzschia australis* conducted from 2021 to 2022**. Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2023-08-28 doi:[10.26008/1912/bco-dmo.906949.1](https://doi.org/10.26008/1912/bco-dmo.906949.1) [[view at BCO-DMO](#)]

Relationship Description: Related dataset published in the same results publication (Kelly et al., 2023).

Kelly, K. J., Fu, F., Hutchins, D. A., Bertin, M., Mansour, A., Mancini, L. A., Jenkins, B. D., Chen, L., Kim, A. (2023) **Single-factor temperature experiment physiology and carbonate chemistry from laboratory experiments with *Pseudo-nitzschia australis* conducted from 2021 to 2022**. Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2023-08-28 doi:[10.26008/1912/bco-dmo.906927.1](https://doi.org/10.26008/1912/bco-dmo.906927.1) [[view at BCO-DMO](#)]

Relationship Description: Related dataset published in the same results publication (Kelly et al., 2023).

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Parameters

Parameter	Description	Units
Treatment	Treatment description including N:P ratio (5, 10, and 50), temperature (13 and 19°C), and total nutrient concentration (high and low)	unitless
Replicate	replicate (1..3)	unitless
Growth_rate	Growth rate per day. Chlorophyll a samples were collected at T-initial and T-final and used for determination of growth rates. The following equation was used to calculate specific growth rates: $\text{growth rate} = \ln(T_{\text{final}} - T_{\text{initial}}) / 2$ (where T_{final} and T_{initial} are the chlorophyll a samples collected at their respective times, and 2 is the number of days between sampling).	per day (d-1)
Particulate_DA	Particulate domoic acid. Null values indicate data point is missing as there was an issue during data collection or processing .	nanograms of domoic acid per micromole of carbon (ng DA/umol C)

Instruments

Dataset-specific Instrument Name	Costech 4010 Elemental Analyzer
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	Turner 10AU field fluorometer
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.

Dataset-specific Instrument Name	Prominence UFLC system (Shimadzu, Kyoto, Japan)
Generic Instrument Name	High-Performance Liquid Chromatograph
Dataset-specific Description	Prominence UFLC system (Shimadzu, Kyoto, Japan) coupled to a SCIEX 4500 QTRAP mass spectrometer (AB Sciex, Framingham, MA, USA)
Generic Instrument Description	A High-performance liquid chromatograph (HPLC) is a type of liquid chromatography used to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir of the mobile phase, a pump, an injector, a separation column, and a detector. Compounds are separated by high pressure pumping of the sample mixture onto a column packed with microspheres coated with the stationary phase. The different components in the mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase.

Dataset-specific Instrument Name	SCIEX 4500 QTRAP mass spectrometer (AB Sciex, Framingham, MA, USA)
Generic Instrument Name	Mass Spectrometer
Dataset-specific Description	Prominence UFLC system (Shimadzu, Kyoto, Japan) coupled to a SCIEX 4500 QTRAP mass spectrometer (AB Sciex, Framingham, MA, USA)
Generic Instrument Description	General term for instruments used to measure the mass-to-charge ratio of ions; generally used to find the composition of a sample by generating a mass spectrum representing the masses of sample components.

Dataset-specific Instrument Name	Olympus BX51 microscope
Generic Instrument Name	Microscope - Optical
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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Project Information

MCA: Developing transcriptomics as a tool to investigate toxic diatom responses to ocean heatwave and upwelling events (Toxic diatoms and heatwaves)

Coverage: University of Southern California

NSF Award Abstract:

The diatom *Pseudo-nitzschia* forms large, toxic harmful algal blooms along the U.S. West Coast, killing wildlife and harming valuable ocean fisheries. Understanding the causes of these blooms and predicting their occurrence, both now and under future changing climate conditions, is critical to coastal environmental and economic health. Puzzlingly, these blooms seem to happen during periods when coastal seawater upwelling results in cold, nutrient-rich, low pH sea surface conditions, and also during times when heat wave events cause warm, nutrient-poor, high pH conditions. These two extremes are forecast to get even more intense with climate change. This project is experimentally testing how *Pseudo-nitzschia* responds to upwelling and heat wave events using measurements of cell growth, toxin production, and gene expression. Broader impacts of this project include training the principal investigator in new gene expression methods, graduate and undergraduate research training, high school research mentoring experiences, and outreach and communications activities aimed at the commercial fishing industry. Societal benefits include obtaining a better understanding of the causes of damaging toxic algal blooms, and how they may change in the future coastal ocean.

The toxic diatom *Pseudo-nitzschia* causes annual harmful blooms along the US West Coast, a region where wind-driven upwelling brings rich nutrient supplies into the euphotic zone. However, this region is also experiencing unprecedented episodic ocean heatwave events linked to global warming. Thus, future climate trends in this region suggest an exaggeration of current physio-chemical extremes between colder, more nutrient-rich, low pH upwelling, and warmer, more nutrient-depleted, higher pH heatwaves. Surprisingly, toxic *Pseudo-nitzschia* spp. can bloom under both upwelling and heatwave conditions, despite opposite trends in key environmental controls like nutrients, temperature, and carbonate chemistry. This project is testing how

this happens by first obtaining full response curves for each of the individual factors, temperature, pCO₂, phosphorus, nitrogen, and silicon for two Pseudo-nitzschia isolates. Then, these variables are combined in holistic upwelling and heatwave scenario incubation experiments, to compare how growth and toxicity is affected in both cultures and natural blooms of Pseudo-nitzschia. The PI is assessing toxic diatom responses in these experiments using her existing expertise in algal physiology, as well as by expanding her professional horizons to develop new skills in transcriptome bioinformatics in partnership with Dr. Bethany Jenkins from the University of Rhode Island. Experiments are conducted to test the physiological responses of Pseudo-nitzschia to changes in nutrient concentrations, temperature and pCO₂ during simulated upwelling or heatwave occurrences, and measure expression of key metabolic pathway genes such as toxin synthesis pathways. This project is helping to understand and interpret the surprising niche flexibility of toxic Pseudo-nitzschia in a changing ocean, and at the same time offers the PI a new avenue forward for her future career development.

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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Funding

Funding Source	Award
National Oceanic and Atmospheric Administration (NOAA)	NA18OAR4170094
NSF Division of Ocean Sciences (NSF OCE)	OCE-2120619
National Institutes of Health (NIH)	NIH-P20GM103430

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