

# Cluster (combined temperature, nutrient concentration, and CO<sub>2</sub>) results from laboratory experiments with *Pseudo-nitzschia australis* conducted from 2021 to 2022

**Website:** <https://www.bco-dmo.org/dataset/906949>

**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)

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## Abstract

These raw data contain physiological data collected from laboratory experiments with *Pseudo-nitzschia australis*. This dataset includes results of cluster experiments of combined temperature, nutrient concentration, and CO<sub>2</sub> in order to reflect upwelling, heatwaves, and extreme heatwaves in the natural environment. 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) was 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.

These experiments were a cluster of combined temperature, nutrient concentration, and CO<sub>2</sub> in order to reflect upwelling, heatwaves, and extreme heatwaves in the natural environment. LTCN was a treatment to test the interactive effects.

Upwelling: 13°C, high CO<sub>2</sub>, high nutrients

Heatwave: 19°C, low CO<sub>2</sub>, low nutrients

Extreme heatwave: 21°C, low CO<sub>2</sub>, low nutrients

LTCN (low temperature, CO<sub>2</sub>, and nitrogen): 13°C, low CO<sub>2</sub>, low nutrients

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). <sup>14</sup>C-bicarbonate was added to 45 mL sub-cultures at T<sub>24</sub> 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 T<sub>final</sub>. Seawater from undisturbed culture bottles was removed with a sterile syringe, ejected into pre-evacuated borosilicate Exetainers, and poisoned with 5% MgCl<sub>2</sub>. 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. Experimental seawater pCO<sub>2</sub> and total alkalinity were calculated from measured DIC and pH using CO<sub>2</sub>SYS version 2.1 software (Lewis and Wallace, 1998).

Problems/Issues:

The following samples were removed from analysis, as there were issues during data collection or processing:

One DA sample was removed from analysis in the pre-industrial treatment in the CO<sub>2</sub> single factor experiment, and from the NP=50, high nutrient, 19°C treatment in the N:P ratio experiment, due to a sampling and/or analytical error. In the cluster experiment, one sample from the extreme heatwave treatment was removed from primary production analyses due to an error made during the assay.

These data points were not included in this dataset.

## BCO-DMO Processing Description

\* File cluster\_experiment.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]

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## Data Files

File
<b>Cluster experiemnt</b> filename: 906949_v1_p-australis-cluster.csv(Comma Separated Values (.csv), 1.24 KB) MD5:3151a6ba704b158582ba33bfbeefa6cf Primary data table for dataset 906949 version 1. Replicate data for cluster 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 pCO<sub>2</sub>, N<sub>2</sub> 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 CO<sub>2</sub> 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) **CO<sub>2</sub> experiment physiology and carbonate chemistry from laboratory experiments with *Pseudonitzschia 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 [[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) **N:P ratio experiment physiology and carbonate chemistry laboratory experiments with *Pseudonitzschia 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.906858.1 [[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 *Pseudonitzschia 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 [[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 type (Upwelling= 13°C, high CO <sub>2</sub> , high nutrients; Heatwave= 19°C, low CO <sub>2</sub> , low nutrients; Extreme heatwave= 21°C, low CO <sub>2</sub> , low nutrients; LTCN (low temperature, CO <sub>2</sub> , and nitrogen) = 13°C, low CO <sub>2</sub> , low nutrients).	unitless

Replicate	replicate number (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 $T_{\text{final}}$ and $T_{\text{initial}}$ 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_perC	Particulate domoic acid. The amount of intracellular domoic acid normalized to particulate organic carbon.	nanograms of domoic acid per micromole of carbon (ng DA/umol C)
Particulate_DA_perCell	Particulate domoic acid. The amount of domoic acid contained in each cell.	nanograms of domoic acid per cell (ng DA/cell)
DA_production_rate	Domoic acid production rate. Domoic acid production rates were calculated by multiplying specific growth rates by DA quotas. This value provides an estimate of how toxic a bloom might be, based on the ability of Pseudo-nitzschia to increase cell abundances and produce high DA quotas (per mol POC).	nanograms of domoic acid per micromole of carbon per day (ng DA/umol C/day)
Net_primary_productivity	Net primary production per day. The amount of carbon fixed in a 24 hour period, accounting for the amount removed by respiration. Normalized primary production (carbon fixed) to cellular carbon.	per hour (h-1)
Nitrogen_use_efficiency	Nitrogen use efficiency. Calculated by normalizing net carbon fixation rates to particulate organic nitrate. Null values indicate data point is missing as there was an issue during data collection or processing.	micromoles of carbon per micromoles of nitrogen per hour (umol C/umol N/hr)
Measured_DIC	Measured dissolved inorganic carbon (DIC). The amount of aqueous carbon dissolved in seawater.	micromoles per kilogram (umol/kg)
Measured_pH	Measured pH	total pH scale
Calculated_bulk_alkalinity	Calculated bulk alkalinity. The buffering capacity of seawater comprised on weak acids and their conjugate bases. This was calculated using CO2sys.	micromoles per kilogram (umol/kg)
Calculated_pCO2	Calculated pCO2. The partial pressure of carbon dioxide in seawater. This was calculated using CO2sys.	microatmospheres (uatm)

## Instruments

<b>Dataset-specific Instrument Name</b>	Costech 4010 Elemental Analyzer
<b>Generic Instrument Name</b>	Elemental Analyzer
<b>Generic Instrument Description</b>	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.

<b>Dataset-specific Instrument Name</b>	Turner 10AU field fluorometer
<b>Generic Instrument Name</b>	Fluorometer
<b>Generic Instrument Description</b>	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.

<b>Dataset-specific Instrument Name</b>	Prominence UFLC system (Shimadzu, Kyoto, Japan)
<b>Generic Instrument Name</b>	High-Performance Liquid Chromatograph
<b>Dataset-specific Description</b>	Prominence UFLC system (Shimadzu, Kyoto, Japan) coupled to a SCIEX 4500 QTRAP mass spectrometer (AB Sciex, Framingham, MA, USA)
<b>Generic Instrument Description</b>	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.

<b>Dataset-specific Instrument Name</b>	Wallac System 1400 liquid scintillation counter
<b>Generic Instrument Name</b>	Liquid Scintillation Counter
<b>Generic Instrument Description</b>	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 to quantify the activity of particulate emitting ( $\beta$ and $\alpha$ ) radioactive samples, it can also detect the Auger electrons emitted from $^{51}\text{Cr}$ and $^{125}\text{I}$ samples. Liquid scintillation counters are instruments assaying alpha and beta radiation by quantitative detection of visible light produced by the passage of rays or particles through a suitable scintillant incorporated into the sample.

<b>Dataset-specific Instrument Name</b>	SCIEX 4500 QTRAP mass spectrometer (AB Sciex, Framingham, MA, USA)
<b>Generic Instrument Name</b>	Mass Spectrometer
<b>Generic Instrument Description</b>	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.

<b>Dataset-specific Instrument Name</b>	Olympus BX51 microscope
<b>Generic Instrument Name</b>	Microscope - Optical
<b>Generic Instrument Description</b>	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".

<b>Dataset-specific Instrument Name</b>	Mettler Toledo SevenCompact pH meter
<b>Generic Instrument Name</b>	pH Sensor
<b>Dataset-specific Description</b>	Mettler Toledo SevenCompact pH meter (calibrated using a three-point calibration curve and total pH scale)
<b>Generic Instrument Description</b>	An instrument that measures the hydrogen ion activity in solutions. The overall concentration of hydrogen ions is inversely related to its pH. The pH scale ranges from 0 to 14 and indicates whether acidic (more $\text{H}^+$ ) or basic (less $\text{H}^+$ ).

<b>Dataset-specific Instrument Name</b>	Picarro cavity ring-down spectrophotometer
<b>Generic Instrument Name</b>	Spectrometer
<b>Generic Instrument Description</b>	A spectrometer is an optical instrument used to measure properties of light over a specific portion of the electromagnetic spectrum.

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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, pCO<sub>2</sub>, 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 pCO<sub>2</sub> 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)	<a href="#">NA18OAR4170094</a>
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-2120619</a>

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