Physiological data from Zn and CO2 culture experiments on the diatom Phaeodactylum tricornutum

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Project
» Parsing the biogeochemistry of marine carbonic anhydrases (Carbonic Anhydrase)

Contributors	Affiliation	Role
Saito, Mak A.	Woods Hole Oceanographic Institution (WHOI)	Co-Principal Investigator
Subhas, Adam V.	Woods Hole Oceanographic Institution (WHOI)	Co-Principal Investigator
McIlvin, Matthew R.	Woods Hole Oceanographic Institution (WHOI)	Scientist
Burdige, Emily	Woods Hole Oceanographic Institution (WHOI)	Student, Contact
Hayden, Matthew G.	Woods Hole Oceanographic Institution (WHOI)	Technician
Soenen, Karen	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
Phaeodactylum tricornutum was grown under 6 different experimental treatments all carried out in biological triplicate. In a CO2 experiment, cultures were grown with replete Zn under varying pCO2 (200, 400, and 750 ppm CO2). In a Zn experiment, cultures were grown under ambient pCO2 (no modification to bubbling) with variable Zinc concentrations (3 nM, 30 nM, and 100 nM [Zn]T) Here, we assess the growth response via flow cytometry measurements, carbonate chemistry composition of the seawater using measurements of dissolved inorganic carbon and total alkalinity, and carbonic anhydrase activity using membrane inlet mass spectrometry. The data collected for this project are used to assess the plasticity of the model diatom Phaeodactylum tricornutum's CCMs, specifically carbonic anhydrase (CA) enzymes and bicarbonate transporters.

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Methods & Sampling

Growth rates: "Growth rates were calculated via a linear regression of the natural log of cell counts during their exponential growth. To monitor growth, the cell densities of all cultures were measured using a flow cytometer (BD Accuri C6 Plus) and gated according to their Forward Scatter and PerCP-A (Peridinin-Chlorophyll-Protein A) concentration. At cell densities exceeding 104 cells mL-1, the aliquot taken for flow cytometry was diluted down for an accurate cell concentration measurement."

Measurements of seawater carbonate chemistry profile: "Seawater samples were taken for total alkalinity (TA, µmol kg-1) and DIC (µmol kg-1) were taken at the termination of the experiment to examine the carbonate chemistry profile of the seawater at the same time point at which samples were taken for CA activity proteomics. Samples were passed through a 0.2 µm filter (Whatman) to remove biomass and stored in 13 mL borosilicate vials where they were poisoned with mercuric chloride (0.02 % final concentration), sealed without

headspace, and stored in the dark at room temperature prior to measurement. TA was measured using an open-system Gran titration on 1.5 mL samples brought to a total volume of 5 mL with Milli-Q water in technical duplicate using a Metrohm 805 Dosimat and a robotic Titrosampler (Borer et al., 2026). The precision of TA measurements assessed by the reproducibility of the standard was ± 2.5 µmol kg−1. DIC was measured on 1.5 mL samples in technical duplicate using an Apollo SciTech AS-C6L DIC analyzer equipped with a LI-COR 7815 CO2 detector (Wang et al., 2017). The precision of DIC measurements as calculated by the mean difference between technical duplicates was ± 4.2 µmol kg−1. Measurements of TA and DIC were calibrated against in-house standards using seawater collected from Vineyard Sound, which were previously calibrated against seawater-certified reference materials (Batch #205) (Dickson, 2026). Measured values of DIC and TA were used as input parameters along with T, Sal, Total P, and Total Si in CO2SYS (Lewis et al., 1998) (ver. 2.3; using constants from Mehrbach et al. (1973) refit by Dickson and Millero (1987)) to calculate pH, [HCO3-], [CO32-], [CO2]aq, and ppm CO2."

Carbonic anhydrase activity: "Carbonic anhydrase activity was assayed according to an 18O depletion method utilizing a membrane inlet mass spectrometer (MIMS), which measures concentrations of dissolved gases (Burlacot et al., 2020). A Pfeiffer QMG 220 mass spectrometer (closed-type ion source, 1–100 AMU mass range) was fitted with custom-made inlet consisting of a 6 inch long, 1/1600 O.D. stainless steel needle with 1 inch of perforations at the tip. Silastic tubing (0.23 mm thick) was gently slid over the needle to cover the perforations and was fixed by a knot at the tip of the needle. The needle functioned in tandem with the mass spec's high vacuum (operating at 4×10−6 mbar) to pull dissolved gases from the sample or standard solution, without introduction of water, into the quadrupole mass spectrometer for detection based on their mass to charge (m/z) ratio.

The 18O depletion method tracks the ion currents of six different CO2 isotopes over time as a double-labeled spike solution (NaH13C18O3, m/z 49) interacts with a background buffer solution (described above). To prepare the spike solution, a 13C-labeled sodium bicarbonate salt (85 mg; MilliporeSigma 870081-58-1) was equilibrated in 1 mL of 18O-labeled water (≥ 97% labeled 18O; MilliporeSigma 14314-42-2) for 24 hr. For every sample, 15 µL of spike was pipetted into the 5 mL aliquot of a sample slurry solution (phosphate buffer medium (0.14 M sodium phosphate, pH 8) and resuspended cell pellet). The reaction was run in a conical reaction vial (MilliporeSigma Z115150) with a stir bar (MilliporeSigma 23227) gently agitating the solution and was held in a thermostat water jacket kept at 20°C. Utilizing the MIMS rapid response time, the interaction of the spike and the buffer are closely followed.

The addition of the spike results in an initial increase in m/z 49, which is immediately diluted by the buffer's intense background 16O signal. The fractional abundance of 18O in 13CO2 is used to calculate a rate of equilibration. Taking the slope of the fractional abundance in log space versus time yields the rate of CO2 hydration/dehydration (Subhas et al., 2019; Uchikawa & Zeebe, 2012).

Bovine carbonic anhydrase (BCA) was used to create a calibration between known concentrations of BCA and the measured rates of CA activity. Standard curves were used to assess instrument performance but were not used to calculate an effective CA concentration for samples, as the activity of a known concentration of bovine carbonic anhydrase is incomparable to phytoplankton CAs (Krishnamurthy et al., 2008). Additionally, "blank" measurements of buffer containing no standard addition of BCA were run every analytical session. The mean plus 1σ standard deviation of all the blanks provided a conservative estimate of the variability in these measurements. This value was subtracted from the sample's measured CA activity to achieve the blank subtracted CA activity. The data in this manuscript are reported as blank subtracted CA activity normalized to the number of cells analyzed for the respective sample, with units of reactions sec-1 cell-1."

Data Processing Description

Replicate measurements as well as calculated average and standard deviations are reported.

BCO-DMO Curation Notes

This section documents curation actions performed prior to publication review with the submitter, and additional information relevant to understanding and reusing this dataset. It distinguishes changes made to the submitted (meta)data from unresolved issues and/or enhancements that improve future reuse and interoperability.

CURATION ACTIONS PERFORMED ON DATA
- Split the submitted file (Ptricornutum_phsyiology_BCODMO.csv) into two tables: 1010840_v1_physiology (replicate-level data) and 1010840_v1_physiology_statistics (treatment means and standard deviations).
- Reshaped the replicate-level data from wide to long format, so replicate number is a column in 1010840_v1_physiology.
- Removed the per-replicate columns from the statistics table, since they are in the replicate-level table. The statistics table keeps only averages and standard deviations.
- Collapsed repeated whitespace in treatment names to a single space in both tables.
- Missing values are blank or “nd” in both tables.c
- Standardized column names in both tables.

CURATION ACTIONS PERFORMED ON METADATA

- BCO-DMO's standard metadata entry and text formatting steps were performed. See: https://www.bco-dmo.org/how-to/standard-curation-edits

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Related Publications

Borer, B., Subhas, A. V., Hayden, M. G., Woosley, R. J., & Babbin, A. R. (2026). Microbially enhanced dissolution of calcite in sinking marine particles. Proceedings of the National Academy of Sciences, 123(11). https://doi.org/10.1073/pnas.2510025123
Methods

Burdige, E. A., Saito, M. A., Hayden, M. G., McIlvin, M. R., & Subhas, A. V. (2026). Carbon concentrating mechanism and growth response of the diatom P. tricornutum to changes in Zn and carbonate chemistry. https://doi.org/10.64898/2026.05.21.726966
Results

Burlacot, A., Burlacot, F., Li-Beisson, Y., & Peltier, G. (2020). Membrane Inlet Mass Spectrometry: A Powerful Tool for Algal Research. Frontiers in Plant Science, 11. https://doi.org/10.3389/fpls.2020.01302
Methods

Dickson, A. G., & Millero, F. J. (1987). A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep Sea Research Part A. Oceanographic Research Papers, 34(10), 1733–1743. doi:10.1016/0198-0149(87)90021-5
Methods

Dickson, A., National Oceanic and Atmospheric Administration (n.d.). CRM Batches: Information on Batches of CO2 in Seawater Reference Material. Ocean Carbon and Acidification Data System. Available from https://www.nodc.noaa.gov/ocads/oceans/Dickson_CRM/batches.html
Methods

Krishnamurthy, V. M., Kaufman, G. K., Urbach, A. R., Gitlin, I., Gudiksen, K. L., Weibel, D. B., & Whitesides, G. M. (2008). Carbonic Anhydrase as a Model for Biophysical and Physical-Organic Studies of Proteins and Protein−Ligand Binding. Chemical Reviews, 108(3), 946–1051. https://doi.org/10.1021/cr050262p
Methods

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
Methods

Mehrbach, C., Culberson, C. H., Hawley, J. E., & Pytkowicx, R. M. (1973). Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnology and Oceanography, 18(6), 897–907. doi:10.4319/lo.1973.18.6.0897
Methods

Subhas, A. V., Adkins, J. F., Dong, S., Rollins, N. E., & Berelson, W. M. (2019). The carbonic anhydrase activity of sinking and suspended particles in the North Pacific Ocean. Limnology and Oceanography, 65(3), 637–651. Portico. https://doi.org/10.1002/lno.11332
Methods

Uchikawa, J., & Zeebe, R. E. (2012). The effect of carbonic anhydrase on the kinetics and equilibrium of the oxygen isotope exchange in the CO2–H2O system: Implications for δ18O vital effects in biogenic carbonates. Geochimica et Cosmochimica Acta, 95, 15–34. doi:10.1016/j.gca.2012.07.022
Methods

Wang, Z. A., Lawson, G. L., Pilskaln, C. H., & Maas, A. E. (2017). Seasonal controls of aragonite saturation states in the Gulf of Maine. Journal of Geophysical Research: Oceans, 122(1), 372–389. doi:10.1002/2016jc012373 https://doi.org/10.1002/2016JC012373
Methods

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Parameters

Parameter	Description	Units
Treatment_Name	experimental treatment names	unitless
zn2_conc	[Zn2+] in the seawater	picomolar Zn2+ (pM)
co2_ppm	ppm CO2 bubbled in the seawater (ambient = not bubbled)	parts per million (ppm)
Replicate	Replicate number (1, 2 or 3)	unitless
Growth_Rate	Growth rate measured for biological replicate	1/day
CA_Activity	carbonic anhydrase activity measured for biological replicate	reactions per sec per cell (rxn/sec/cell)
DIC_Final	Final dissolved inorganic carbon concentration measured for biological replicate	micromol per kilogram seawater (umol/kg)
Alk_Final	Final Total Alkalinity concentration measured for biological replicate	micromol per kilogram seawater (umol/kg)
PH_Final	Final pH calculated for biological replicate	unitless
HCO3	bicarbonate ion concentration calculated for biological replicate	micromol per kilogram seawater (umol/kg)
CO3	carbonate ion concentration calculated for biological replicate	micromol per kilogram seawater (umol/kg)
CO2_aq	aqueous carbon dioxide concentration calculated for biological replicate	micromol per kilogram seawater (umol/kg)
CO2	partial pressure of carbon dioxide calculated for biological replicate	parts per million (ppm)

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Instruments

Dataset-specific Instrument Name	Apollo SciTech AS-C6L DIC analyzer
Generic Instrument Name	Apollo SciTech AS-C6L Dissolved Inorganic Carbon (DIC) analyzer
Dataset-specific Description	Dissolved inorganic carbon was measured using an Apollo SciTech AS-C6L DIC analyzer equipped with a LI-COR 7815 CO2 detector.
Generic Instrument Description	An instrument designed for the analysis of dissolved inorganic carbon in samples from various aquatic environments. It comprises of a laser-based CO2 detector (LI-7815), a digital syringe pump, a mass flow controller, CO2 stripping reactor, an electronic cooling system and a computer communication assembly (RS-485, USB). The AS-C6L supersedes the earlier AS-C3 model, which used non-dispersive infra-red CO2 detection (LI-7000, discontinued). The AS-C6L improves on the AS-C3 by incorporating a multi-sampler of one set of standards plus 8 samples, and uses improved Apollo SciTech software. The AS-C6L is suitable for use in either shipboard or land-based laboratories. It maintains a precision of +/-0.1 % for seawater (or +/-2 umol/kg), enables sample volumes ranging from 0.5 - 3.5 ml per analysis, and an analytical rate of approximately 3 minutes.

Dataset-specific Instrument Name	BD Accuri C6 Plus
Generic Instrument Name	BD Biosciences Accuri C6 Plus flow cytometer
Dataset-specific Description	Cell counts were measured using a BD Accuri C6 Plus. Samples were analyzed in a 96-well plate. An average cell count between two technical replicates was reported for each sample. The instrument was calibrated using the BD CS&T RUO beads with a known median fluorescence intensity (MFI) and distribution (rCV) to monitor performance.
Generic Instrument Description	A flow cytometer designed to detect fluorochromes (including FITC, PE, APC), polymer dyes, and fluorescent proteins (including GFP, YFP, mCherry). The instrument is equipped with a blue and red laser, two light scatter detectors, and four fluorescence detectors with user-interchangeable filters. A non-pressurised peristaltic pump system drives the fluidics. The system monitors the sample volume pulled per run and can calculate absolute counts or sample concentration per microliter (uL). In the standard configuration (3-blue/1-red), three detectors read fluorescence emissions from fluorochromes excited by the blue laser, while a fourth detector reads emissions from fluorochromes excited by the red laser. The optional Selectable Laser Module allows the system to operate in 2-blue/2-red and 4-blue configurations. The minimum detectable particle size and sample volume are 0.5 micrometers (um) and 0.5 uL respectively. The maximum number of events is 1 million with an acquisition rate of up to 10,000 events/second.

Dataset-specific Instrument Name	Pfeiffer QMG 220 mass spectrometer
Generic Instrument Name	Membrane Inlet Mass Spectrometer
Dataset-specific Description	Carbonic anhydrase activity measurements were made with a membrane inlet mass spectrometer, which uses a quadrupole mass spectrometer for detection based on ions mass to charge (m/z) ratio.
Generic Instrument Description	Membrane-introduction mass spectrometry (MIMS) is a method of introducing analytes into the mass spectrometer's vacuum chamber via a semipermeable membrane.

Dataset-specific Instrument Name	Metrohm 805 Dosimat and a robotic Titrosampler
Generic Instrument Name	Metrohm 805 Dosimat
Dataset-specific Description	Total alkalinity was measured using a Metrohm 805 Dosimat and a robotic Titrosampler. A pH calibration was run prior to any analytical session in order to assess electrode sensitivity. The slope of this calibration needed to be > 95% to ensure accurate data reporting.
Generic Instrument Description	The Metrohm 805 Dosimat is a dispensing instrument for titrating and dosing operations in the laboratory. The 805 Dosimat is controlled by Touch control or PC control software. The instrument controls the dosing of liquids, which are attached via an exchange unit. Metrohm recommends using the Metrohm 806 Exchange units which come with 1, 5, 10, 20, or 50 milliliter (mL) dosing cylinders. The instrument can read and overwrite data from the exchange unit. It has a resolution of 20,000 steps per cylinder volume and a dosing/filling time of 18 seconds. The 805 Dosimat and the corresponding 806 Exchange Unit are suitable as a buret not only for simply dosing auxiliary solutions but also for titrations. Additional information is available from the instrument manufacturer: https://www.metrohm.com/en_au/products/2/8050/28050010.html

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Project Information

Parsing the biogeochemistry of marine carbonic anhydrases (Carbonic Anhydrase)

Coverage: Woods Hole Oceanographic Institution, Bermuda Atlantic Timeseries Station

Although carbon dioxide (CO2) is found both in the atmosphere and in sea water, it does not react quickly with water. This presents a problem for marine organisms that need to wait for CO2 to react with water before using it for photosynthesis. Therefore, organisms use an enzyme to greatly speed up this reaction. The enzyme, known as carbonic anhydrase (CA), is so useful that it is found across the entire tree of life. However, the role of carbonic anhydrase in the CO2 cycle has been hard to measure. In this project, a team of investigators from the Woods Hole Oceanographic Institution will use a combination of instrument techniques and laboratory experiments to investigate the role of different types of CA in marine organisms. The team will identify the different types of CA and their inherent catalytic rates. The team will then identify the metal cofactors present in these enzymes, which are key for enzyme function. This work will deepen our understanding of the biochemical basis for carbon dioxide cycling in marine phytoplankton, and will provide key insights into one of the most ubiquitous and fastest enzymes on the planet. The team will collaborate with Professor Indu Sharma and her students from Hampton University which has just launched a new marine science major program. Team members will visit Hampton University for a workshop on careers in

oceanography. In Year 2, Professor Sharma and her students will accompany the team on a research expedition to the Bermuda-Atlantic Time Series and will help design a sampling campaign focused on the shared research interests of Professor Sharma and the investigators.

Carbonic anhydrases (CAs) are ubiquitous enzymes involved in almost every carbon transformation in the marine environment. Every CA uses a metal cofactor in its active site, and the expression of CAs is tied to the uptake and utilization of trace metals such as zinc and cobalt in the surface ocean. We are starting to understand that organisms express many different forms of CA, all of which serve distinct physiological roles; exhibit unique catalytic rates; and can use different metal cofactors at the enzyme active site. In this proposal, the team will exploit this enzymatic diversity to investigate carbonic anhydrase kinetics in marine diatoms. By combining an isotope-based enzyme activity assay with quantitative proteomics, they will document the inherent kinetics of multiple CA isoforms. In addition, they will identify the metal cofactors associated with the most abundant CAs, therefore providing a direct linkage between enzyme activity and trace metal utilization.

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
NSF Division of Ocean Sciences (NSF OCE)	OCE-2123055

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