Net calcification of coral fragments collected as part of a study of pCO2 variability on the reef-building coral Pocillopora damicornis conducted at Heron Island Research Station, Heron Island, southern Great Barrier Reef in 2021

Website: https://www.bco-dmo.org/dataset/885664
Data Type: Cruise Results
Version: 1
Version Date: 2022-12-20

Project
» Influence of environmental pH variability and thermal sensitivity on the resilience of reef-building corals to acidification stress (Coral Resilience)

Contributors	Affiliation	Role
Barott, Katie	University of Pennsylvania (Penn)	Principal Investigator
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Abstract
This dataset contains the overall change in net calcification of the coral fragments (% change in buoyant weight per day). These data were collected as part of a study of pCO2 variability on the reef-building coral Pocillopora damicornis conducted at Heron Island Research Station, Heron Island, southern Great Barrier Reef in 2021 (Brown et al., 2022). Abstract for all data from the study (Brown et al., 2022) including this dataset: Ocean acidification is a growing threat to coral growth and the accretion of coral reef ecosystems. Corals inhabiting environments that already endure extreme diel pCO2 fluctuations, however, may represent acidification resilient populations capable of persisting on future reefs. Here, we examined the impact of pCO2 variability on the reef-building coral Pocillopora damicornis originating from reefs with contrasting environmental histories (variable reef flat vs. stable reef slope) following reciprocal exposure to stable (218 ± 9) or variable (911 ± 31) diel pCO2 amplitude (µtam) in aquaria over eight weeks. This study measured: growth (net calcification, extension, CaCO3 density) and physiology (dark respiration, light-enhanced dark respiration, host soluble protein, mycosporine-like amino acids, net photosynthesis, photosynthetic efficiency, endosymbiont density, chlorophyll a concentration, intracellular pH) of P. damicornis across treatment and origin. See all datasets related to this publication (https://www.bco-dmo.org/related-resource/885684).

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Coverage

Spatial Extent: **Lat**:-23.27 **Lon**:151.55
Temporal Extent: 2021-01-06 - 2021-04-06

Methods & Sampling

This methodology describes this dataset and other datasets from this experiment. See "Related Datasets" section for data access and more details of each related dataset.

Physiological analyses

Coral survivorship was assessed visually daily, and only one coral fragment died during the experiment. Net calcification, surface area (a proxy for extension; Rathbone (2021)), volume, and dark-adapted photosynthetic efficiency (Fv/Fm) of coral fragments were measured six times during the experiment (~2 week intervals) via buoyant weight and photogrammetry using previously described methods (Davies, 1989; Brown et al. 2021; Ferrari et al., 2016) (Brown et al. (2022) Supp Methods, Figure S4, Figure S5). At the end of the experiment, metabolic rates (net photosynthesis, dark respiration and light-enhanced dark respiration) were assessed via changes in oxygen evolution using oxygen optodes connected to an OXY-10 (PreSens) optical analyzer (Brown et al. (2019) Supp Methods). Upon completion of these living analyses, half of the coral fragments were flash frozen in liquid nitrogen and stored at -80°C. Subsequent laboratory analyses were done on these 48 specimens. For these analyses, corals (n = 12) were water-piked on ice to remove coral tissue from the skeleton using 50 mL of 0.1 M phosphate buffered saline solution. The tissue slurry was centrifuged at 4°C once for 5 min at 2500 *g* to sufficiently separate host tissue and the intracellular endosymbiont cells. Host tissue was analyzed for host-soluble protein concentration and mycosporine-like amino acids (MAAs) concentrations spectrophotometrically (Whitaker and Granum, 1980). Endosymbiont densities were determined from cell counts of three aliquots using a hemocytometer (Brown et al., 2019). Host protein concentration and endosymbiont cell densities were standardized to surface area (cm2), which was determined using the single wax-dipping technique (Holmes, 2008), whereas MAAs were normalized to host protein content. Endosymbiont photopigments were extracted in 100% acetone for 24 hours and concentration of chlorophyll *a* was determined via absorbance at 630, 663, and 750 nm using the equations in (Jeffrey and Humphrey, 1975). Pigment concentrations were standardized to both surface area and endosymbiont densities. Wax-dipping was also used to determine calcium carbonate (CaCO3) bulk density, where the skeleton was sealed with a coat of wax, dry weighed, and then buoyant weighed (Tambutté et al., 2015). The difference between dry weight and buoyant weight was calculated to determine the bulk volume, which was subtracted from the dry weight to yield bulk density. The other half of the fragments were transported alive from Heron Island to the University of Queensland, Brisbane to assess intracellular acid-base status and acidification resilience following established methods (Innis et al., 2021). Briefly, cells were loaded with SNARF-1AM and imaged using a confocal microscope (Zeiss LSM 710) via excitation at 561 nm, with SNARF-1 fluorescence emission acquired in two channels (585 and 640 ± 10 nm) simultaneously (see full details in Supp Methods of Brown et al., 2022).

Physiological analyses

Net calcification of coral fragments was measured six times across the experiment (22 Jan, 30 Jan, 12 Feb, 26 Feb, 12 Mar and 19 Mar of 2021) using the buoyant weight technique (Rathbone et al., 2021; Camp et al., 2018). At the same time, surface area and volume were quantified via non-invasive three-dimensional photogrammetry, with fragment reconstructions created from a set of ≥50 photographs using the program Autodesk ReCap Photo (Camp et al., 2018; Brown et al., 2022 Figure S4). On the night prior to growth measurements, dark-adapted photochemical efficiency (Fv/Fm) was quantified using a Diving-PAM (Walz GmbH) approximately 1 hour after sunset. Measurements were made using the Diving-PAM 5-mm diameter fibre-optic probe at a standardized distance 5 mm above the coral tissue after F0 stabilized (n=3 per fragment). At the end of the experiment, metabolic rates were assessed via changes in oxygen evolution using oxygen optodes connected to an optical analyzer (OXY-10, PreSens) (Kenkel and Matz, 2016). Coral fragments were analyzed at the end of the experimental period between 08:00 and 18:00 within 140 cm3 clear acrylic chambers on top of a magnetic stirrer to allow for continuous mixing. Corals were dark-adapted for at least 30 min prior to each assay, which followed a light program of 20 min of darkness (0 µmol quanta m-2 s-1) to measure dark respiration, 25 min of midday light levels (~500 µmol quanta m-2 s-1) to determine maximum net photosynthesis and 15 min of darkness (0 µmol quanta m-2 s-1) to determine light enhanced dark respiration. Seawater conditions were replicated to those experienced in the tanks by: (1) using seawater collected from treatment tanks to provide initial pCO2 concentrations and (2) using a water bath to maintain respective treatment temperatures within the incubation chambers. Tank water was filtered using a 0.22µm filter and oxygen content of the seawater was lowered to 70% using N2 to avoid hyperoxia. Chambers were completely drained and cleaned with a soft sponge in between trials.

Assessment of coral intracellular acid–base homeostasis

The other half of the fragments were transported alive from Heron Island Research Station (HIRS) to the University of Queensland, Brisbane to assess intracellular acid-base status and acidification resilience following established methods. Corals were held in an indoor, closed system (186 L per treatment; n = 2 tanks per treatment) that replicated stable and variable pCO2 conditions similar to the controllers at HIRS via the addition

of CO2 or CO2-free air using Apex controllers and probes (Neptune Systems). PAR followed a 12 hr:12 hr day:night cycle, with mean PAR ~125 µmol quanta m−2 s−1. Corals were held for a maximum of two weeks, and were selected for analysis randomly. Coral cells were isolated by submerging the fragment in a shallow dish of 50mL filtered seawater (FSW) and gently brushing with a soft toothbrush. Cells were filtered through at 100 µM cell strainer into a clean 50mL tube and centrifuged for 4 min at 350 x g to pellet the cells. The supernatant was decanted, and the pellet was resuspended in 2 mL FSW. Cells were loaded with SNARF- 1 AM by adding 1 µL SNARF-1 AM (20 mM) and 1 µL pluronic acid (20% w/v) to 2 mL cells for 30 min in darkness. Isolated cells were spun down using a benchtop microfuge for 5 sec, discarding the supernatant and resuspending in ambient filtered seawater (pHe 8.0) or in acidified seawater (pHe 7.4) in darkness to measure cellular response to acidosis. Cells were imaged using a confocal microscope (Zeiss LSM 710) via excitation at 561 nm, with SNARF- 1 AM fluorescence emission acquired in two channels (585 and 640 ± 10 nm) simultaneously. A total of 8–10 cells containing algal symbionts (symbiocytes) and 8–10 cells without symbionts (non-symbiocytes) were imaged for each coral fragment. To measure acid stress response, SNARF-1 AM loaded cells were imaged over time (0, 15, 30, 45, 60, 75 minutes after acid stress) in darkness. SNARF-1 AM fluorescence emission was quantified in ImageJ by drawing two regions of interest per cell within the coral cytoplasm. A region drawn in the surrounding medium was used to subtract background fluorescence, and for each region of interest, a fluorescence ratio was calculated and converted to pH using a calibration curve generated as previously described (Oliver and Palumbi, 2011). The rate of pHi recovery was calculated as the slope of pHi from 15 to 60 minutes post-acidification.

For more detailed information, please see: Brown et al. (2022).

Data Processing Description

See results publication Brown et al., 2022 for details of statistical analyses performed using these data. All statistical analyses were done using R version 4.0.0 software (R Core Team, 2021), and graphical representations were produced using the package ggplot2 (Wickham, 2016).

The analysis code package for 'Environmental memory gained from exposure to extreme pCO2 variability promotes coral cellular acid-base homeostasis" published as Brown (2022, doi: 10.5281/zenodo.7373705) which is a publication of github repository https://github.com/imkristenbrown/pCO2-variability-promotes-coral-cellular-acid-base-homeostasis.

The code package includes the following R-markdown files and supplemental files related to this dataset:

'Calcification, physiology and pHi.Rmd' = R markdown file with code for analyses and figures for calcification, physiology and intracellular pH (pHi) data.

'Calcification by BW overall percent change.csv' = Overall change in net calcification (% change in buoyant weight per day). This is the source data file that was imported for this BCO-DMO dataset https://www.bco-dmo.org/dataset/885664.

'pHi all.csv' = Intracellular pH (pHi) data. This is the source data file that was imported for related BCO-DMO dataset https://www.bco-dmo.org/dataset/885646.

'Heron pHi coral physiology R.csv' = Coral physiology and intracellular pH (pHi) data. This is the source data file that was imported for related BCO-DMO dataset https://www.bco-dmo.org/dataset/885659.

'Percent change in SA and vol weekly.csv' = Weekly change in surface area (% change in surface area per day) and volume (% change in volume per day)

'Respirometry analysis.csv' = Photosynthesis and respiration rates data

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Data Files

File
calcification_by_bw.csv (Comma Separated Values (.csv), 4.32 KB) MD5:d640f2eb0b27ec8f3ce1830391413906
Primary data file for dataset ID 885664

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Related Publications

Brown, K. T. (2022). Barott Lab/Heron pHi [Data set]. Zenodo. https://doi.org/10.5281/ZENODO.7373705 https://doi.org/10.5281/zenodo.7373705
Software

Brown, K. T., Bender-Champ, D., Kenyon, T. M., Rémond, C., Hoegh-Guldberg, O., & Dove, S. (2019). Temporal effects of ocean warming and acidification on coral–algal competition. Coral Reefs, 38(2), 297–309. https://doi.org/10.1007/s00338-019-01775-y
Methods

Brown, K. T., Bender-Champ, D., Achlatis, M., Zande, R. M., Kubicek, A., Martin, S. B., Castro-Sanguino, C., Dove, S. G., & Hoegh-Guldberg, O. (2020). Habitat-specific biogenic production and erosion influences net framework and sediment coral reef carbonate budgets. Limnology and Oceanography, 66(2), 349–365. Portico. https://doi.org/10.1002/lno.11609
Methods

Brown, K. T., Mello-Athayde, M. A., Sampayo, E. M., Chai, A., Dove, S., & Barott, K. L. (2022). Environmental memory gained from exposure to extreme pCO2 variability promotes coral cellular acid–base homeostasis. Proceedings of the Royal Society B: Biological Sciences, 289(1982). https://doi.org/10.1098/rspb.2022.0941
Results

Camp, E. F., Schoepf, V., Mumby, P. J., Hardtke, L. A., Rodolfo-Metalpa, R., Smith, D. J., & Suggett, D. J. (2018). The Future of Coral Reefs Subject to Rapid Climate Change: Lessons from Natural Extreme Environments. Frontiers in Marine Science, 5. https://doi.org/10.3389/fmars.2018.00004
Methods

Davies, P.S. (1989). Short-term growth measurements of corals using an accurate buoyant weighing technique. Marine Biology, 101(3), 389–395. doi:10.1007/bf00428135 https://doi.org/10.1007/BF00428135
Methods

Ferrari, R., McKinnon, D., He, H., Smith, R., Corke, P., González-Rivero, M., Mumby, P., & Upcroft, B. (2016). Quantifying Multiscale Habitat Structural Complexity: A Cost-Effective Framework for Underwater 3D Modelling. Remote Sensing, 8(2), 113. https://doi.org/10.3390/rs8020113
Methods

Holmes, G. (2008). Estimating three-dimensional surface areas on coral reefs. Journal of Experimental Marine Biology and Ecology, 365(1), 67–73. https://doi.org/10.1016/j.jembe.2008.07.045
Methods

Innis, T., Allen-Waller, L., Brown, K. T., Sparagon, W., Carlson, C., Kruse, E., Huffmyer, A. S., Nelson, C. E., Putnam, H. M., & Barott, K. L. (2021). Marine heatwaves depress metabolic activity and impair cellular acid-base homeostasis in reef-building corals regardless of bleaching susceptibility. https://doi.org/10.1101/2021.02.23.432550
Methods

Jeffrey, S. W., & Humphrey, G. F. (1975). New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. Biochemie Und Physiologie Der Pflanzen, 167(2), 191–194. doi:10.1016/s0015-3796(17)30778-3 https://doi.org/10.1016/S0015-3796(17)30778-3
Methods

Kenkel, C. D., & Matz, M. V. (2016). Gene expression plasticity as a mechanism of coral adaptation to a variable environment. Nature Ecology & Evolution, 1(1). https://doi.org/10.1038/s41559-016-0014
Methods

Oliver, T. A., & Palumbi, S. R. (2011). Do fluctuating temperature environments elevate coral thermal tolerance? Coral Reefs, 30(2), 429–440. https://doi.org/10.1007/s00338-011-0721-y
Methods

R Core Team (2021). R: A language and environment for statistical computing. R v4.0.0. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/
Software

Rathbone, M., Brown, K. T., & Dove, S. (2021). Tolerance to a highly variable environment does not infer resilience to future ocean warming and acidification in a branching coral. Limnology and Oceanography, 67(1), 272–284. Portico. https://doi.org/10.1002/lno.11991
Methods

Tambutté, E., Venn, A. A., Holcomb, M., Segonds, N., Techer, N., Zoccola, D., Allemand, D., & Tambutté, S. (2015). Morphological plasticity of the coral skeleton under CO2-driven seawater acidification. Nature Communications, 6(1). https://doi.org/10.1038/ncomms8368
Methods

Whitaker, J. R., & Granum, P. E. (1980). An absolute method for protein determination based on difference in absorbance at 235 and 280 nm. Analytical Biochemistry, 109(1), 156–159. https://doi.org/10.1016/0003-2697(80)90024-x https://doi.org/10.1016/0003-2697(80)90024-X
Methods

Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York. ISBN 978-3-319-24277-4, https://ggplot2.tidyverse.org. https://doi.org/10.1007/978-3-319-24277-4
Methods

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Related Datasets

IsRelatedTo

Brown, K. T., Barott, K. (2023) **Intracellular pH (pHi) data collected as part of a study of pCO2 variability on the reef-building coral Pocillopora damicornis conducted at Heron Island Research Station, Heron Island, southern Great Barrier Reef in 2021.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2022-12-20 doi:10.26008/1912/bco-dmo.885646.1 [view at BCO-DMO]
Relationship Description: Data from the same experiment.

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Parameters

Parameter	Description	Units
Origin	Origin (Flat | Slope | Experiment)	unitless
Treatment	Treatment (Stable | Variable)	unitless
Coral_ID	Coral Identifier	unitless
Colony	Colony identifier	unitless
Cal	Calcification (percent change)	percent (%)
Cal_day	Calcification rate (percent change per day)	percent (%) change per day (d-1)

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Project Information

Influence of environmental pH variability and thermal sensitivity on the resilience of reef-building corals to acidification stress (Coral Resilience)

Coverage: Kaneohe Bay, Oahu, HI; Heron Island, Queensland, Australia

NSF Award Abstract:
Coral reefs are incredibly diverse ecosystems that provide food, tourism revenue, and shoreline protection for coastal communities. The ability of coral reefs to continue providing these services to society is currently threatened by climate change, which has led to increasing ocean temperatures and acidity that can lead to the death of corals, the animals that build the reef framework upon which so many species depend. This project examines how temperature and acidification stress work together to influence the future health and survival of corals. The scientists are carrying out the project in Hawaii where they have found individual corals with different sensitivities to temperature stress that are living on reefs with different environmental pH conditions. This project improves understanding of how an individual coral's history influences its response to multiple stressors and helps identify the conditions that are most likely to support resilient coral communities. The project will generate extensive biological and physicochemical data that will be made freely available. Furthermore, this project supports the education and training of undergraduate and high school students and one postdoctoral researcher in marine science and coral reef ecology. Hands-on activities for high school students are being developed into a free online educational resource.

This project compares coral responses to acidification stress in populations experiencing distinct pH dynamics (high diel variability vs. low diel variability) and with distinct thermal tolerances (historically bleaching sensitive vs. tolerant) to learn about how coral responses to these two factors differ between coral species and within populations. Experiments focus on the two dominant reef builders found at these stable and variable pH reefs: Montipora capitata and Porites compressa. Individuals of each species exhibiting different thermal sensitivities (i.e., bleached vs. pigmented) were tagged during the 2015 global coral bleaching event. This system tests the hypotheses that 1) corals living on reefs with larger diel pH fluctuations have greater resilience to acidification stress, 2) coral resilience to acidification is a plastic trait that can be promoted via acclimatization, and 3) thermally sensitive corals have reduced capacity to cope with pH stress, which is exacerbated at elevated temperatures. Coral cells isolated from colonies from each environmental and bleaching history are exposed to acute pH stress and examined for their ability to recover intracellular pH in vivo using confocal microscopy, and the expression level of proteins predicted to be involved in this recovery (e.g., proton transporters) is examined via Western blot and immunolocalization. Corals from each pH history are exposed to stable and variable seawater pH in a controlled aquarium setting to determine the level of plasticity of acidification resilience and to test for pH acclimatization in this system. Finally, corals with different levels of thermal sensitivity are exposed to thermal stress and recovery, and their ability to regulate pH is examined over time. The results of these experiments help identify reef conditions that promote coral resilience to ocean acidification against the background of increasingly common thermal stress events, while advancing mechanistic understanding of coral physiology and symbiosis.

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-1923743

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