Micromorphological analyses of Pocillopora damicornis Spine RADs on samples collected from reef of Heron Island, southern Great Barrier Reef from Jan 2021 to Feb 2021

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
Brown, Kristen	University of Queensland	Co-Principal Investigator
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Abstract
Corals residing in habitats that experience frequent seawater pCO2 variability may possess an enhanced capacity to cope with ocean acidification. Yet, we lack a clear understanding of the molecular toolkit enabling acclimatization to environmental extremes, and how life-long exposure to pCO2 variability influences biomineralization. We examined the gene expression responses and micro-skeletal characteristics of Pocillopora damicornis originating from the reef flat and reef slope of Heron Island, southern Great Barrier Reef. The reef flat (454 ± 3.0) and reef slope (418 ± 1.9) had similar mean seawater pCO2 (µatm; mean ± SE), but the reef flat experienced twice the mean daily pCO2 amplitude (range of 797v. 399 µatm day-1, respectively). A controlled mesocosm experiment was conducted over eight weeks, exposing P. damicornis from the reef slope and reef flat to stable (218±9) or variable (911±31) diel pCO2 fluctuations (µatm; mean ± SE). This dataset includes the data and analyses for the overall skeletal micromorphological analyses of P. damicornis, including the origin reef environment, treatment, colony ID, feature of interest, image magnification, image name, observer, replicate, number of RADs, and area of RADs.

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Coverage

Location: Heron Island Research Station, Heron Island, southern Great Barrier Reef (23 27°S, 151 55°E).
Spatial Extent: **Lat**:-23.27 **Lon**:151.55
Temporal Extent: 2021-01-06 - 2021-04-06

Methods & Sampling

Sample Collection

The experiment was performed during the austral summer from mid-January to late March 2021 at Heron Island Research Station (HIRS), southern Great Barrier Reef (23 27°S, 151 55°E). Heron Reef is composed of five distinct geomorphological habitats characterized by diverse benthic communities and biogeochemical conditions. Fragments of the coral P. damicornis were collected from the reef flat and slope locations within the same depth range (1–3 m) on 14 and 15 January 2021. Four fragments were collected from each individual colony (genetic clones), totalling 96 fragments from 24 colonies (n = 12 per habitat). For more information about sample collection methods and treatment, see Brown et al., 2022.

Skeletal micromorphological analysis

The limited amount of new CaCO3 deposition observed during the 8-week exposure (~15–30% for each fragment; (Brown et al., 2022)) precluded our resolution to detect changes in net calcification or CaCO3 density of newly formed skeleton that were attributable to experimental pCO2 treatment conditions. To better resolve changes in biomineralization resulting from the seawater pCO2 variability treatments, a total of 16 coral fragments (n=4 per origin per treatment) were selected for skeletal micromorphological analyses. All tissue was removed from the skeletons by soaking the fragments in 10% sodium hypochlorite for 24 hr, rinsing with deionized (DI) water, and drying. Areas of CaCO3 deposition that occurred during the experiment were identified by comparing images at the start and end of the 8-week experiment. These deposits of new CaCO3 were carefully chipped off of the experimental fragments using a razor blade and imaged using a scanning electron microscope (SEM; Quanta 600 FEG Mark II Environmental Scanning Electron Microscope, Field Electron and Ion Company). Using the SEM, fragments were imaged across scales with magnification maintained between samples: an overall view of the skeleton (56x), individual whole calyxes (124x), spine structures between (141x) and inside (164x) the calyxes, and the rapid accretion deposits (RADs) on the spines (1013x). Several features of interest previously used to investigate coral biomineralization (Scucchia et al., 2023; Scucchia, Malik, Zaslansky, et al., 2021) were quantified using ImageJ (v1.53c) (Schneider et al., 2012), including: number of corallites, distance between corallites (i.e., coenosteum width), corallite diameter, circularity of the corallite, number of spines within calyx, spine length and maximum spine width (on spines both between and inside the calyx), number of RADs, and size of RADs.

The significant interaction between treatment and origin was explored on all micromorphological features using linear mixed effects models, with colony as a random effect. The significance of fixed effects and their interactions was determined using an analysis of variance with a type III error structure using the Anova function in car package (Fox et al., 2012). Significant interactive effects were followed by pairwise comparison of estimate marginal means using the emmeans package with Tukey HSD adjusted p values (Lenth et al., 2018). Data were tested for homogeneity of variance and normality of distribution through graphical analyses of residual plots for all models. All statistical analyses were done using R version 4.0.3 software (R Core Team, 2020), and graphical representations were produced using the package ggplot2 (Wickham, 2016).

Data Processing Description

See related code package Dellaert et al. (2024) "imkristenbrown/Heron-Pdam-gene-expression: pCO2 variability and biomineralization", doi: 10.5281/zenodo.14041606. This code package was used for results publication Brown et al. (2024).

BCO-DMO Processing Description

All 4 datasets submitted in same ticket were processed in same data pipeline, but steps have been broken out separately in the processing sections. Additionally 4 of 5 data files required implied data to filled into blank values. To do this in the BCO-DMO system, they are imported twice and joined.

-Imported "Spine.csv" into the BCO-DMO system as rad_metadata, filtered dataset to include only rows with "Number of RADs on spine" values and removed all other fields
-Imported "Spine.csv" into the BCO-DMO system as Spine and joined to rad_metadata on the "Image name" to populate "Number of RADs on spine" in all fields for the specified image
-Changed parameter names to comply with BCO-DMO naming conventions

-Exported file as "942939_v1_pocillopora_damicornis_rad_spine.csv"

Data Files

File
942939_v1_pocillopora_damicornis_rad_spine.csv (Comma Separated Values (.csv), 71.61 KB)   MD5:539f138e269dd682b8aaad19a03f1462   Primary data file for dataset ID 942939, version 1

Supplemental Files

File	
SEM_Image_inventory.tsv	(Tab Separated Values (.tsv), 48.35 KB) MD5:57cd0dabf607f5072cffc692055eb857
Inventory of images in the zip file containing all the images used in analysis with checksum information	
SEM_Images.zip	(ZIP Archive (ZIP), 1.18 GB) MD5:d41291d969c64c4fb4ee1ae90aa40586
Zip file containing images used in the skeletal micromorphological analysis.	

Related Publications

Brown, K. T., Dellaert, Z., Martynek, M. P., Durian, J., Mass, T., Putnam, H. M., & Barott, K. L. (2024). Extreme Environmental Variability Induces Frontloading of Coral Biomineralisation Genes to Maintain Calcification Under pCO2 Variability. Molecular Ecology, 34(2). Portico. https://doi.org/10.1111/mec.17603
Results

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
Methods

Fox J et al. (2012) Package 'car': Companion to Applied Regression. R package version 2.0. Vienna: R Foundation for Statistical Computing. Available from https://cran.r-project.org/package=car
Software

Lenth, R. et al. (2018). emmeans: Estimated Marginal Means, aka Least-Squares Means. Estimated Marginal Means, aka Least-Squares Means.R package version 1.3. Vienna: R Foundation for Statistical Computing. Available from https://cran.r-project.org/package=emmeans
Software

R Core Team (2020). R: A language and environment for statistical computing. R v4.0.3. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/
Software

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. Nature Methods, 9(7), 671–675. https://doi.org/10.1038/nmeth.2089
Software

Scucchia, F., Malik, A., Zaslansky, P., Putnam, H. M., & Mass, T. (2021). Combined responses of primary coral polyps and their algal endosymbionts to decreasing seawater pH. Proceedings of the Royal Society B: Biological Sciences, 288(1953). https://doi.org/10.1098/rspb.2021.0328
Methods

Scucchia, F., Zaslansky, P., Boote, C., Doheny, A., Mass, T., & Camp, E. F. (2023). The role and risks of selective adaptation in extreme coral habitats. Nature Communications, 14(1). https://doi.org/10.1038/s41467-023-39651-7
Methods

Wickham, H. (2016). Data Analysis. Ggplot2, 189–201. https://doi.org/10.1007/978-3-319-24277-4_9
Software

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Related Datasets

IsRelatedTo

Barott, K., Brown, K., Putnam, H. (2025) **Gene expression of Pocillopora damicornis collected from reef of Heron Island, southern Great Barrier Reef from Jan 2021 to Feb 2021.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-11-21 doi:10.26008/1912/bco-dmo.942938.1 [view at BCO-DMO]
Relationship Description: Datasets from the same study published in Brown et al. (2024) and utilized the same code package (doi:10.5281/zenodo.14041606).

Brown, K., Dellaert, Z., Putnam, H., Barott, K. (2025) **Micromorphological analyses of Pocillopora damicornis calyxes collected from reef of Heron Island, southern Great Barrier Reef from Jan 2021 to Feb 2021.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2025-03-28 doi:10.26008/1912/bco-dmo.942962.1 [view at BCO-DMO]
Relationship Description: Datasets from the same Pocillopora damicornis skeletal micromorphological analysis.

Brown, K., Dellaert, Z., Putnam, H., Barott, K. (2025) **Micromorphological analyses of Pocillopora damicornis overall skeleton collected from reef of Heron Island, southern Great Barrier Reef from Jan 2021 to Feb 2021.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2025-03-28 doi:10.26008/1912/bco-dmo.942939.1 [view at BCO-DMO]
Relationship Description: Datasets from the same Pocillopora damicornis skeletal micromorphological analysis.

Brown, K., Dellaert, Z., Putnam, H., Barott, K. (2025) **Micromorphological analyses of Pocillopora damicornis spine structures collected from reef of Heron Island, southern Great Barrier Reef from Jan 2021 to Feb 2021.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2025-03-28 doi:10.26008/1912/bco-dmo.942948.1 [view at BCO-DMO]
Relationship Description: Datasets from the same Pocillopora damicornis skeletal micromorphological analysis.

Software

Zoe Dellaert, Kristen Brown, & Hollie Putnam. (2024). *imkristenbrown/Heron-Pdam-gene-expression: pCO2 variability and biomineralization* (Version v1.0.0) [Computer software]. Zenodo. https://doi.org/10.5281/ZENODO.14041606 https://doi.org/10.5281/zenodo.14041606

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Parameters

Parameter	Description	Units
Origin	Habitat of origin (flat v. slope)	unitless
Treatment	Treatment (pCO2 variability (stable v. variable))	unitless
Colony_ID	Colony ID	unitless
Feature_of_interest	Feature of interest (Macro (M), polyp (P), Spine in calyx (SC), Spine between calyx (SBC), Spine (S))	unitless
Image_magnification	Image magnification	unitless
Image_name	Image name	unitless
Observer	Observer	unitless
Replicate	Replicate	unitless
RADs_num	Number of RADs on spine	unitless
RAD_area	Area of RAD	micrometers (um)

Instruments

Dataset-specific Instrument Name	Quanta 600 FEG Mark II Environmental Scanning Electron Microscope, Field Electron and Ion Company
Generic Instrument Name	Scanning Electron Microscope
Dataset-specific Description	These deposits of new CaCO3 were carefully chipped off of the experimental fragments using a razor blade and imaged using a scanning electron microscope (SEM; Quanta 600 FEG Mark II Environmental Scanning Electron Microscope, Field Electron and Ion Company).
Generic Instrument Description	A scanning electron microscope (SEM) scans a focused electron beam over a surface to create an image. The electrons in the beam interact with the sample, producing various signals that can be used to obtain information about the surface topography and composition.

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.

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1923743