Gene expression of Pocillopora damicornis collected from reef of Heron Island, southern Great Barrier Reef from Jan 2021 to Feb 2021

Website: https://www.bco-dmo.org/dataset/942938
Data Type: Other Field Results, experimental
Version: 1
Version Date: 2024-11-05

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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Putnam, Hollie	University of Rhode Island (URI)	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, from mid-January to late-March 2021, 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 gene expression data for these samples.

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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**:-23.45 **Lon**:151.9167
Temporal Extent: 2021-01-14 - 2021-02-06

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.

RNA and DNA Extractions

Samples for nucleic acid extraction were preserved in RNAlaterTM stabilization solution and stored at -80ºC until extraction. Genomic DNA and total RNA were extracted concurrently using the Zymo Quick-DNA/RNA Miniprep Plus Kit (Zymo Research #D7003) using the following modifications to the manufacturer's protocol. Samples were thawed on ice and two small fragments (5 mm x 5 mm) were removed from the stabilization solution using sterile forceps. Immediately, excess stabilization solution was removed with the corner of a KimWipe and fragments were submerged in a new 1.5 mL screw-cap tube containing 0.5mm glass beads (Fisher Scientific, #50-212-143) and 800 µl of DNA/RNA shield (Zymo Research, #R1100-50). Samples were homogenized by vortex at max speed for 1–2 minutes. Following homogenization, 400 µl of homogenate was removed and centrifuged for 3 mins at 9000 rcf. The supernatant was transferred to a new tube and mixed with 30 µl Proteinase K digestion buffer and 15 µl Proteinase K (Zymo Research #D7003). This mixture was incubated at room temperature for 15 minutes followed by another centrifugation step for 3 mins at 9000 rcf. The supernatant was combined with an equal volume of DNA/RNA lysis buffer (Zymo Research #D7003). Extraction was thereafter performed as described by the manufacturer's protocol, including the optional DNase I treatment. DNA and RNA concentration were quantified using the Qubit dsDNA and RNA Broad Range kits (Invitrogen #Q10211) and integrity was assayed using non-denaturing gel electrophoresis (1.5% agarose gel, 60 minutes at 60 V). Purity was assessed using Nanodrop.

Species Identification

Twenty of the 24 colonies were previously identified as P. damicornis (Brown et al., 2022). For the four colonies that were not identified due to difficulties with PCR amplification, the mitochondrial open reading frame (mtORF) region was amplified from new genomic DNA extracts via PCR as described by (Burgess et al., 2021; Johnston et al., 2018) using primers from (Flot et al., 2008): FatP6.1 (5′-TTTGGGSATTCGTTTAGCAG-3′) and RORF (5′-SCCAATATGTTAAACASCATGTCA-3′). PCR master mixes contained 12.55 µL of EmeraldAmp GT PCR Master Mix (TaKaRa Bio USA Inc. Cat # RR310B), 0.32 µL of forward and reverse primers as listed, 1 µL of template DNA, and 10.80 µL of nuclease free water totaling 25 µL final volume. Negative controls were included as master mix without template DNA. mtORF was amplified using a polymerase chain reaction (PCR) profile of a single denaturation set of 94°C for 60 s followed by 30 cycles of 94°C for 30 s for denaturation, 53°C for 30 s for annealing, and 72°C for 75 s for extension and a final incubation of 72°C for 5 min. PCR products were assessed with a 1.5% agarose gel in TAE for 30 mins at 80 V with an expected band size of approx. 1000 bp. PCR products were cleaned using ethanol precipitation. 1/10th of the volume of 3M sodium acetate (Fisher Cat. AAJ61928AE) was added to each PCR product, followed by an addition of 3 times the total volume of the mixture of ice-cold 100% ethanol. The mixture was incubated overnight at -20 ºC and DNA was precipitated by centrifugation at 15,000 rcf for 30 mins at room temperature. The pellet was washed with 70% ethanol twice, dried, and resuspended in 30 µl of 1M Tris-HCl, pH 8.0 (Fisher Cat. 15568025). DNA quantity was assessed using Broad Range dsDNA Qubit and Nanodrop. Sanger sequencing using the same primers utilized during PCR amplification was performed at the URI Genomics and Sequencing center using Applied Biosystems BigDye Terminator v3.1. Geneious Prime (Version 2023.2.1) was used to align the four sequences (Muscle 5.1 alignment, PPP algorithm, default parameters) to known P. damicornis (NCBI Accession Numbers: JX994077, KJ720219, JX994086, JX624991, KF583925, KF583946, EU374235, KJ720218, KP698587, KM215098, KX538982, KJ720226, KX538983, KF583950, JX994087, KJ720235, JX985618, KJ690905, JX625025, and KX538984) and P. acuta (NCBI Accession Numbers: JX994073, JX624999, KF583928, KF583935, EU374226, FJ424111, KJ720240, KM215075, KJ690906, KP698585, KX538985, KX538986, KM215104, and KJ720241) mtORF sequences and a Neighbor-Joining tree was built from the alignment. The samples sequenced in this study were identified as P. damicornis.

Library Preparation and Tag Seq Analysis

Extracted total RNA was prepared for TagSeq (Lohman et al., 2016). Library preparation and sequencing of the 48 samples was conducted at the University of Texas Austin, Genomic Sequencing and Analysis Facility. Sequencing was completed targeting standard coverage of 3–5 million 100-bp single-end reads per sample (Illumina NovaSeq 600 SR100). We used fastp to trim raw TagSeq reads of TruSeq 1 Illumina adapters, poly-A and poly-G sequences, and remove low-quality reads (i.e., reads with >40% of bases with Phred score <30) and low-complexity reads (i.e., <50%) (Chen et al., 2018). Before and after filtering, MultiQC was used to

assess filtering success (Ewels et al., 2016). After successful trimming and filtering, filtered reads were mapped to the Pocillopora damicornis genome (Cunning et al., 2018) and P. acuta genome (Stephens et al., 2022) using HISAT2 using the downstream-transcriptome-assembly mode for unpaired reads (Kim et al., 2019). Mapping rate was approximately 150% higher for the P. acuta genome than the P. damicornis genome (average of 72.48% compared to 29.10%), despite all corals used in the experiments confirmed as P. damicornis (Brown et al., 2022). Accordingly, downstream analyses were performed using the P. acuta genome. Mapped reads were quantified and assembled using StringTie (Pertea et al., 2016) and a gene count matrix was generated using the Stringtie script prepDE.py for downstream analysis. All code and data are publicly available (https://github.com/imkristenbrown/Heron-Pdam-gene-expression/, Dellaert et al. (2024), doi: 10.5281/zenodo.14041606). All raw TagSeq data can be accessed at the Sequence Read Archive (SRA) (https://www.ncbi.nlm.nih.gov/sra/PRJNA934298).

Quality control of gene expression data

All analyses were conducted using R version 4.0.3 software (R Core Team, 2020). First, all genes not detected (i.e., 100% of the samples showed counts of 0) across any of our samples (n=48) were removed, leaving data for 24,220 genes of the 33,730 genes in the P. acuta genome. Next, low-expression genes, defined as any gene that did not have at least 10 counts in 25% of the samples, were removed using the function pOverA in the package genefilter (Gentleman R, Carey V, Huber W, Hahne F, 2023). This retained genes that were expressed in at least one of the four different conditions, leaving a robust dataset of counts for 9,056 genes. Gene counts were normalized and transformed using variance stabilizing transformation (herein referred to as 'vst-normalized gene expression') in the package DESeq2 (Love et al., 2014). After examination of vst-normalized gene expression plots, two outliers were identified (two samples of the same genotype, RF16). These outliers were also identified as having high percentage of sequence duplication based on FastQC of raw fastq sequence files and were subsequently removed from the analysis. Following the removal of these two samples, pOverA filtering and variance stabilizing transformation was re-run, and the final dataset for statistical analysis included a robust dataset of counts for 9,012 genes from 46 samples. The resulting sample group sizes (biological replicates) for gene expression analysis were: flat-stable (n=11), flat-variable (n=11), slope-stable (n=12) and slope-variable (n=12).

Co-expression Analysis

Weighted Gene Co-expression Network Analysis (WGCNA, (Langfelder & Horvath, 2008)) was carried out using the R package WGCNA (version 1.72-5) to identify co-expression patterns based on native habitat of origin (flat vs. slope) or 8 weeks of exposure to pCO2 treatments (stable vs. variable). A signed adjacency similarity matrix of vst-normalized gene expression was constructed for all gene pairs across samples using the adjacency function (using a soft power of 5), then converted into a topological overlap dissimilarity matrix using the function TOMsimilarity (Langfelder & Horvath, 2008). Next, a hierarchical clustering of genes based on topological overlap was performed using the function hclust, followed by module identification using the function cutreeDynamic (hybrid method, deepSplit = 1) in the package dynamicTreeCut (Langfelder et al., 2008), retaining modules with at least 30 genes and merging highly similar modules using the function mergeCloseModules with a cut height of 0.15 (eigengenes correlated at R > 0.85). Trait data (categorical and physiological metrics) were then related to the expression of modules and clustered based on eigengene correlation using the package complexHeatmap (Gu et al., 2016). The complete list of the caterogrical and physiological metrics included was: net calcification, CaCO3 density, net photosynthesis, light-enhanced dark respiration, dark respiration, photosynthesis:respiration ratio, protein concentration, symbiont density, chlorophyll a concentration, mycosporine-like amino acid shinorine, rate of pHi recovery (symbiocyte and non-symbiocyte), acidosis magnitude (symbiocyte and non-symbiocyte), as well as categorical factors treatment, origin and genotype.

Gene ontology (GO) enrichment was explored for WGCNA modules using the EggNog (Cantalapiedra et al., 2021) and KEGG functional annotation files from the P. acuta genome (Stephens et al., 2022). Of the 9,012 genes in the dataset, there was no GO annotation for 4,448 of the genes and no KEGG annotation for 3,828 of the genes. Gene length information was calculated from the "gff3" file of the P. acuta genome (Stephens et al., 2022). An "over-represented p-value" for each GO term was calculated using the Wallenius method in the package goseq (Young et al., 2012) and only the biological process (BP) ontology was investigated. GO terms were considered to be overrepresented if they had a p-value of less than 0.01. An adjusted p-value using the Benjamini & Hochberg ("BH") method of the p.adjust function in R was calculated for each GO term, and is available in the supporting information of the results paper. The overrepresented GO term set was further reduced using a similarity matrix with a threshold of 0.7 to collapse similar GO terms for plotting them by their parent terms using the package rrvgo (Sayols, 2023).

Differentially expressed genes and gene expression frontloading

A linear mixed effects model was used to analyze differential gene expression by origin and treatment, with colony as a random effect, using the package glmmSeq (Lewis et al., 2021). First, a dispersion vector and size factors were calculated for each gene using DESeq2 on the raw gene count matrix (i.e., vst-normalized gene counts were not used as input for glmmSeq analysis). Then, the interactive effects of origin and treatment were explored on gene expression data (raw count data with dispersions provided), with colony genotype as a random effect. For one gene, the model did not converge, causing the final dataset for glmmSeq to contain 9,011 genes. Adjusted p-values were calculated using the package qvalue (Storey JD, Bass AJ, Dabney A, Robinson D, 2023). Genes with an adjusted p-value (q-value) less than 0.05 were considered to be differentially expressed.

Because habitat of origin was a major driver of the gene expression responses, we further tested for frontloading of transcripts (constitutive levels of expression) following the methodology of (Gurr et al., 2022). We calculated two ratios: a control ratio (stable flat / stable slope) and a fold change ratio (variable/stable flat / variable/stable slope) (Gurr et al., 2022). "Frontloaded" transcripts are defined as having: (i) a greater expression in the flat habitat of origin in the variable treatment compared to the slope origin corals in the stable treatment (control ratio > 1) and (ii) a smaller fold change from variable to stable in the flat origin compared to the slope origin (fold change ratio < 1) (Barshis et al., 2013). A fold change ratio of less than 1 indicates that these transcripts underwent less of an expression change from the stable to variable treatment in the corals from the flat habitat, suggesting "frontloading" of expression to cope with a more variable environment (Barshis et al., 2013), since these genes were constitutively higher expressed in the flat origin corals under a stable pH treatment (control ratio > 1). These calculations were made based on the glmmseq estimated mean expression of each gene in each treatment:origin combination based on the fitted model.

Gene ontology enrichment was performed for genes differentially expressed by origin, treatment, and the interaction as well as for frontloaded genes using the same method as described for the WGCNA modules. Enrichment of these gene sets was tested against the 9,011 genes in the glmmSeq dataset. Full datafiles are available in the supporting information of the results paper.

Expression of putative biomineralization-related genes

The expression of coral biomineralization-related genes from the literature (Scucchia, Malik, Putnam, et al., 2021; Scucchia, Malik, Zaslansky, et al., 2021) was compared to both the differential expression and frontloaded results. A BLASTp search (using BLAST+ 2.9.0-iimpi-2019b) was performed against a database of the P. acuta genome to identify the best match (e-value < 0.01) of each of these coral biomineralization-related genes in the genome of P. acuta. The top hit for each biomineralization-related gene was used (Stephens et al., 2022).

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).

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Related Publications

Barshis, D. J., Ladner, J. T., Oliver, T. A., Seneca, F. O., Traylor-Knowles, N., & Palumbi, S. R. (2013). Genomic basis for coral resilience to climate change. Proceedings of the National Academy of Sciences, 110(4), 1387–1392. doi:10.1073/pnas.1210224110
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
Methods

Burgess, S. C., Johnston, E. C., Wyatt, A. S. J., Leichter, J. J., & Edmunds, P. J. (2021). Response diversity in corals: hidden differences in bleaching mortality among cryptic Pocillopora species. Ecology, 102(6). doi:10.1002/ecy.3324
Methods

Cantalapiedra, C. P., Hernández-Plaza, A., Letunic, I., Bork, P., & Huerta-Cepas, J. (2021). eggNOG-mapper v2: Functional Annotation, Orthology Assignments, and Domain Prediction at the Metagenomic Scale. Molecular Biology and Evolution, 38(12), 5825–5829. https://doi.org/10.1093/molbev/msab293
Software

Chen, S., Zhou, Y., Chen, Y., & Gu, J. (2018). fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics, 34(17), i884–i890. https://doi.org/10.1093/bioinformatics/bty560
Software

Cunning, R., Bay, R. A., Gillette, P., Baker, A. C., & Traylor-Knowles, N. (2018). Comparative analysis of the Pocillopora damicornis genome highlights role of immune system in coral evolution. Scientific Reports, 8(1). https://doi.org/10.1038/s41598-018-34459-8
Methods

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Software

Flot, J.-F., Magalon, H., Cruaud, C., Couloux, A., & Tillier, S. (2008). Patterns of genetic structure among Hawaiian corals of the genus Pocillopora yield clusters of individuals that are compatible with morphology. Comptes Rendus Biologies, 331(3), 239–247. doi:10.1016/j.crvi.2007.12.003
Methods

Gentleman R, Carey V, Huber W, Hahne F (2023). *genefilter: methods for filtering genes from high-throughput experiments*. R package version 1.82.1. Bioconductor. https://doi.org/10.18129/B9.BIOC.GENEFILTER https://doi.org/10.18129/B9.bioc.genefilter
Software

Gu, Z., Eils, R., & Schlesner, M. (2016). Complex heatmaps reveal patterns and correlations in multidimensional genomic data. Bioinformatics, 32(18), 2847–2849. https://doi.org/10.1093/bioinformatics/btw313
Software

Gurr, S. J., Trigg, S. A., Vadopalas, B., Roberts, S. B., & Putnam, H. M. (2022). Acclimatory gene expression of primed clams enhances robustness to elevated pCO2. Molecular Ecology, 31(19), 5005–5023. Portico. https://doi.org/10.1111/mec.16644
Methods

Johnston, E. C., Forsman, Z. H., & Toonen, R. J. (2018). A simple molecular technique for distinguishing species reveals frequent misidentification of Hawaiian corals in the genus Pocillopora. PeerJ, 6, e4355. doi:10.7717/peerj.4355
Methods

Kim, D., Paggi, J. M., Park, C., Bennett, C., & Salzberg, S. L. (2019). Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. Nature Biotechnology, 37(8), 907–915. https://doi.org/10.1038/s41587-019-0201-4
Methods

Langfelder, P., & Horvath, S. (2008). WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics, 9(1). doi:10.1186/1471-2105-9-559
Methods

Langfelder, P., Zhang, B., & Horvath, S. (2008). Defining clusters from a hierarchical cluster tree: the Dynamic Tree Cut package for R. Bioinformatics, 24(5), 719–720. https://doi.org/10.1093/bioinformatics/btm563
Software

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Software

Lohman, B. K., Weber, J. N., & Bolnick, D. I. (2016). Evaluation of TagSeq, a reliable low-cost alternative for RNAseq. Molecular Ecology Resources, 16(6), 1315–1321. doi:10.1111/1755-0998.12529
Methods

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology, 15(12). doi:10.1186/s13059-014-0550-8
Software

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https://doi.org/[10.1093/nar/gkm321](https://doi.org/10.1093/nar/gkm321)

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Methods

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Software

Sayols, S. (2023). *rrvgo: a Bioconductor package for interpreting lists of Gene Ontology terms*. microPublication Biology. https://doi.org/10.17912/MICROPUB.BIOLOGY.000811
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Software

Scucchia, F., Malik, A., Putnam, H. M., & Mass, T. (2021). Genetic and physiological traits conferring tolerance to ocean acidification in mesophotic corals. Global Change Biology, 27(20), 5276–5294. Portico. https://doi.org/[10.1111/gcb.15812](https://doi.org/10.1111/gcb.15812)
Methods

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](https://doi.org/10.1098/rspb.2021.0328)
Methods

Stephens, T. G., Lee, J., Jeong, Y., Yoon, H. S., Putnam, H. M., Majerová, E., & Bhattacharya, D. (2022). Correction to: High-quality genome assemblies from key Hawaiian coral species. (2022). GigaScience, 12. https://doi.org/[10.1093/gigascience/giad027](https://doi.org/10.1093/gigascience/giad027)
Methods
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Methods

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Software

Young, M. D., Wakefield, M. J., Smyth, G. K., & Oshlack, A. (2012). goseq: Gene Ontology testing for RNA-seq datasets. R Bioconductor, 8, 1–25. https://bioconductor.org/packages/goseq/
Software

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Related Datasets

IsRelatedTo

Barott, K., Brown, K., Putnam, H. (2024) **Pocillopora damicornis skeletal micromorphological analysis: Calyxes.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-11-05 http://lod.bco-dmo.org/id/dataset/942962 [[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).

Barott, K., Brown, K., Putnam, H. (2024) **Pocillopora damicornis skeletal micromorphological analysis: Overall skeleton.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-11-05 http://lod.bco-dmo.org/id/dataset/942939 [[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).

Barott, K., Brown, K., Putnam, H. (2024) **Pocillopora damicornis skeletal micromorphological analysis: Spine RADs.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-11-05 http://lod.bco-dmo.org/id/dataset/942955 [[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).

Barott, K., Brown, K., Putnam, H. (2024) **Pocillopora damicornis skeletal micromorphological analysis: Spine structures.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-11-05 http://lod.bco-dmo.org/id/dataset/942948 [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).

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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University of Pennsylvania. Pocillopora damicornis, Heron Island Pocillopora damicornis gene expression. 2023/02. In: BioProject [Internet]. Bethesda, MD: National Library of Medicine (US), National Center for Biotechnology Information; 2011-. Available from: http://www.ncbi.nlm.nih.gov/bioproject/PRJNA934298. NCBI:BioProject: PRJNA934298. https://www.ncbi.nlm.nih.gov/bioproject/PRJNA934298

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Parameters

Parameters for this dataset have not yet been identified

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Instruments

Dataset-specific Instrument Name	Illumina NovaSeq 600 SR100
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	Sequencing was completed targeting standard coverage of 3–5 million 100-bp single-end reads per sample (Illumina NovaSeq 600 SR100).
Generic Instrument Description	General term for a laboratory instrument used for deciphering the order of bases in a strand of DNA. Sanger sequencers detect fluorescence from different dyes that are used to identify the A, C, G, and T extension reactions. Contemporary or Pyrosequencer methods are based on detecting the activity of DNA polymerase (a DNA synthesizing enzyme) with another chemoluminescent enzyme. Essentially, the method allows sequencing of a single strand of DNA by synthesizing the complementary strand along it, one base pair at a time, and detecting which base was actually added at each step.

Dataset-specific Instrument Name	Centrifuge
Generic Instrument Name	Centrifuge
Dataset-specific Description	Following homogenization, 400 µl of homogenate was removed and centrifuged for 3 mins at 9000 rcf.
Generic Instrument Description	A machine with a rapidly rotating container that applies centrifugal force to its contents, typically to separate fluids of different densities (e.g., cream from milk) or liquids from solids.

Dataset-specific Instrument Name	incubator
Generic Instrument Name	Incubator
Dataset-specific Description	This mixture was incubated at room temperature for 15 minutes followed by another centrifugation step for 3 mins at 9000 rcf.
Generic Instrument Description	A device in which environmental conditions (light, photoperiod, temperature, humidity, etc.) can be controlled. Note: we have more specific terms for shipboard incubators (https://www.bco-dmo.org/instrument/629001) and in-situ incubators (https://www.bco-dmo.org/instrument/494).

Dataset-specific Instrument Name	vortex
Generic Instrument Name	Shaker
Dataset-specific Description	Samples were homogenized by vortex at max speed for 1–2 minutes.
Generic Instrument Description	A Shaker is a piece of lab equipment used to mix, blend, or to agitate substances in tube(s) or flask(s) by shaking them, which is mainly used in the fields of chemistry and biology. A shaker contains an oscillating board which is used to place the flasks, beakers, test tubes, etc.

Dataset-specific Instrument Name	Nanodrop
Generic Instrument Name	Thermo Scientific NanoDrop spectrophotometer
Dataset-specific Description	Purity was assessed using Nanodrop.
Generic Instrument Description	Thermo Scientific NanoDrop spectrophotometers provide microvolume quantification and purity assessments of DNA, RNA, and protein samples. NanoDrop spectrophotometers work on the principle of ultraviolet-visible spectrum (UV-Vis) absorbance. The range consists of the NanoDrop One/OneC UV-Vis Spectrophotometers, NanoDrop Eight UV-Vis Spectrophotometer and NanoDrop Lite Plus UV Spectrophotometer.

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