Expression of heat shock protein 70 (HSP70) in larvae at 11 days post-fertilization (DPF) from experiments investigating heat priming in Nematostella vectensis

Website: https://www.bco-dmo.org/dataset/923415
Data Type: experimental
Version: 1
Version Date: 2024-03-26

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

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Abstract
Across diverse taxa, sublethal exposure to abiotic stressors early in life can lead to benefits such as increased stress tolerance upon repeat exposure. This phenomenon, known as hormetic priming, is largely unexplored in early life stages of marine invertebrates, which are increasingly threatened by anthropogenic climate change. To investigate this phenomenon, larvae of the sea anemone and model marine invertebrate Nematostella vectensis were exposed to control (18 °C) or elevated (24 °C, 30 °C, 35 °C, or 39 °C) temperatures for 1 hour at 3 days post-fertilization (DPF), followed by return to control temperatures (18 °C). The animals were then assessed for growth, development, metabolic rates, and heat tolerance at 4, 7, and 11 DPF. To investigate a possible molecular mechanism for the observed changes in heat tolerance, the expression of heat shock protein 70 (HSP70) was quantified at 11 DPF. The study's findings suggest heat priming may augment the climate resilience of marine invertebrate early life stages via the modulation of key developmental and physiological phenotypes, while also affirming the need to limit further anthropogenic ocean warming. This dataset includes data pertaining to the expression of heat shock protein 70 (HSP70) in larvae at 11 days post-fertilization (DPF). See related datasets for other results from these experiments. These data and results are published in Glass et al. (2023) (DOI: 10.7717/peerj.16574).

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Coverage

Location: Laboratory at the University of Pennsylvania

Temporal Extent: 2023-05-08 - 2023-08-02

Dataset Description

This dataset includes data pertaining to the expression of heat shock protein 70 (HSP70) in larvae at 11 days post-fertilization (DPF). See related datasets for other results from these experiments. These data and results are published in Glass et al. (2023) (DOI: 10.7717/peerj.16574).

Methods & Sampling

Summary:
Development data were collected via visual inspection of animals under brightfield illumination; animals were scored according to developmental stage (planula or post-planula). Dose-response curve data for larval heat tolerance were collected by exposing animals to heat ramps peaking at temperatures between 39-43°C, followed by visual scoring of mortality after 48 hours. Heat shock protein 70 (HSP70) expression was determined via Western blotting according to standard protocols. Sizes of larvae and juveniles (body column lengths) were measured from calibrated images of animals collected via brightfield microscopy. Respiration rates were determined using a Presens SensorDish Reader. Protein content of larvae was determined via a Bradford assay according to standard protocols.

The following methods are excerpted from Glass et al. (2023) (DOI: 10.7717/peerj.16574).

Adult collection, culture, and sexual reproduction
Adult *Nematostella vectensis* sea anemones were collected from a salt marsh in Brigantine, New Jersey in the fall of 2020. Following transport to the laboratory, anemones were kept in 12 parts per thousand (ppt) artificial seawater (1/3-ASW; Spectrum Brands, Madison, WI, USA) at 18 degrees Celsius (°C) in a dark incubator (Boekel Scientific, Feasterville Trevose, PA, USA). Animals were fed twice per week with *Artemia nauplii* (Brine Shrimp Direct, Ogden, UT, USA) with water changes occurring every 2 weeks for approximately 2.5 years. Spawning was induced using a standard method for *N. vectensis* (Hand & Uhlinger, 1992; Fritzenwanker & Technau, 2002; Stefanik, Friedman & Finnerty, 2013). As culture containers housed both male and female anemones, eggs were left to fertilize during spawning and then transferred to a plastic dish with ~25 milliliters (mL) new 1/3-ASW. Fertilized embryos were then held at 18 °C in the dark for 3 days.

Priming treatment and larval culture
At 3 days post-fertilization (DPF), 200 swimming planula larvae were pipetted into each of 15 conical tubes (15 mL), for a total of three replicate tubes of 200 larvae (N = 600 larvae priming temperature^{-1}) for each of the five priming temperature treatments: 18 °C (control), 24 °C, 30 °C, 35 °C, and 39 °C. Tubes were placed in water baths (Thermo Fisher Scientific, Waltham, MA, USA) set to each treatment temperature for 1 hour. Following the treatment period, tubes containing larvae were poured into petri dishes (15 mL capacity) and a partial water change was performed by aspirating and replacing ~7.5 mL (~50%) 1/3-ASW; dishes were then held at 18 °C in the dark for the remainder of the experiment. The day after the priming treatment, one dish of larvae primed at 39 °C displayed 100% mortality along with dense overgrowth of an unidentified microbe, and was therefore removed from the experiment. All other larvae were left unfed and in the same water for the remainder of the short-term experiment (11 days in total). For the long-term experiment, this entire procedure was repeated for a second cohort of larvae, which were held at 18 °C for 6 weeks following priming at 3 DPF. In both experiments, control animals were kept at 18 °C for the entire experiment duration, having gone through the priming treatment but without a change in temperature.

Image collection and quantification of growth and development
Each day following the priming treatment (4-11 DPF), dishes containing larvae were individually removed from the incubator and photographed in a single, haphazardly chosen region under a dissecting microscope (Leica MZ12; Leica, Wetzlar, Germany) with a camera attachment (Retiga R3 CCD), after which they were returned to the incubator. Each photograph contained at least 20 larvae, and microscope settings were unchanged between dishes; a ruler was also photographed each day using the same microscope settings. To quantify larval growth, images were analyzed in FIJI (Schindelin et al., 2012); the ruler image was used to set the scale, and then the line tool was used to individually measure the lengths along the longest axis of at least 20 larvae per photograph (N = 40-60 larvae priming temperature^{-1} time point^{-1}). Next, developmental progression was quantified from the images by counting the number of larvae clearly past the planula stage (i.e., not a homogenous oval shape), which was divided by the total number of larvae in each image and then converted to a percentage of "post-planula" larvae (N = 17-129 larvae priming temperature^{-1} time point^{-1}).

Respiration and total protein measurements
At 4, 7, and 11 DPF, 15 larvae per culture dish (N = 30-45 larvae priming temperature^{-1} time point^{-1}) were transferred to a 24-well plate (N = 1 well dish^{-1}) with oxygen sensor spots (Loligo Systems, Viborg, Denmark). All wells were filled to capacity (80 microliters (µL)) with 1/3-ASW, and 3-4 wells were also filled with culture water without larvae to serve as controls. The plate was sealed with an adhesive plate cover and placed on a calibrated PreSens SensorDish Reader (Precision Sensing, Regensburg, Germany) at room temperature (~21 °C) under ambient lighting. The oxygen concentration (micromoles oxygen per liter (µmol O2 L^{-1})) in each well was recorded every 15 seconds for 1 hour, during which conditions remained normoxic (>180 µmol O2 L^{-1}). Following respiration measurements, larvae in each well (N = 15 larvae) were transferred to 1.5 mL tubes, seawater was removed, and the tubes were frozen and stored at -80 °C. Tubes containing larvae were later thawed on ice, and 60 µL of 1x tris-NaCl-EDTA lysis buffer supplemented with dithiothreitol, protease inhibitor cocktail (Thermo Fisher Scientific, Waltham, MA, USA), and phosphatase inhibitors (Roche, Basel, Switzerland), were added to each tube. Next, larvae were lysed in a water bath sonicator (Diagenode UCD-200; Diagenode Inc., Denville, NJ, USA) at 4 °C for 5 min with a 30:60 seconds on:off cycle. Following sonication, tubes were centrifuged (1,500 × g for 5 min at 4 °C) and the protein concentrations of the supernatants were determined in triplicate using a Bradford assay with a bovine serum albumin standard curve. Due to defects in the plastic of the 1.5 mL tubes used for total protein assays, six of the 42 protein samples were lost during processing. However, at least one sample was processed for each time point and priming temperature combination, with the exception of 24 °C at 7 DPF. To determine respiration rates, the rate of oxygen consumption for each well was determined as the slope of a linear best-fit line of the oxygen level in the well over time, and the average rate for the control wells was subtracted from the wells containing larvae. The absolute values of the slopes were then converted to pmol O2 minute^{-1} µg protein^{-1} by multiplying by 1,000 and the volume of the wells (80 µL), and dividing by the total protein.

Heat tolerance measurements
Larval heat tolerance was determined at 4, 7, and 11 DPF using previously established methods (Rivera et al., 2021; Glass et al., 2023). Specifically, six larvae from each culture dish were exposed to each of the following peak temperatures: 39 °C, 40 °C, 41 °C, 42 °C, or 43 °C, yielding 2–3 dose-response curves (N = 30 larvae curve^{-1}) per priming temperature. Specifically, individual larvae were pipetted into wells of a 96-well PCR plate with 100 µL of 1/3-ASW, and plates were sealed with an adhesive plate cover to prevent evaporation during the assay. Thermocyclers (Thermo Fisher Scientific, Waltham, MA, USA) were used to generate the heat ramps, and were programmed as follows: (i) 1 min at 25 °C; (ii) 4 min at 30 °C; (iii) 4 min at 38 °C; (iv) 1 h at peak temperature (39-43 °C); (v) 4 min at 38 °C; (vi) 4 min at 30 °C; and (vii) infinite hold at 22 °C. Following completion of the heat ramp, the plates were uncovered and placed at 18 °C for 48 h, after which larvae were scored as dead (visible tissue lysis) or alive (no visual abnormalities; often swimming). For each dose-response curve, the proportion of larvae surviving at each peak temperature was calculated by dividing the number of larvae surviving by the total number of larvae treated. Data pertaining to proportion survival were used to calculate lethal temperature 50s (LT50s) for each dose-response curve as detailed below.

Western blotting for heat shock protein 70 (HSP70)
Following the extraction of proteins and quantification via a Bradford assay, equal amounts (micrograms (µg)) of protein in lysis buffer from each group of larvae collected at 11 DPF (N = 2-3 groups of 15 larvae priming temperature^{-1}) were combined with Laemlli buffer (Bio-Rad, Hercules, CA, USA), denatured at 70 °C for 15 minutes, and loaded at a target amount of 2.02 µg protein well^{-1} into a 4-12% tris-glycine gel. Next, electrophoresis was performed for 30 minutes at 60 volts (V) followed by 1 hour at 120 V, and proteins were then transferred to a polyvinylidene fluoride membrane (100 V for 100 minutes at 4 °C). Following transfer, the membrane was blocked for 1 hour in blocking buffer (5% w/v bovine serum albumin in tris-buffered saline (TBS) with 0.1% v/v Tween-20 (TBST)) and incubated with 0.55 µg mL^{-1} polyclonal antibodies for HSP70 (Novus Biologicals, Centennial, CO, USA), which were chosen based on the target epitope's ability to bind to all five known isoforms of *N. vectensis* HSP70 (Waller et al., 2018; Knighton et al., 2019). Next, the membrane was washed (3 × 10 minutes with TBST) and a secondary antibody (anti-rabbit IgG with horseradish peroxidase) was added for 1 hour before final washing (3 × 10 min with TBST followed by 1 × 10 min with TBS), treatment with chemiluminescence reagents (Thermo Fisher Scientific, Waltham, MA, USA), and imaging on an Amersham Imager 600 (General Electric, Boston, MA, USA). After initial imaging, the membrane was probed for β-tubulin using 2.5 µg mL^{-1} monoclonal antibodies (Cell Signaling Technology, Danvers, MA, USA) and reimaged.

Long-term growth and heat tolerance experiments
In order to characterize the persistence of heat priming effects on growth and heat tolerance, a second cohort of larvae was produced 2 weeks after the initial spawning by the same adult population and exposed to the same priming treatment at 3 DPF as described above. Following priming, larvae were kept in culture through 6 weeks post-fertilization (WPF) at 18 °C in the dark, where they progressed to the juvenile stage and began to grow tentacles. Animals were fed twice per week (i.e., every 3-4 days) after settlement (~7 DPF) with homogenized *Artemia nauplii*. Specifically, 3 mL of live *Artemia* nauplii were homogenized in a 15 mL conical

using a rotostator at 20,000 rpm for 10 seconds, and 200 µL of the resulting slurry was added to each culture dish. Within 2-3 hours after feeding, a partial water change was performed for each dish by aspirating and replacing 5-7 mL (33-50%) of 1/3-ASW. Images were collected weekly as described above to characterize long-term effects of priming on growth, and the number of tentacles possessed by each juvenile in the images was also quantified. After 6 weeks, juvenile heat tolerance was determined via a single-temperature heat challenge at 42 °C due to fewer juveniles in the long-term priming experiment vs the short-term experiment. Specifically, eight juveniles from each culture dish (N = 24 juveniles priming temperature^{-1}) were exposed to a heat ramp peaking at 42 °C for 1 hour then returned to 18 °C. While juveniles were intended to be monitored at intervals over 48 hours to generate dose-response curves over time, >60% mortality for all groups at 21 hours precluded this procedure, so the assay was concluded at that time. Finally, the number of surviving juveniles was divided by the number treated to obtain the proportion surviving.

Data Processing Description

Summary:
For respiration data, the average oxygen consumption rate for the blank wells was subtracted from the larval rates, which were then converted to nanomoles of oxygen per minute per larva (nmol O_2 minute^{-1} larva^{-1}.

For heat tolerance data, the percentage of larvae surviving after exposure to each peak temperature was calculated as the number of larvae surviving divided by the total number of larvae exposed to each temperature.

The following methods are excerpted from Glass et al. (2023) (DOI: 10.7717/peerj.16574).

Data analysis
All data were analyzed using R version 4.2.1 (R Core Team, 2022) in RStudio (RStudio Team, 2020). First, proportion survival data from larval heat tolerance assays were used to create dose-response curves represented by two-parameter log-logistic functions using the package drc (Ritz et al., 2015), and an LT50 was determined for each curve using the package chemCal (Ranke, 2022). Data pertaining to growth (body column length and juvenile tentacle number), development (post-planula larvae), respiration rates, and LT50s were analyzed using linear mixed-effect models with priming temperature, DPF/WPF, and their interaction as effects and culture dish as a random effect.

For total protein, the interaction between priming temperature and DPF was not significant, so a linear model relating protein to the additive combination of priming temperature and DPF was used for this metric.

Data pertaining to normalized HSP70 expression at 11 DPF were also analyzed using a linear model with priming temperature as a fixed effect. Additionally, to investigate the correlation between HSP70 expression and LT50 at 11 DPF, a linear model was built with HSP70 expression as a fixed effect. For juvenile survival following heat shock at 6 WPF, a linear model was created with priming temperature as a fixed effect.

BCO-DMO Curation Notes

- Imported original file "HSP70_data.csv" into the BCO-DMO system.
- Renamed fields to comply with BCO-DMO naming conventions.
- Saved the final file as "923415_v1_n_vectensis_hsp70_data.csv".

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Data Files

File	
923415_v1_n_vectensis_hsp70_data.csv	(Comma Separated Values (.csv), 715 bytes) MD5:910ada72ff378cbed883c15566c87e4c
Primary data file for dataset ID 923415, version 1	

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Related Publications

Fritzenwanker, J. H., & Technau, U. (2002). Induction of gametogenesis in the basal cnidarian Nematostella vectensis (Anthozoa). Development Genes and Evolution, 212(2), 99–103. https://doi.org/10.1007/s00427-002-0214-7
Methods

Glass, B. H., Jones, K. G., Ye, A. C., Dworetzky, A. G., & Barott, K. L. (2023). Acute heat priming promotes short-term climate resilience of early life stages in a model sea anemone. PeerJ, 11, e16574. Portico. https://doi.org/10.7717/peerj.16574
Results

Hand, C., & Uhlinger, K. R. (1992). The Culture, Sexual and Asexual Reproduction, and Growth of the Sea Anemone Nematostella vectensis. The Biological Bulletin, 182(2), 169–176. https://doi.org/10.2307/1542110
Methods

Knighton, L. E., Nitika, Waller, S. J., Strom, O., Wolfgeher, D., Reitzel, A. M., & Truman, A. W. (2019). Dynamic remodeling of the interactomes of Nematostella vectensis Hsp70 isoforms under heat shock. Journal of Proteomics, 206, 103416. https://doi.org/10.1016/j.jprot.2019.103416
Methods

RStudio Team (2022) RStudio: Integrated Development for R. Version 4.2.1,. RStudio, Inc., Boston, MA. http://www.rstudio.com/
Software

Ranke J. 2022. chemCal: calibration functions for analytical chemistry. https://cran.r-project.org/web/packages/chemCal/index.html
Software

Ritz, C., Baty, F., Streibig, J. C., & Gerhard, D. (2015). Dose-Response Analysis Using R. PLOS ONE, 10(12), e0146021. https://doi.org/10.1371/journal.pone.0146021
Methods

Rivera, H. E., Chen, C.-Y., Gibson, M. C., & Tarrant, A. M. (2021). Plasticity in parental effects confers rapid larval thermal tolerance in the estuarine anemone Nematostella vectensis. Journal of Experimental Biology, 224(5). https://doi.org/10.1242/jeb.236745
Methods

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., … Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. Nature Methods, 9(7), 676–682. doi:10.1038/nmeth.2019
Methods

Stefanik, D. J., Friedman, L. E., & Finnerty, J. R. (2013). Collecting, rearing, spawning and inducing regeneration of the starlet sea anemone, Nematostella vectensis. Nature Protocols, 8(5), 916–923. https://doi.org/10.1038/nprot.2013.044
Methods

Waller, S. J., Knighton, L. E., Crabtree, L. M., Perkins, A. L., Reitzel, A. M., & Truman, A. W. (2018). Characterizing functional differences in sea anemone Hsp70 isoforms using budding yeast. Cell Stress and Chaperones, 23(5), 933–941. https://doi.org/10.1007/s12192-018-0900-7
Methods

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Related Datasets

IsRelatedTo

Glass, B., Barott, K. (2024) **Data pertaining to dose-response curves (DRC) quantifying survival of larvae after exposure to heat ramps from experiments investigating heat priming in Nematostella vectensis.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-03-26 doi:10.26008/1912/bco-dmo.923386.1 [view at BCO-DMO]
Relationship Description: These datasets result from the same set of experiments.

Glass, B., Barott, K. (2024) **Data pertaining to the development of larvae past the planula stage from experiments investigating heat priming in Nematostella vectensis.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-03-25 doi:10.26008/1912/bco-dmo.923284.1 [view at BCO-DMO]
Relationship Description: These datasets result from the same set of experiments.

Glass, B., Barott, K. (2024) **Heat tolerance (survival) of juveniles at 6 WPF following heat shock from experiments investigating heat priming in Nematostella vectensis.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-03-26 doi:10.26008/1912/bco-dmo.923497.1 [view at BCO-DMO]
Relationship Description: These datasets result from the same set of experiments.

Glass, B., Barott, K. (2024) **Lethal temperature 50s (LT50s) displayed by larvae derived from dose-response curves after heat shock from experiments investigating heat priming in Nematostella vectensis.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-03-26 doi:10.26008/1912/bco-dmo.923586.1 [view at BCO-DMO]
Relationship Description: These datasets result from the same set of experiments.

Glass, B., Barott, K. (2024) **Long-term body column lengths and tentacle numbers of larvae and juveniles from experiments investigating heat priming in Nematostella vectensis.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-03-26 doi:10.26008/1912/bco-dmo.923447.1 [view at BCO-DMO]
Relationship Description: These datasets result from the same set of experiments.

Glass, B., Barott, K. (2024) **Respiration rates and protein content of larvae from experiments investigating heat priming in Nematostella vectensis.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-03-27 doi:10.26008/1912/bco-dmo.923674.1 [view at BCO-DMO]
Relationship Description: These datasets result from the same set of experiments.

Glass, B., Barott, K. (2024) **Sizes of larvae from 4-11 days post-fertilization from experiments investigating heat priming in Nematostella vectensis.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-03-27 doi:10.26008/1912/bco-dmo.923616.1 [view at BCO-DMO]
Relationship Description: These datasets result from the same set of experiments.

Different Version

Glass, B., Jones, K., Ye, A., Dworetzky, A., & Barott, K. (2023). *Data and code for: Acute heat priming promotes short-term climate resilience of early life stages in a model sea anemone* (Version 6) [Data set]. Dryad. https://doi.org/10.5061/DRYAD.F4QRFJ724 https://doi.org/10.5061/dryad.f4qrfj724

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Parameters

Parameter	Description	Units
Priming_temperature_C	Temperature at which larvae were primed for 1 hour at 3 DPF	degrees Celsius
Group	Arbitrary number assigned to the replicate dishes in which larvae were cultured	unitless
Days_post_fertilizaiton	Number of days post-fertilization	unitless
HSP70	Mean gray value for Western blot band representing HSP70 as determined via ImageJ	unitless
Tubulin	Mean gray value for Western blot band representing tubulin as determined via ImageJ	unitless
Normalized_HSP70	HSP70/tubulin	unitless
LT50_C	Lethal temperature 50 for larvae at 11 DPF	degrees Celsius

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Instruments

Dataset-specific Instrument Name	Retiga R3 microscope camera
Generic Instrument Name	Camera
Dataset-specific Description	Retiga R3 microscope camera (QImaging, Canada) for brightfield microscopy images
Generic Instrument Description	All types of photographic equipment including stills, video, film and digital systems.

Dataset-specific Instrument Name	centrifuge
Generic Instrument Name	Centrifuge
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 (Boekel Scientific, Feasterville Trevose, PA, USA)
Generic Instrument Name	In-situ incubator
Generic Instrument Description	A device on a ship or in the laboratory that holds water samples under controlled conditions of temperature and possibly illumination.

Dataset-specific Instrument Name	dissecting microscope (Leica MZ12; Leica, Wetzlar, Germany)
Generic Instrument Name	Microscope - Optical
Generic Instrument Description	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".

Dataset-specific Instrument Name	PreSens SensorDish Reader
Generic Instrument Name	Oxygen Microelectrode Sensor
Dataset-specific Description	PreSens SensorDish Reader (Precision Sensing, Regensburg, Germany) for respiration measurements (calibrated according to manufacturer's instructions)
Generic Instrument Description	Any microelectrode sensor that measures oxygen.

Dataset-specific Instrument Name	oxygen sensor spots (Loligo Systems, Viborg, Denmark)
Generic Instrument Name	Oxygen Sensor
Generic Instrument Description	An electronic device that measures the proportion of oxygen (O2) in the gas or liquid being analyzed

Dataset-specific Instrument Name	ruler
Generic Instrument Name	ruler
Generic Instrument Description	A device used for measuring or for drawing straight lines, consisting of an elongated piece of rigid or semi-rigid material marked with units for measurement. Device that allows one or more physical dimensions of a sample or specimen to be determined by visible comparison against marked graduations in units of measurement of dimension length.

Dataset-specific Instrument Name	MiniAmp thermal cyclers
Generic Instrument Name	Thermal Cycler
Dataset-specific Description	MiniAmp thermal cyclers (Thermo Fisher Scientific, Waltham, MA, USA) for thermal tolerance heat ramps
Generic Instrument Description	A thermal cycler or "thermocycler" is a general term for a type of laboratory apparatus, commonly used for performing polymerase chain reaction (PCR), that is capable of repeatedly altering and maintaining specific temperatures for defined periods of time. The device has a thermal block with holes where tubes with the PCR reaction mixtures can be inserted. The cycler then raises and lowers the temperature of the block in discrete, pre-programmed steps. They can also be used to facilitate other temperature-sensitive reactions, including restriction enzyme digestion or rapid diagnostics. (adapted from http://serc.carleton.edu/microbelife/research_methods/genomics/pcr.html)

Dataset-specific Instrument Name	water bath sonicator (Diagenode UCD-200; Diagenode Inc., Denville, NJ, USA)
Generic Instrument Name	ultrasonic cell disrupter (sonicator)
Generic Instrument Description	Instrument that applies sound energy to agitate particles in a sample.

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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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