Physiology measurements on collected coral pairs from Kaneohe Bay, Oahu, HI between July and December 2019

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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
Innis, Teegan	University of Pennsylvania (Penn)	Contact
Heyl, Taylor	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager
Rauch, Shannon	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
Rates of photosynthesis and respiration were measured in 10 pairs of visually bleached and non-bleached colonies of Montipora capitata and Porites compressa in Kaneohe Bay, Oahu, HI between July and December 2019.

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Coverage

Spatial Extent: **N**:21.4518 **E**:-157.796 **S**:21.4506 **W**:-157.797
Temporal Extent: 2019-07 - 2020-01

Methods & Sampling

At the peak of a marine heatwave in 2015, 10 pairs of visually bleached and non-bleached colonies of *Montipora capitata* and *Porites compressa* adjacent to each other on the reef were identified, gps-marked and tagged with cattle tags. Before, during, and after the 2019 marine heatwave, these pairs were photographed with size and color standards. At each time point, 1 fragment (~4 centimeters) and 1 biopsy (~2 centimeters) were collected from each colony. The fragments were then transferred in ambient seawater to an outdoor flow-through seawater table for physiological measurements and the biopsies were snap-frozen in liquid nitrogen for future molecular assays

The volume of each coral fragment was measured via the water displacement method.

Photochemical efficiency was measured on each coral fragment approximately 1 hour after sunset on the same day of collection. Measurements were made using the Diving-PAM 5-millimeter diameter fiber-optic probe at a standardized 5 millimeters above the coral tissue after F0 stabilized. The Diving-PAM settings were set to a

measuring light intensity of 5, gain of 2, and saturation pulse intensity of 5.

Within 3 days of collection, rates of photosynthesis and respiration were measured on each coral fragment by quantifying oxygen evolution and consumption. Each coral fragment was placed in a 250 milliliter (mL) sealed chamber filled with ambient seawater surrounded by a temperature-controlled water jacket to maintain a constant temperature (ambient: 25-27°C). Seawater in the chambers was constantly mixed using a magnetic stir bar. Temperature and dissolved oxygen concentrations were measured using a Pt100 temperature probe and PSt7 oxygen optode (PreSens), respectively, inserted through a port in the lid of each chamber. Oxygen optodes were calibrated on each measurement day with a 0% oxygen solution (0.01 gram per milliliter (g mL-1) NaSO3) and 100% air saturated seawater. Oxygen evolution rates were measured at steady increments of light (112–726 micromole: per second and square meter; µmol m-2 s-1), increasing light intensity only after a steady slope was achieved for all fragments for at least 10 minutes. After the maximum light level, the lights were turned off and oxygen consumption rates were measured until a steady slope was achieved for at least 10 minutes. Temperature and oxygen data were recorded every 3 seconds. Corals were then snap frozen and stored at -80°C until further processing.

Tissue was removed from each fragment using an airbrush containing phosphate-buffered saline solution. The resulting tissue slurry was homogenized at 25,000 rpm for 10 seconds using tissue and aliquoted for subsequent assays.

For symbiont cell counts, tissue homogenate was homogenized at 25,000 rpm for 10 seconds followed by needle shearing with a 22-gauge needle. Algal cells were then pelleted by centrifugation at 7,000 g for 5 minutes and resuspended in 0.1% sodium dodecyl sulfate (SDS) in 0.22 micrometer filtered seawater. Symbiont concentrations were determined by flow cytometry. Symbiont cells were excited with a blue laser (488 nanometers) and identified by analyzing forward scatter and red autofluorescence in GuavaSoft 3.4 with the same gating for all samples.

Chlorophyll was extracted in 100% acetone. Tissue homogenate was centrifuged at 14,000 rpm for 3 minutes at 4°C and the supernatant was removed. The remaining pellet was incubated in 100% acetone for 32-48 hours in the dark at -20°C. The samples were then centrifuged at 14,000 rpm for 3 minutes at 4°C. The supernatant was transferred to a 96-well flat bottom glass plate and absorbance was quantified by measuring absorbance at 630 nanometers, 663 nanometers and 750 nanometers on a plate reader.

Soluble protein content was analyzed via the Bradford method using Coomassie Plus Bradford assay reagent. The crude tissue homogenate was analyzed to obtain a measure of holobiont protein. For the host fraction, symbionts were removed from the crude homogenate by centrifugation at 10,000 g for 10 minutes at 4°C, and the resulting supernatant was analyzed. The samples were mixed with the reagent on a plate shaker for 30 seconds, incubated for 10 minutes at room temperature and again mixed on a plate shaker for 30 seconds. The sample absorbance was then measured at 595 nanometers on a plate reader.

Ash-free dry weight was measured from a known volume of each homogenate dried at 60°C for 24 hours until a constant weight was achieved. After the dry weight was recorded, the homogenates were burned in a muffle furnace at 450°C for 6 hours. The samples were allowed to cool in the furnace before being weighed and the ash weight recorded.

Total antioxidant capacity was measured using the OxiSelect Total Antioxidant Capacity Assay Kit. The tissue homogenate was first centrifuged at 10,000 g for 10 minutes at 4°C prior to loading on the 96-well plate. Net maximum absorbance values were measured at 490 nanometers on a plate reader at the initial and final time points.

After tissue was removed, skeletons were soaked in 10% bleach for approximately 12 hours and then dried at 60°C for approximately 12 hours until a constant weight was reached. Surface area was determined by the single wax dipping method (Veal et al. 2010). Each skeleton fragment was pre-weighed before being dipped in paraffin wax, after which the final weight was recorded. The change in weight due to wax addition was compared against a standard curve of dipped wooden dowels of known surface area to calculate the skeletal surface area of each fragment.

Data Processing Description

Coral colony bleaching scores were determined visually on a 5-point scale from photographs with 1 being fully pigmented and 5 being fully bleached as in McClanahan 2004.

Photosynthetic and respiration rates were calculated from volumetric oxygen production and consumption rates (i.e., micromole oxygen per minute per liter: µmol O2 L-1 min-1) by multiplying the oxygen concentration changes and volume of water in each chamber (chamber volume – coral volume in liters) and accounting for background oxygen flux rates by subtracting the rate of the corresponding seawater-only control chamber. Photosynthesis-irradiance (PI) curves were generated by curve fitting to the Platt model (Platt, Gallegos, and Harrison 1980) in order to extract alpha, Ik, and Pmax. Respiration was calculated from the dark period following the PI curve maximum step. Gross photosynthetic rates were determined by subtracting the oxygen consumption rates from oxygen production for each fragment.

Chlorophyll content was calculated from the equations in (Jeffrey and Humphrey 1975) for dinoflagellates in 100% acetone, correcting for the 0.6 centimeters path length of the 96-well quartz plate:
Chl a = (11.43(A663 - A750/PL) – 0.64(A630 – A750/PL))/(mL homogenate)

The difference between the ash weight and the dry weight was calculated to determine the AFDW of each fragment.

The difference between initial and final readings of antioxidant capacity was used to calculate TAC in micromoles (µM) against the uric acid standard curve. Uric acid equivalents were converted to copper reducing equivalents (CRE).

BCO-DMO processing description:
- Converted dates to ISO date format (yyyy-mm-dd);
- Adjusted field/parameter names to comply with BCO-DMO naming conventions;
- Added the "Site", "Pair", "Bleach", "Latitude", and "Longitude" columns from the "CollectionSummary" data file to the "SummaryData" file;
- Changed longitude from degrees West (positive values) to degrees East (negative values);
- Added a conventional header with dataset name, PI names, version date.

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Data Files

File	
coral_physio_summary.csv	(Comma Separated Values (.csv), 44.05 KB) MD5:2eb881d1b86fa5aa4afdebe0c7f49cdd
Primary data file for dataset ID 852054	

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Related Publications

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

McClanahan, T. R. (2004). The relationship between bleaching and mortality of common corals. Marine Biology, 144(6), 1239–1245. doi:10.1007/s00227-003-1271-9
Methods

Platt, T., C. L. Gallegos, and W. G. Harrison. 1980. “Photoinhibition of Photosynthesis in Natural Assemblages of Marine Phytoplankton.” Journal of Marine Research, 16.
Methods

Veal, C. J., Carmi, M., Fine, M., & Hoegh-Guldberg, O. (2010). Increasing the accuracy of surface area estimation using single wax dipping of coral fragments. Coral Reefs, 29(4), 893–897. doi:10.1007/s00338-010-0647-9
Methods

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Parameters

Parameter	Description	Units
ColonyID	Coral colony genotype	unitless
Species	Coral colony species	unitless
Date	Coral colony collection date; format: YYYY-MM-DD	date
Site	Coral colony reef	unitless
Latitude	Coral colony latitude location	decimal degrees North
Longitude	Coral colony longitude location	decimal degrees East
Pair	Coral colony pair	unitless
Bleach	Coral colony historical bleaching susceptibility 1 = pigmented, 5 = bleached	unitless
Yield	Photochemical efficiency	unitless
FragVol	Coral fragment volume	milliliters
BleachScore	Coral colony bleaching score	1= pigmented, 5=bleached
SA	Coral fragment surface area	centimeters squared
AFDW	Coral fragment ash-free dry weight	grams
BM	Coral fragment biomass	milligrams per centimeters squared
SymFrag	Coral fragment symbiont	cells
ProtFrag	Coral fragment protein content	milligrams protein
ProtHostFrag	Coral fragment host protein content	milligrams protein
ChlaFrag	Coral fragment chlorophyll a content	micrograms chlorophyll a
TAChost	Coral fragment total antioxidant capacity	micromolar coral reef equivalents
alpha	PI curve initial slope	unitless
ik	PI curve saturation point	photosynthetically active radiation (PAR); micromoles per square meter per second (umol m-2 s-1)
NPumolmin	Coral Fragment net photosynthesis rate	micromoles of oxygen per minute
Rumolmin	Coral fragment respiration rate	micromoles of oxygen per minute
GPumolmin	Coral fragment gross photosynthesis rate	micromoles of oxygen per minute

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Instruments

Dataset-specific Instrument Name	Millipore Guava flow-cytometer (Guava easyCyte 5HT)
Generic Instrument Name	Flow Cytometer
Generic Instrument Description	Flow cytometers (FC or FCM) are automated instruments that quantitate properties of single cells, one cell at a time. They can measure cell size, cell granularity, the amounts of cell components such as total DNA, newly synthesized DNA, gene expression as the amount messenger RNA for a particular gene, amounts of specific surface receptors, amounts of intracellular proteins, or transient signalling events in living cells. (from: http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm)

Dataset-specific Instrument Name	Diving PAM (Walz GmbH, Germany)
Generic Instrument Name	Fluorometer
Dataset-specific Description	Diving PAM (Walz GmbH, Germany): An underwater fluorometer used to study in situ photosynthesis by chlorophyll fluorescence analysis. This Dive PAM was used for measuring F0, Fm and Fv/Fm (photochemical efficiency)
Generic Instrument Description	A fluorometer or fluorimeter is a device used to measure parameters of fluorescence: its intensity and wavelength distribution of emission spectrum after excitation by a certain spectrum of light. The instrument is designed to measure the amount of stimulated electromagnetic radiation produced by pulses of electromagnetic radiation emitted into a water sample or in situ.

Dataset-specific Instrument Name	EcoTech Radion XR30w Pro
Generic Instrument Name	LED light
Dataset-specific Description	The EchoTech Radon is an LED aquarium light. The Pro features 42 energy-efficient LEDs and full spectrum output from ultraviolet to hyper-red. The Radion XR30w Pro LED lighting system expands on the technology found in the Radion XR30w. Six channels and 42 high-efficiency LEDs (including ultraviolet) represent the best in purpose-designed LED lighting.
Generic Instrument Description	A light-emitting diode (LED) is a semiconductor light source that emits light when current flows through it. Electrons in the semiconductor recombine with electron holes, releasing energy in the form of photons.

Dataset-specific Instrument Name	PSt7 oxygen optode (PreSens)
Generic Instrument Name	Oxygen Sensor
Dataset-specific Description	PreSens, OXY-10 ST: Software and oxygen optode device for measuring metabolic rates
Generic Instrument Description	An electronic device that measures the proportion of oxygen (O2) in the gas or liquid being analyzed

Dataset-specific Instrument Name	BioTek PowerWave XS2
Generic Instrument Name	plate reader
Dataset-specific Description	Plate reader used in measuring chlorophyll absorbance
Generic Instrument Description	Plate readers (also known as microplate readers) are laboratory instruments designed to detect biological, chemical or physical events of samples in microtiter plates. They are widely used in research, drug discovery, bioassay validation, quality control and manufacturing processes in the pharmaceutical and biotechnological industry and academic organizations. Sample reactions can be assayed in 6-1536 well format microtiter plates. The most common microplate format used in academic research laboratories or clinical diagnostic laboratories is 96-well (8 by 12 matrix) with a typical reaction volume between 100 and 200 uL per well. Higher density microplates (384- or 1536-well microplates) are typically used for screening applications, when throughput (number of samples per day processed) and assay cost per sample become critical parameters, with a typical assay volume between 5 and 50 µL per well. Common detection modes for microplate assays are absorbance, fluorescence intensity, luminescence, time-resolved fluorescence, and fluorescence polarization. From: http://en.wikipedia.org/wiki/Plate_reader, 2014-09-0-23.

Dataset-specific Instrument Name	BioTek ELx808
Generic Instrument Name	plate reader
Dataset-specific Description	Plate reader used in measuring protein and antioxidant concentrations
Generic Instrument Description	Plate readers (also known as microplate readers) are laboratory instruments designed to detect biological, chemical or physical events of samples in microtiter plates. They are widely used in research, drug discovery, bioassay validation, quality control and manufacturing processes in the pharmaceutical and biotechnological industry and academic organizations. Sample reactions can be assayed in 6-1536 well format microtiter plates. The most common microplate format used in academic research laboratories or clinical diagnostic laboratories is 96-well (8 by 12 matrix) with a typical reaction volume between 100 and 200 uL per well. Higher density microplates (384- or 1536-well microplates) are typically used for screening applications, when throughput (number of samples per day processed) and assay cost per sample become critical parameters, with a typical assay volume between 5 and 50 µL per well. Common detection modes for microplate assays are absorbance, fluorescence intensity, luminescence, time-resolved fluorescence, and fluorescence polarization. From: http://en.wikipedia.org/wiki/Plate_reader, 2014-09-0-23.

Dataset-specific Instrument Name	Olympus TG-5
Generic Instrument Name	Underwater Camera
Dataset-specific Description	Underwater camera for photographing whole coral colonies and individual fragments
Generic Instrument Description	All types of photographic equipment that may be deployed underwater including stills, video, film and digital systems.

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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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