Coral physiology parameters acquired during a heatwave experiment done September to November 2018 using reef building corals collected in Kāne'ohe Bay, O'ahu, Hawai'i.

Website: https://www.bco-dmo.org/dataset/884544
Data Type: experimental
Version: 1
Version Date: 2023-01-31

Project
» NSFOCE-BSF: COLLABORATIVE RESEARCH: Elucidating adaptive potential through coral holobiont functional integration (Holobiont Integration)

Contributors	Affiliation	Role
Putnam, Hollie	University of Rhode Island (URI)	Co-Principal Investigator
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Abstract
Two common reef-building corals, Montipora capitata and Pocillopora acuta, were collected from six sites in Kāne'ohe Bay, O'ahu, Hawai'i. Fragments were allowed to acclimate in experimental tanks for two weeks prior to exposure to one of the following four treatments: Ambient Temperature Ambient pCO2 (ATAC), Ambient Temperature High pCO2 (ATHC), High Temperature Ambient pCO2 (HTAC), and High Temperature High pCO2 (HTHC). The treatment period lasted for a two month period, starting on September 22nd, 2018 and lasting through November 17th, 2018. Following the stress period, coral fragments were exposed to a two-month recovery period in ambient conditions. Throughout the entire four-month experiment, fragments were randomly sampled from each tank and treatment for the following physiology parameters: gross photosynthesis, respiration, net photosynthesis (gross photosynthesis - respiration), photosynthesis:respiration ratio, chlorophyll concentration (pigment a and c2), symbiont and host tissue biomass, symbiont:host tissue biomass ratio, host soluble protein, host total antioxidant capacity, and endosymbiont density. Net photosynthesis, chlorophyll concentration, and symbiont tissue biomass were normalized to host surface area (cm-2) and endosymbiont density (cell-1).

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Coverage

Spatial Extent: **N**:21.477194 **E**:-157.786861 **S**:21.429417 **W**:-157.833667
Temporal Extent: 2018-09-22 - 2018-11-17

Methods & Sampling

Corals sampled at six reefs within Kāne'ohe Bay, O'ahu, Hawai'i:

1.) USA: Hawaii HIMB: 21.436056, -157.786861
2.) USA: Hawaii Reef.11.13: 21.450806, -157.794944
3.) USA: Hawaii Reef.35.36: 21.473889, -157.833667
4.) USA: Hawaii Reef.18: 21.450806, -157.811139
5.) USA: Hawaii Lilipuna.Fringe: 21.429417, -157.791111
6.) USA: Hawaii Reef.42.43: 21.477194, -157.826889

Experiment conducted at the Hawai'i Institute of Marine Biology.

Photosynthesis and Respiration Rates: Prior to experimental exposures, 10 coral fragments (5 per species) were used to generate a photosynthesis-irradiance curve to determine saturating irradiance for assessing rates of photosynthesis. Fragments were exposed to 10 light levels: 0, 15, 30, 60, 91, 136, 227, 416, 529, and 756 µmol m-2 s-1 generated by two LED lights (Aqua Illumination Hydra FiftyTwo) hung above the incubation chambers (described below). Rates of oxygen consumption or evolution were extracted using curve fitting of a non-linear least squares fit for a non-rectangular hyperbola (NLLS; Marshall & Biscoe 1980, Heberling 2013) was used to identify PI curve characteristics of each species. This model is as follows: = ϕPAR+√(φPPFD + Pmax)2-4ΘφPAR Pmax2Θ-Rd . Theta was set at 0.6 for *M. capitata* and 0.64 for *P. acuta*. Photosynthesis-irradiance curves were performed four times throughout the experiment on fragments of both species in the HTHC treatment to determine that there were no significant changes in Ik that occured during bleaching, and thus no need to change light settings used to measure respiration and photosynthetic rates.

Fragments were placed in individual respiration chambers (~610mL), with individual temperature (Pt1000 temperature sensor, PreSens) and fiber-optic oxygen probes (Oxygen Dipping Probes DP-PSt7, accuracy = ± 0.05% O2, PreSens) connected to a 10-channel oxygen meter (OXY-10 ST, accuracy = ± 1.0 °C, resolution = 0.1 °C, PreSens), to evaluate photosynthesis under saturating light conditions, as determined by PI curves described above, and light enhanced dark respiration (LEDR; Edmunds and Davies 1988). The respirometry setup consisted of 10 chambers with stirbars. Samples were measured in a series of runs that consisted of eight fragments (n=4 per species and n=2 blank chambers) per run, and exposed to PAR irradiance of 590 ± 7.16 µmol photons m-2 s-1 for 15 minutes to assess photosynthetic rates. Immediately afterwards, these fragments were exposed to dark conditions (0 µmol photons m-2 s-1) for 20 minutes to assess LEDR. Following respirometry, fragments were immediately snap-frozen in liquid nitrogen and stored at -80°C.

Sample Collection and Endosymbiont Density: Coral tissue was removed from the coral skeletons by airbrushing (Iwata Eclipse HP-BCS) with ice-cold 1X Phosphate Buffer Saline (PBS) solution, separating the soft tissue from the calcium carbonate skeleton and the total volume was recorded. The tissue slurry was then homogenized for 15 seconds at high speed (PRO Scientific Bio-Gen PRO200 Homogenizer). Of the total tissue slurry, 500 µL was taken for endosymbiont density, 500 µL for host soluble protein and host total antioxidant capacity, 1 mL for chlorophyll concentration assays, and 5 mL for ash-free dry weight. The skeleton was rinsed in freshwater and soaked in 20% bleach for 24 hours and dried at room temperature for 24 hours. The single dip wax-dipping technique (Veal et al. 2010) was used to determine surface area of each dried fragment skeleton. Symbiodiniaeceae cell concentration was quantified with 6 technical replicate counts on a haemocytometer (Hausser Scientific Bright-Line Counting Chamber) after homogenizing the tissue slurry for 1 minute. The average count (cells mL-1) was standardized to homogenate volume and surface area to produce cell density values in cells cm-2.

Host soluble protein and total antioxidant capacity: To quantify host soluble protein and host total antioxidant capacity the host tissue was isolated from symbionts by centrifugation at 10,000g for 10 minutes at 4°C. Soluble host protein of the supernatant was measured using the Pierce BCA Protein Assay Kit (Pierce Biotechnology, Waltham, Massachusetts, Catalog #23225) with two duplicates per sample measured by a 96 well microplate reader (BioTek Synergy HTX Multi-Mode Reader) at a wavelength of 562 nm. Resulting values were compared to a bovine serum albumin standard curve and standardized to homogenate volume and surface area to obtain host soluble protein values (mg cm-2). Host total antioxidant capacity was quantified in duplicates per sample with the Cell BioLabs OxiSelect Total Antioxidant Capacity (TAC) Assay Kit (Catalog #STA-360) manufacturer's instructions and compared to a uric acid standard curve to obtain Copper Reducing Equivalents (CRE) in mmol L-1. CRE values were standardized to total soluble protein for final values in units of CRE µmol mg-1.

Chlorophyll concentration: To isolate symbiont cells for chlorophyll concentration measurements, a 1 mL aliquot of tissue homogenate was centrifuged at 13,000 g for 3 min and the supernatant was discarded. 1 mL of acetone was added to the symbiont pellet and incubated in the dark at 4°C for 24 hours. Samples and acetone blanks were read in duplicate (200 µL each) on a 96-well microplate reader at λ = 630 and 663 nanometers. Chlorophyll pigments a and c2 were calculated with the following dinoflagellate equations, respectively: 11.43E663 - 0.64E630 and 27.09E630 - 3.63E663 (Jeffrey and Humphrey 1975). Concentration values were multiplied by 0.584 to correct for the path length of the plate, and standardized by both surface

area (µg cm-2) and cell density (µg cells-1).

Tissue Biomass: To quantify biomass as ash-free dry weight (AFDW), 5 mL of tissue slurry was centrifuged at 13,000 g for 3 minutes. 4 mL of the supernatant host tissue was removed and placed in pre-weighed burned aluminum pan and the symbiont pellet was resuspended in 1 mL PBS solution and added to separate pre-weighed burned aluminum pans. The samples were placed in a drying oven for 24 hours at 60°C (Thermo Scientific Heratherm General Protocol Oven, Catalog #51028112), weighed, and then placed in a muffle furnace for 4 hours at 500°C (Thermo Scientific Lindberg Blue M Muffle Furnace, Catalog #BF51728C-1). AFDW (mg cm-2) of the host and symbiont fractions were calculated as the post-drying oven weight (dry weight) - post-muffle furnace weight and final values were normalized to surface area. Symbiont to host (S:H) AFDW ratios were calculated by dividing the symbiont AFDW by the host AFDW.

BCO-DMO Processing Description

2023-09-08 [D.Gerlach]
- Corrected parameter mapping to be 'proteins' for amounts, rather than 'protein' name.
- Added NODC topic of 'biota'

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Data Files

File
heatwave_physiology.csv (Comma Separated Values (.csv), 126.90 KB) MD5:0909db05d1bc502e3c38fab71bf44d91 Primary data file for dataset ID 884544

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Related Publications

Anthony, K., & Hoegh-Guldberg, O. (2003). Kinetics of photoacclimation in corals. Oecologia, 134(1), 23–31. https://doi.org/10.1007/s00442-002-1095-1
Methods

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

Edmunds, P. J., & Davies, P. S. (1988). Post-illumination stimulation of respiration rate in the coral Porites porites. Coral Reefs, 7(1), 7–9. https://doi.org/10.1007/bf00301975 https://doi.org/10.1007/BF00301975
Methods

Edmunds, P. J., Gates, R. D., & Gleason, D. F. (2003). The tissue composition of Montastraea franksi during a natural bleaching event in the Florida Keys. Coral Reefs, 22(1), 54–62. https://doi.org/10.1007/s00338-003-0278-5
Methods

Heberling, J. M., & Fridley, J. D. (2013). Resource-use strategies of native and invasive plants in Eastern North American forests. New Phytologist, 200(2), 523–533. Portico. https://doi.org/10.1111/nph.12388
Methods

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

Jokiel, P.L., Maragos, J.E., & Franzisket, L. (1978). Coral growth: buoyant weight technique. Coral Reefs:

Research Methods.
Methods

MARSHALL, B., & BISCOE, P. V. (1980). A Model for C3Leaves Describing the Dependence of Net Photosynthesis on Irradiance. Journal of Experimental Botany, 31(1), 29–39. https://doi.org/10.1093/jxb/31.1.29
Methods

Olito, C., White, C. R., Marshall, D. J., & Barneche, D. R. (2017). Estimating monotonic rates from biological data using local linear regression. Journal of Experimental Biology. https://doi.org/10.1242/jeb.148775
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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Related Datasets

IsRelatedTo

Strand, E., Putnam, H. (2023) **Carbonate Chemistry Parameters from a heatwave experiment done September to November 2018 using reef building corals collected in Kāne'ohe Bay, O'ahu, Hawai'i.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2023-01-31 doi:10.26008/1912/bco-dmo.884411.1 [view at BCO-DMO]
Relationship Description: Dataset is part of same experiment.

Strand, E., Putnam, H. (2023) **Coral growth rate measured during a heatwave experiment done September to November 2018 using reef building corals collected in Kāne'ohe Bay, O'ahu, Hawai'i.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2023-01-31 doi:10.26008/1912/bco-dmo.884530.1 [view at BCO-DMO]
Relationship Description: Dataset is part of the same experiment.

Strand, E., Putnam, H. (2023) **Coral survivorship tracked during a heatwave experiment done September to November 2018 using reef building corals collected in Kāne'ohe Bay, O'ahu, Hawai'i.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2023-01-31 doi:10.26008/1912/bco-dmo.884551.1 [view at BCO-DMO]
Relationship Description: Dataset is part of the same experiment.

Strand, E., Putnam, H. (2023) **Experiment Tank Conditions from a heatwave experiment done September to November 2018 using reef building corals collected in Kāne'ohe Bay, O'ahu, Hawai'i.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2023-01-31 doi:10.26008/1912/bco-dmo.884417.1 [view at BCO-DMO]
Relationship Description: Dataset is part of same experiment.

Strand, E., Putnam, H. (2023) **Photosynthetic irradiance capacity of coral fragments measured during a heatwave experiment done September to November 2018 using reef building corals collected in Kāne'ohe Bay, O'ahu, Hawai'i.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2023-01-31 doi:10.26008/1912/bco-dmo.884537.1 [view at BCO-DMO]
Relationship Description: Dataset is part of the same experiment.

Strand, E., Putnam, H. (2023) **Physiology color score extracted from pictures taken during a heatwave experiment done September to November 2018 using reef building corals collected in Kāne'ohe Bay, O'ahu, Hawai'i.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2022-11-23 doi:10.26008/1912/bco-dmo.884208.1 [view at BCO-DMO]
Relationship Description: Dataset is part of same experiment.

Strand, E., Putnam, H. (2023) **Temperature data measured during a heatwave experiment done September to November 2018 using reef building corals collected in Kāne'ohe Bay, O'ahu, Hawai'i.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2023-01-31 doi:10.26008/1912/bco-dmo.884738.1 [view at BCO-DMO]
Relationship Description: Dataset is part of the same experiment.

Parameters

Parameter	Description	Units
Site_Name	The reef location where that sample was collected (6 options total)	unitless
Site_Latitude	Latitude of coral sampling site, south is negative	decimal degrees
Site_Longitude	Longitude of coral sampling site, west is negative	decimal degrees
Plug_ID	The individual coral fragment ID number	unitless
Species	Coral host species: Montipora capitata or Pocillopora acuta	unitless
ANALYSIS	Physiology for all entries indicating that fragment was sampled for physiology	unitless
Timepoint	The sampling time point; at what point during the experiment was that fragment sampled	unitless
Treatment	Temperature and pCO2 treatment exposure indication. Ambient Temperature Ambient pCO2 (ATAC), Ambient Temperature High pCO2 (ATHC), High Temperature Ambient pCO2 (HTAC), or High Temperature High pCO2 (HTHC).	unitless
Tank	The tank number that coral fragment was sampled from (12 options; 3 tanks per treatment)	unitless
Temperature	Temperature treatment indication: high or ambient	unitless
CO2	pCO2 treatment indication: high or ambient	unitless
Pgross_umol_cm2_hr	Gross photosynthesis measurement normalized to host surface area. Units are change in oxygen levels	umol/cm2/hr
Pnet_umol_cm2_hr	Net photosynthesis measurement normalized to host surface area. Units are change in oxygen levels	umol/cm2/hr
PR	Ratio of Net photosynthetic rates (Pnet_umol.cm2.hr; u mol cm-2 h-1) to Respiration rates (Rdark_umol.cm2.hr; u mol cm-2 h-1)	umol/cm2/hr
Rdark_umol_cm2_hr	Respiration rates measurement normalized to host surface area. Units are change in oxygen levels	umol/cm2/hr
Pnet_cell	Net photosynthesis measurement normalized to endosymbiont density. Units are change in oxygen levels	umol/cm2/hr
chla_ug_cm2	Chlorophyll-a concentration measurement normalized to host surface area	ug cm-2
chla_ug_cells	Chlorophyll-a concentration measurement normalized to endosymbiont density	ug cell-1
AFDW_mg_cm2_Sym	Symbiont Tissue Biomass Ash-Free Dry Weight (AFDW) measurement normalized to host surface area (AFDW)	mg cm-2
AFDW_mg_cm2_Host	Host Tissue Biomass Ash-Free Dry Weight (AFDW) measurement normalized to host surface area (AFDW)	mg cm-
AFDW_mg_cells_Sym	Symbiont Tissue Biomass Ash-Free Dry Weight (AFDW) measurement normalized to endosymbiont density (AFDW)	mg cell-1
Ratio_S_H	Ratio of Symbiont Tissue Biomass : Host Tissue Biomass	mg cm-2
prot_mg_cm2	Host soluble protein normalized to host surface area	mg cm-2
cre_umol_mgprot	Host total antioxidant capacity in Copper Reducing Equivalents normalized to host soluble protein	umol mg protein-1
haemo_cells_cm2	Endosymbiont density normalized to host surface area	cells cm-2
vol_mL	Volume of homogenate collected	milliliters (ml)
surface_area_cm2	The calculated surface area based on the standard curve used	units

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Instruments

Dataset-specific Instrument Name	
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	Thermo Scientific Heratherm General Protocol Oven, Catalog #51028112
Generic Instrument Name	Drying Oven
Generic Instrument Description	a heated chamber for drying

Dataset-specific Instrument Name	Hausser Scientific Bright-Line Counting Chamber
Generic Instrument Name	Hemocytometer
Generic Instrument Description	A hemocytometer is a small glass chamber, resembling a thick microscope slide, used for determining the number of cells per unit volume of a suspension. Originally used for performing blood cell counts, a hemocytometer can be used to count a variety of cell types in the laboratory. Also spelled as "haemocytometer". Description from: http://hlsweb.dmu.ac.uk/ahs/elearning/RITA/Haem1/Haem1.html.

Dataset-specific Instrument Name	PRO Scientific Bio-Gen PRO200 Homogenizer
Generic Instrument Name	Homogenizer
Generic Instrument Description	A homogenizer is a piece of laboratory equipment used for the homogenization of various types of material, such as tissue, plant, food, soil, and many others.

Dataset-specific Instrument Name	Thermo Scientific Lindberg Blue M Muffle Furnace, Catalog #BF51728C-1
Generic Instrument Name	muffle furnace
Generic Instrument Description	A muffle furnace or muffle oven (sometimes retort furnace in historical usage) is a furnace in which the subject material is isolated from the fuel and all of the products of combustion, including gases and flying ash. A type of jacketed enclosure that is used to heat a material to significantly high temperatures while keeping it contained and fully isolated from external contaminants, chemicals or substances. Muffle furnaces are usually lined with stainless steel, making them largely corrosion-resistant.

Dataset-specific Instrument Name	BioTek Synergy HTX Multi-Mode Reader
Generic Instrument Name	plate reader
Dataset-specific Description	96 well microplate reader (BioTek Synergy HTX Multi-Mode Reader)
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.

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Project Information

NSFOCE-BSF: COLLABORATIVE RESEARCH: Elucidating adaptive potential through coral holobiont functional integration (Holobiont Integration)

Website: https://sites.rutgers.edu/coralbase/

Coverage: Hawaii, Rhode Island, New Jersey, Israel

NSF Abstract:

The remarkable success of coral reefs is explained by interactions of the coral animal with its symbiotic microbiome that is comprised of photosynthetic algae and bacteria. This total organism, or "holobiont", enables high ecosystem biodiversity and productivity in coral reefs. These ecosystems are, however, under threat from a rapidly changing environment. This project aims to integrate information from the cellular to organismal level to identify key mechanisms of adaptation and acclimatization to environmental stress. Specific areas to be investigated include the role of symbionts and of epigenetics (molecular "marks" on coral DNA that regulate gene expression). These aspects will be studied in Hawaiian corals to determine whether they explain why some individuals are sensitive or resistant to environmental perturbation. Results from the proposed project will also provide significant genomic resources that will contribute to fundamental understanding of how complex biological systems generate emergent (i.e., unexpected) properties when faced with fluctuating environments. Broader impacts will extend beyond scientific advancements to include postdoctoral and student training in Science, Technology, Engineering and Mathematics (STEM). Data generated in the project will be used to train university students and do public outreach through live videos of experimental work, and short stop-action animations for topics such as symbiosis, genomics, epigenetics, inheritance, and adaptation. The research approaches and results will be shared with the public in Hawaii through the Hawaii Institute of Marine Biology education department and presentations at Hawaiian hotels, as well as at Rutgers University through its 4-H Rutgerscience Saturdays and 4-H Rutgers Summer Science Programs.

Symbiosis is a complex and ecologically integrated interaction between organisms that provides emergent properties key to their survival. Such is the case for the relationship between reef-building corals and their microbiome, a meta-organism, where nutritional and biogeochemical recycling provide the necessary benefits

that fuel high reef productivity and calcification. The rapid warming and acidification of our oceans threatens this symbiosis. This project addresses how relatively stress resistant and stress sensitive corals react to the environmental perturbations of increased temperature and reduced pH. It utilizes transcriptomic, epigenetic, and microbial profiling approaches, to elucidate how corals respond to environmental challenges. In addition to this profiling, work by the BSF Israeli partner will implement powerful analytical techniques such as network theory to detect key transcriptional hubs in meta-organisms and quantify biological integration. This work will generate a stress gene inventory for two ecologically important coral species and a (epi)genome and microbiome level of understanding of how they respond to the physical environment. Acknowledgment of a role for epigenetic mechanisms in corals overturns the paradigm of hardwired genetic control and highlights the interplay of genetic and epigenetic variation that may result in emergent evolutionary and ecologically relevant properties with implications for the future of reefs. Furthermore, clarifying the joint contribution of the microbiome and host in response to abiotic change will provide an important model in metazoan host-microbiome biotic interactions.

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-1756623

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