# Photochemical yield and color score data from short-term heat stress assays performed with with corals collected from sites around Heron Island, southern Great Barrier Reef in Sept and Oct of

Website: https://www.bco-dmo.org/dataset/926887 Data Type: experimental Version: 1 Version Date: 2024-05-07

#### Project

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

Contributors	Affiliation	Role
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#### Abstract

Variable temperature regimes that expose corals to sub-lethal heat stress have been recognized as a mechanism to increase coral thermal tolerance and lessen coral bleaching. However, there is a need to better understand which thermal regimes maximize coral stress hardening. Here, standardized thermal stress assays were used to determine the relative thermal tolerance of three divergent genera of corals (Acropora, Pocillopora, Porites) originating from six reef sites representing an increasing gradient of annual mean diel temperature fluctuations of 1–3°C day-1. Bleaching severity and dark-acclimated photochemical yield (i.e., Fv/Fm) were quantified following exposure to five temperature treatments ranging from 23.0 to 36.3°C (see Related Datasets for temperature data). This data set contains photochemical yield and color score data used to determine effective dose 50 (ED50, thermal tolerance). It also includes images used for analysis of coral color, a proxy for bleaching severity.

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# Coverage

Location: Heron Island Research Station, Heron Island, southern Great Barrier Reef (23 27°S, 151 55°E) Spatial Extent: Lat:-23.27 Lon:151.55 Temporal Extent: 2022-09-15 - 2022-10-30

# **Dataset Description**

See the "Related Datasets" section for other closely related data that was also part of the study published in Brown et al., 2024.

#### Methods & Sampling

Acute heat stress experiment

A standardized temperature profile was used to measure heat tolerance in corals (e.g., (Voolstra et al. 2020; Cunning et al. 2021; Marzonie et al. 2022; Evensen et al. 2023)) with minor modification in temperature profiles. The climatological maximum monthly mean (MMM) of Heron Reef is 27.3°C (Weeks et al. 2008). A pilot experiment with all three genera indicated no difference in Fv/Fm between MMM, MMM+3°C and MMM+6°C, so the latter two treatments were increased to more accurately assess the decline in performance, such that the five treatments used here included ambient, MMM, MMM+4°C, MMM+6.5°C, and MMM+9°C. Generally, experiments began at ~12:00 with a 3-hr ramp to respective treatment temperatures (23°C, 27.3°C, 31.3°C, 33.8°C, 36.3°C), a 3-hr hold, and a 1-hr ramp down to MMM (Evensen et al. 2023) (Figure S1 of Brown et al., 2024). Lights were turned off at the onset of the 1-hr ramp down to correspond with sunset. Due to experimental constraints (space, equipment, and time), only two treatments (n = 1 tank) were performed per day and each site was done in isolation. Accordingly, a complete assay took two days per site, with treatments tested each day selected randomly (Table S1, Figure S1 of Brown et al., 2024). A fragment from each coral colony was randomly placed into each treatment, so that all colonies were present in each treatment. Temperatures were controlled using an Apex controller (Neptune Systems). Apex temperature probes were calibrated against a high-precision temperature probe (HANNA HI-98190; accuracy: ±0.4°C at 25°C; resolution:  $\pm 0.10$  °C) at the onset of the experiment. Temperatures were also recorded using cross-calibrated temperature loggers (HOBO UA-001-64, accuracy: ± 0.29°C at 25°C). Photosynthetically active radiation (PAR) was static and controlled using aquarium lights (NICREW HyperReef LED, Shenzhen NiCai Technology Co.), averaging 250 µmol m-2 sec-1.

Physiological responses to acute heat stress

At the end of the ramp and after 1 h of darkness (~19:00), corals were assessed for dark-adapted photochemical yield (Fv/Fm) using a Diving-PAM (Walz GmbH) 5-mm diameter fiber-optic probe at a standardized distance (5 mm) above the coral tissue after Fo stabilized. Two random spots on either side of a single fragment were measured to obtain average measures of Fv/Fm. All readings with Fo values that were less than 110 were removed to avoid any false detections (Marzonie et al., 2022). The following morning at 07:00 corals were photographed with a color standard (WDKK Waterproof Color Chart, DGK Color Tools) to assess the effect of temperature on coral color, a proxy for relative chlorophyll density and bleaching severity(Winters et al. 2009; Voolstra et al. 2020).

Image analysis of coral color, a proxy for bleaching severity

Coral color was determined from each photograph in a semi-automated manner. Each photograph was first cropped to a standard size to remove excess background via a custom automated batch script in Adobe Photoshop (Version 21.1.2). Photographs were then loaded into Imagel (v1.53c (Schneider et al. 2012)), and the performance of 16 built-in segmentation models were tested on a sub-sample of coral images to remove the background of the cropped image, which was turned to black, thus leaving only the coral fragment. The segmentation model that best segmented all coral fragments from the background effectively with limited coral fragment cut off (Model Li) was then implemented on all images, which were batch processed using a custom image segmentation macro script modified from (Strock 2021). Once segmented, the script then extracted red pixel intensity of the fragment in RGB, HSB, and LAB color spaces. Finally, the mean red pixel intensity of the red color standard from the original (unsegmented) images was extracted from a region of interest drawn by hand in Imagel. Pixel intensities of the coral and corresponding red standard were then converted to a 'darkness' score by subtracting the red channel 'brightness' from the maximum value (255). The mean red channel darkness of each coral was then normalized by dividing by the mean red pixel darkness of the red color standard from the same photograph. These red-normalized color values were then used to calculate the changes in bleaching severity between species, sites and treatments. For visualization, the red-normalized color values were divided by the mean color under ambient (MMM) conditions for that species.

#### Organism identifiers (Genus, Lifesciences Identifier [LSID]):

Pocillopora, urn:lsid:marinespecies.org:taxname:206938 Porites, urn:lsid:marinespecies.org:taxname:206485 Acropora, urn:lsid:marinespecies.org:taxname:205469

#### **Data Processing Description**

For more detailed information on analysis and results, please see: Brown, et al. (2024).

#### **BCO-DMO Processing Description**

\* Tables imported into BCO-DMO data system from provided files "All sites yield data.csv" and "Color scores all HB2.csv.

\* After discussion with data submitter, color score data was joined into the all sites yield table using the colonyID as a key(colony id, treatment).

\*\* Missing data values are displayed differently based on the file format you download. They are blank in csv files, "NaN" in MatLab files, etc.

\* Column names adjusted to conform to BCO-DMO naming conventions designed to support broad re-use by a variety of research tools and scripting languages. [Only numbers, letters, and underscores. Can not start with a number]

\* file "ed50\_revised.csv" renamed "ed50.csv" and attached to dataset as supplemental file.

#### **Problem Description**

N/A

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### **Related Publications**

Brown, K. T., Martynek, M. P., & Barott, K. L. (2024). Local habitat heterogeneity rivals regional differences in coral thermal tolerance. Coral Reefs. https://doi.org/<u>10.1007/s00338-024-02484-x</u> *Results* 

Cunning, R., Parker, K. E., Johnson-Sapp, K., Karp, R. F., Wen, A. D., Williamson, O. M., Bartels, E., D'Alessandro, M., Gilliam, D. S., Hanson, G., Levy, J., Lirman, D., Maxwell, K., Million, W. C., Moulding, A. L., Moura, A., Muller, E. M., Nedimyer, K., Reckenbeil, B., ... Baker, A. C. (2021). Census of heat tolerance among Florida's threatened staghorn corals finds resilient individuals throughout existing nursery populations. Proceedings of the Royal Society B: Biological Sciences, 288(1961). https://doi.org/<u>10.1098/rspb.2021.1613</u> *Methods* 

Evensen, N. R., Parker, K. E., Oliver, T. A., Palumbi, S. R., Logan, C. A., Ryan, J. S., Klepac, C. N., Perna, G., Warner, M. E., Voolstra, C. R., & Barshis, D. J. (2023). The Coral Bleaching Automated Stress System (CBASS): A low-cost, portable system for standardized empirical assessments of coral thermal limits. Limnology and Oceanography: Methods, 21(7), 421–434. Portico. https://doi.org/<u>10.1002/lom3.10555</u> *Methods* 

#### Methods

Marzonie, M. R., Bay, L. K., Bourne, D. G., Hoey, A. S., Matthews, S., Nielsen, J. J. V., & Harrison, H. B. (2022). The effects of marine heatwaves on acute heat tolerance in corals. Global Change Biology, 29(2), 404–416. Portico. https://doi.org/<u>10.1111/gcb.16473</u> *Methods* 

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

#### Software

Strock, C. (2021). Protocol for extracting basic color metrics from Images in ImageJ/Fiji. Zenodo. https://doi.org/10.5281/ZENODO.5595203 <u>https://doi.org/10.5281/zenodo.5595203</u> *Methods* 

Voolstra, C. R., Buitrago-López, C., Perna, G., Cárdenas, A., Hume, B. C. C., Rädecker, N., & Barshis, D. J. (2020). Standardized short-term acute heat stress assays resolve historical differences in coral thermotolerance across microhabitat reef sites. Global Change Biology, 26(8), 4328-4343. Portico. https://doi.org/<u>10.1111/gcb.15148</u> *Methods* 

#### , Methods

Weeks, S. J., Anthony, K. R. N., Bakun, A., Feldman, G. C., & Guldberg, O. H.-. (2008). Improved predictions of coral bleaching using seasonal baselines and higher spatial resolution. Limnology and Oceanography, 53(4), 1369–1375. Portico. https://doi.org/10.4319/lo.2008.53.4.1369 Methods

#### Methods

Winters, G., Holzman, R., Blekhman, A., Beer, S., & Loya, Y. (2009). Photographic assessment of coral chlorophyll contents: Implications for ecophysiological studies and coral monitoring. Journal of Experimental Marine Biology and Ecology, 380(1–2), 25–35. https://doi.org/<u>10.1016/j.jembe.2009.09.004</u> *Methods* 

Methods

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# **Related Datasets**

#### IsRelatedTo

Brown, K., Barott, K. (2024) **Temperature data from short-term heat stress assays performed with with corals collected from sites around Heron Island, southern Great Barrier Reef in Sept and Oct of 2022.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-05-07 http://lod.bco-dmo.org/id/dataset/926905 [view at BCO-DMO] *Relationship Description: This dataset was part of the same heat-assay experiment.* 

Brown, K., Barott, K. (2024) **Thermal tolerance (ED50) data used to compare previously published regional (Florida Reef Tract, Coral Sea, Red Sea) coral thermal tolerance with Heron Island, Great Barrier Reef values measured in 2022.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-05-07 http://lod.bco-dmo.org/id/dataset/926911 [view at BCO-DMO]

Relationship Description: These datasets were part of the same study published in Brown et al., 2024.

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#### Parameters

Parameter	Description	Units
Site	Site Name ( 'Deep Lagoon', 'Fourth Point', 'Harry\'s Bommie', 'Reef Crest', 'Reef Flat', 'Shallow Lagoon' )	unitless
Genus	Coral genus	unitless
ColonyID	Colony ID (genotype)	unitless
Treatment_nonnum	Treatment label: Ambient, T1, T2, T3, T4 (see methodology for treatment code details).	unitless
Treatment	Numeric treatment identifier	unitless
Replicate	Replicate	unitless
Temperature	Temperature	degrees Celsius (degC)
FO	minimum fluorescence value	unitless
Fm	maximum amount of fluorescence	unitless
Y	Dark-acclimated photochemical yield (Fv/Fm)	unitless
Label	image filename with suffix _RGB (see Supplemental Files for images within Heron_STHS_photos.zip)	unitless
Color_type	colorspace	unitless
Color	color channel (R=red,G=green,B=blue)	unitless
Name	updated filename with color channel information added as a suffix _RGB_## (see Supplemental Files for images within Heron_STHS_photos.zip)	unitless
Areal	area of selected image space	square pixels
Mean_frag	mean pixel intensity across the coral fragment	unitless
StdDev1	standard deviation of pixel intensity	unitless
Mode1	mode of pixel intensity	unitless
MinThr1	minimum pixel intensity of color channel	unitless
MaxThr1	maximum pixel intensity of color channel	unitless
Label_match	updated filename (no suffix or extension). See Supplemental Files for images within Heron_STHS_photos.zip which contain the Label_match with added file extension .jpg	unitless

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Instruments

Dataset- specific Instrument Name	
Generic Instrument Name	Fluorometer
Dataset- specific Description	photochemical yield (Fv/Fm): Diving-PAM (Walz GmbH)
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	temperature loggers (HOBO UA-001-64)
Generic Instrument Name	Onset HOBO Pendant Temperature/Light Data Logger
Dataset- specific Description	Temperatures were controlled using an Apex controller (Neptune Systems). Apex temperature probes were calibrated against a high-precision temperature probe (HANNA HI-98190; accuracy: $\pm 0.4$ °C at 25°C; resolution: $\pm 0.10$ °C) at the onset of the experiment. Temperatures were also recorded using cross-calibrated temperature loggers (HOBO UA-001-64, accuracy: $\pm 0.29$ °C at 25°C).
Generic Instrument Description	The Onset HOBO (model numbers UA-002-64 or UA-001-64) is an in-situ instrument for wet or underwater applications. It supports light intensity, soil temperature, temperature, and water temperature. A two-channel logger with 10-bit resolution can record up to approximately 28,000 combined temperature and light measurements with 64K bytes memory. It has a polypropylene housing case. Uses an optical USB to transmit data. A solar radiation shield is used for measurement in sunlight. Temperature measurement range: -20 deg C to 70 deg C (temperature). Light measurement range: 0 to 320,000 lux. Temperature accuracy: +/- 0.53 deg C from 0 deg C to 50 deg C. Light accuracy: Designed for measurement of relative light levels. Water depth rating: 30 m.

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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 aguarium 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)	<u>OCE-1923743</u>

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