Anemone photochemistry assessed via active chlorophyll a fluorescence by pulse amplitude modulation (PAM) fluorometry after acute heating across a range of temperatures (28–36°C) in a controlled Coral Bleaching Acute Stress System (CBASS)

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Data Type: experimental
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Project
» EAGER: Collaborative Research: Bleaching phenotypes of acute vs. chronic coral bleaching susceptibility and resilience: towards a standardized coral resilience diagnostic (EAGER-CBASS)

Contributors	Affiliation	Role
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Abstract
In January 2023, the model sea anemone Exaiptasia diaphana was infected with two different strains of symbiotic algae that were originally grown under either a pre-acclimated ambient control temperature (28°C), or an elevated thermal selection temperature (31 or 32°C) for 80 generations. Once infected with these algal cultures, these anemones were photoacclimated to low and high light used to test the photochemical response after acute heating across a range of temperatures (28–36°C) in a controlled Coral Bleaching Acute Stress System (CBASS). After six hours of heating, anemone photochemistry was assessed via active chlorophyll a fluorescence by pulse amplitude modulation (PAM) fluorometry. These data were used to evaluate the thermal tolerance of wild-type and thermally selected symbionts, and to assess if the thermal heating dose-response from this symbiosis is comparable to similar acute heating protocols used to evaluate symbiotic reef building corals. These data were collected in the laboratory of Dr. Mark Warner at the University of Delaware, School of Marine Science and Policy.

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Coverage

Location: Lewes, Delaware, USA
Temporal Extent: 2022-10-01 - 2023-04-28

Methods & Sampling

In the fall of 2022, Aposymbiotic (lacking Symbiodiniaceae) sea anemones, *Exaiptasia diaphana* were used for initial algal infections by two strains of algae, *Symbiodinium necroappetens* and *Breviolum minutum*.

For both algal strains, previous work (not related to this project) had established different lines of thermally naïve (i.e., wild-type, WT hereafter) and thermally selected (TS hereafter) cell lines. Briefly, each alga was first placed into uni-cellular culture and then subjected to a long-term thermal adaptation trial, using the ratchet

heating method. Replicate cultures were subjected to a chronic heating protocol wherein temperature was increased in 1 degree Celsius (°C) increments while monitoring algal growth. A subset of the fastest growing isolates at each temperature were then shifted into the next higher temperature and growth was monitored for approximately one month. This process was repeated until negative growth was noted in each algal strain. Thermally selected algae were then grown at the highest temperature for which growth remained positive for 80 generations (32°C for *S. necroappetens* and 31°C for *B. minutum*). After maintaining cultures at these elevated temperatures for approximately one year, thermally selected cultures were then shifted back to the naïve growth temperature (28 °C) for three months.

Anemones harboring each WT and TS alga were then grown under low and high light conditions in 2022 (50 µmol photons m-2 s-1 and 220 µmol photons m-2 s-1, respectively) for two months prior to acute heating experiments that began in January 2023. All animals were fed brine shrimp and cleaned once per week until experimental work began. To test the response to acute heating, anemones were subjected to four temperatures (28, 32, 34, and 36 °C) for six hours within two Coral Bleaching Automated Stress Systems (CBASS). All acute heating trials were run under the respective growth light level for each set of anemones with the same aquarium LED lights used for the initial photoacclimation period described above. After six hours of heating at each temperature, anemones where shifted into the dark and held for dark acclimation for 25 minutes. Following dark acclimation, the maximum quantum yield of photosystem II (Fv/Fm) was recorded with a pulse amplitude modulation fluorometer (Diving PAM, Walz). Fv/Fm readings were taken from each anemone before (Time zero) and after six hours of heating.

Data Processing Description

Data were offloaded from the PAM fluorometer at the end of each day of use, using the WinControl software. Text files were created to record the maximum quantum yield of photosystem II (Fv/Fm) for each anemone held at low light (LL) and (HL), harboring either thermally naïve (wild type, WT) or thermally selected (SS) symbionts *S. necroappetens* (isolate # 1163) or *B. minutum* (isolate # 64).

BCO-DMO Processing Description

- Imported original file "Dark FvFm only.csv" into the BCO-DMO system.
- Renamed fields to comply with BCO-DMO naming conventions.
- Saved final file as "949804_v1_anemone_acute_heating.csv"

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Parameters

Parameter	Description	Units
temp	temperature in °C (28, 32, 34, 36)	degrees Celsius
light	HL (high light, 220 µmol photons m-2 s-1) or LL (50 µmol photons m-2 s-1)	unitless
phenotype	algal phenotype for each algal genotype: Thermally selected (SS) or Wild type (WT)	unitless
genotype	the algal genotype (species) used: 1163 (S. necroapetens) or 64 (B. minutum)	unitless
dark_b	?	?
dark_h	?	?

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Instruments

Dataset-specific Instrument Name	Coral Bleaching Automated Stress System (CBASS)
Generic Instrument Name	Coral Bleaching Automated Stress System
Dataset-specific Description	A home-built, open-access instrument designed to rapidly and accurately change and hold chamber temperature while holding symbiotic organisms (e.g., reef corals or sea anemones). This system is described in detail in the following open access publication: https://doi.org/10.1002/lom3.10555.
Generic Instrument Description	CBASS, which stands for "Coral Bleaching Automated Stress System", are portable, field-deployable experimental tanks used to apply rapid, acute heat stress challenges. This system is described in: 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/10.1111/gcb.15148

Dataset-specific Instrument Name	Diving PAM Fluorometer (Walz)
Generic Instrument Name	Fluorometer
Dataset-specific Description	Diving PAM Fluorometer (Walz) with a blue LED excitation light and halogen saturation light. Relevant instrument settings: Measuring light: 4, Gain: 6, Saturation Pulse intensity: 12, Saturation Pulse width: 0.6 seconds.
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	LED Aquarium lights
Generic Instrument Name	LED light
Dataset-specific Description	ARKNOAH LED Aquarium Light 165W Full Spectrum.
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.

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Project Information

EAGER: Collaborative Research: Bleaching phenotypes of acute vs. chronic coral bleaching susceptibility and resilience: towards a standardized coral resilience diagnostic (EAGER-CBASS)

Coverage: Red Sea, Thuwal, Saudi Arabia, Eilat Israel

NSF Award Abstract:
The past few years have seen an unprecedented amount of coral bleaching across the globe. Global bleaching events in 2015-17, severely impacting iconic coral reefs in places such as the Great Barrier Reef, Micronesia, Hawaiian Islands, and Caribbean, were the worst recorded in recent human history. When ocean temperatures rise, the symbiosis between reef-building corals and their photosynthetic algae deteriorates, many times resulting in widespread coral die-offs as corals can starve without their symbiotic partners to supply food. These widespread events can have drastic impacts on ocean health and biodiversity, as well as the communities that depend on reefs for fishing, tourism, and protection from storms. Importantly, some corals resist or recover from bleaching better than others. Such variability in coral response to ocean warming could be critical to reef survival in the future, yet the scientific community lacks any standardized diagnostics to rapidly assess bleaching tolerance limits. Here, we plan to: 1) develop a standardized, short-term exposure to assess bleaching limits (analogous to cardiac stress tests for humans), 2) design an experimental system capable of delivering a range of thermal treatments as an open-source, low-cost, highly-portable device that can be readily adapted for bleaching tests in a wide variety of coral habitats, and 3) disseminate the results, instructions, and technologies to the reef research and conservation community through a combination of hands-on workshops, online outreach materials, press releases, and open-access research publications. Widespread dissemination of project products will be achieved via hands-on demonstrations and workshops in

key geographic areas (Middle East, Caribbean, and Indo-Pacific), with a focus on the assembly of the system and operation of the experimental assay using local corals. This project will train both graduate students and a postdoctoral researcher, and brings together a team of national and global researchers in a collaborative investigation to address the international problem of coral bleaching.

With each passing year, coral bleaching has shifted from an issue of serious sporadic concern to a critical widespread threat to reefs across the globe that is increasing in frequency and severity. However, during widespread bleaching events, some scattered corals and reef sections are able to survive better than others. Whether this is due to acclimatization or adaptation in thermal stress tolerance, this variability in response is critical to coral resilience to climate impacts. Currently, the scientific community lacks a standardized approach to rapidly assess coral thermal limits and identify resilient individuals or populations. Present day approaches range from observational surveys of natural bleaching and mortality, to multiple weeks of controlled chronic thermal exposure, to rapid, single or multi-day acute heat shocks. To what degree bleaching response varies across short-term versus longer-term experiments and how these responses compare to natural bleaching patterns is largely unknown. Using a group of coral species representative of a historical range of bleaching susceptibility (e.g., Acropora hemprichii, Pocillopora meandrina, and Porites lobata), research will address this important knowledge gap by experimental evaluation of the bleaching response to acute (0 - 2 day) versus chronic (>4 week) thermal stress. The overarching questions for this study are: how are the acute and chronic coral bleaching responses related, and can investigators predict ecologically relevant bleaching outcomes from the response to a short-term, acute heat-stress? To answer these questions, the research team will: 1) objectively compare acute versus chronic heat-stress exposures and synthesize a variety of response metrics based on core physiological measurements to develop a standardized, short-term thermal assay and diagnostic approach to rapidly assess bleaching, 2) operationalize an experimental system built around an open-source, cost-effective, easily transportable temperature control technology, and 3) distribute the results, experimental procedures, and temperature controlling technologies to the reef research and conservation communities. This project will produce an affordable experimental system and short-term diagnostic capable of determining coral thermal limits in just a few days in almost any location with reliable access to seawater and electricity or a portable generator. The research fills a critical knowledge gap through the development of a standardized set of diagnostic tools to assess coral thermal vulnerability before widespread bleaching events actually occur, so that proactive conservation and management strategies can be implemented ahead of widespread impacts to reef ecosystems.

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-1833215

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