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The Coral Bleaching Automated Stress System (CBASS): A low-cost, portable system for standardized empirical assessments of coral thermal limits

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Abstract

Ocean warming is increasingly affecting marine ecosystems across the globe. Reef-building corals are particularly affected by warming, with mass bleaching events increasing in frequency and leading to widespread coral mortality. Yet, some corals can resist or recover from bleaching better than others. Such variability in thermal resilience could be critical to reef persistence; however, the scientific community lacks standardized diagnostic approaches to rapidly and comparatively assess coral thermal vulnerability prior to bleaching events. We present the Coral Bleaching Automated Stress System (CBASS) as a low-cost, open-source, field-portable experimental system for rapid empirical assessment of coral thermal thresholds using standardized temperature stress profiles and diagnostics. The CBASS consists of four or eight flow-through experimental aquaria with independent water masses, lighting, and individual automated temperature controls capable of delivering custom modulating thermal profiles. The CBASS is used to conduct daily thermal stress exposures that typically include 3-h temperature ramps to multiple target temperatures, a 3-h hold period at the target temperatures, and a 1-h ramp back down to ambient temperature, followed by an overnight recovery period. This mimics shallow water temperature profiles observed in coral reefs and prompts a rapid acute heat stress response that can serve as a diagnostic tool to identify putative thermotolerant corals for in-depth assessments of adaptation mechanisms, targeted conservation, and possible use in restoration efforts. The CBASS is deployable within hours and can assay up to 40 coral fragments/aquaria/day, enabling high-throughput, rapid determination of thermal thresholds for individual genotypes, populations, species, and sites using a standardized experimental framework.

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The past decade has seen an unprecedented amount of coral bleaching across the globe, with the 2014 2017 coral bleaching event regarded as the worst event recorded to date (Eakin et al. 2022). Such mass bleaching events are expected to occur on an annual basis by 2030 2060 in most coral habitats if current anthropogenic emissions continue (Donner et al. 2005; van Hooidonk et al. 2016; Hughes et al. 2018; Kleypas et al. 2021), resulting in the potential loss of 70 90% of corals worldwide by mid-century (van Hooidonk et al. 2016). Critical to reef persistence in the future are species and populations of reef-building corals that can withstand, adapt/acclimatize to, or recover from these widespread bleaching events (van Woesik et al. 2011; Guest et al. 2012;

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Pineda et al. 2013; Palumbi et al. 2014; Dixon et al. 2015; Logan et al. 2021).

Corals with enhanced resilience to thermal stress and/or bleaching are increasingly being identified, both at the scale of individual coral genotypes (Lundgren et al. 2013; Bay and Palumbi 2014; Dixon et al. 2015) and at the scale of entire populations and reef regions (Barshis et al. 2013; Fine et al. 2013; Palumbi et al. 2014; Parker et al. 2020; Cornwell et al. 2021; Voolstra et al. 2021). Identifying resilient populations is crucial not only for conservation efforts, but also for coral restoration projects where naturally resilient genotypes and/or populations can be used for outplanting and broodstock (van Oppen et al. 2017; Morikawa and Palumbi 2019; Humanes et al. 2021; Blanco-Pimentel et al. 2022). Importantly though, there is still no standard procedure to determine coral bleaching or thermal thresholds nor to assess individuals and populations for resilient characteristics, limiting the capacity for cross-species and cross-study comparisons. To date, common approaches to determining the impacts of thermal stress on corals include widespread observational surveys of naturally occurring coral bleaching and mortality (Guest et al. 2012), meta-analyses (Sully et al. 2019, 2022), multiple weeks/months of controlled thermal exposures or years of field transplantation experiments (Fine et al. 2013; Kenkel et al. 2013; Palumbi et al. 2014), and single-day to multiday acute heat shock assays (Oliver and Palumbi 2011; Barshis et al. 2013; Seneca and Palumbi 2015; Cornwell et al. 2021).

Observational surveys provide the most direct measure of coral bleaching in the field, but different reefs, and even different colony locations within reefs, can have different thermal stress exposures, making comparisons of coral bleaching in the field an indirect measure of colony susceptibility. Indeed, this measure of coral bleaching is largely correlated with environment and Symbiodiniaceae genus, with coral genetic make-up explaining only 2 3% of this measure in one study (Fuller et al. 2020). In addition, surveying bleaching events across large scales and monitoring rates of recovery and mortality are costly and opportunistic. By contrast, multiweek/month thermal stress experiments have long been used to compare natural thermal stress events (Jokiel and Coles 1977) across locations, providing some capacity for standardization. Yet, this approach requires expensive seawater systems and personnel capable of sustaining corals and overseeing precise heating treatments for weeks. As a result, such approaches have an annual throughput of a handful of coral populations or species.

In contrast, recent experiments utilizing relatively minimal equipment to conduct acute thermal exposures (over hours to days) have successfully revealed subtle differences in thermal tolerances of corals across small spatial scales (Barshis et al. 2013; Bay and Palumbi 2014; Klepac and Barshis 2020; Naugle et al. 2021; Voolstra et al. 2020) and assayed hundreds of corals in a short timespan (Cornwell et al. 2021; Cunning et al. 2021). Although short-term heat stress is ecologically relevant to corals experiencing acute daily temperature fluctuations, such as corals in lagoon or similar tidal environments (Oliver and Palumbi 2011; Schoepf et al. 2015), how these responses relate to prolonged heat stress and long-term variation in stress tolerance is only beginning to be revealed (Morikawa and Palumbi 2019; Voolstra et al. 2020; Evensen et al. 2022). In light of the increasing breadth of approaches being used to assay corals in recent decades, there have been recent calls to standardized experimental approaches, as well as standardizing data reporting, to improve the comparability across thermal stress experiments conducted on corals (Grottoli et al. 2020; McLachlan et al. 2020).

Another major emerging aspect of coral reef work is that it involves many different communities across many cultures and human economies (Voolstra et al. 2021). Though prior work on comparative coral bleaching tended to involve international research teams moving among locations, a different model of regional research involves mentoring local students, citizen scientists, researchers, and community leaders in the tools and analysis of comparative coral bleaching. In this model, local communities can conduct their own research and directly use this information in local decision making about coral restoration and protection (Stefanoudis et al. 2021). This model emphasizes low-cost, reliable approaches and transparent, understandable data sets to spread the science of coral resilience widely across reef localities. Further, much of the research is conducted in small communities communicating digitally with peer groups in other locations. The goal is to mesh this approach with the extensive efforts driven by major national research agendas.

Here, we present a standardized experimental system called the Coral Bleaching Automated Stress System (CBASS) that integrates precise, customizable temperature controlling and flow-through aquaria. Novel aspects of this system are the low cost (< USD$4000 for eight experimental tanks), the use of an open-source platform (Arduino) for the controller electronics, and the development of standardized thermal profiles and analytical routines (Voolstra et al. 2020, 2021; Evensen et al. 2021, 2022); modifications allow even lower cost using off-the-shelf controllers at the cost of some temperature accuracy. Importantly, the system is highly portable and self-contained, allowing for deployment in almost any area where seawater (either by pump or by hand) and electricity (via electrical grid or portable generator) can be accessed, including remote reef locations, ship-based operations, and/or standard laboratory settings. Finally, we have developed a series of 18-h acute thermal stress profiles to determine standardized thermal threshold proxies delivered by the CBASS through a set of assays. We provide empirical support for the utility of the approach in determining individual-specific thermal thresholds for multiple coral species across a variety of experimental settings and locations, as well as preliminary evidence of concurrence between these

thermal thresholds and expected patterns of increased thermal tolerance in warmer reef regions. It is our hope that this technology and approach can enable widespread, standardized comparisons of coral thermal thresholds and grant insight into the mechanisms allowing corals to persist under a warming climate, though additional cross-comparisons between acute stress responses and responses to natural warming events will be needed.

Materials and procedures

System overview

The CBASS consists of a modular design that includes: (1) four experimental flow-through aquaria with individual heating, chilling, and water circulation devices (Figs. 1, 2); (2) a temperature controller capable of delivering four unique, dynamic thermal profiles in 18-h increments (Fig. 4); and (3) four light-emitting diode (LED) light panels attached to adjustable lighting frames (Fig. 1). The aquaria can further be divided into two separate experimental tanks, which, when combined with an additional set of temperature controllers, can produce an eight-tank system with eight unique thermal profiles (Cunning et al. 2021) or four replicated thermal profiles (Evensen et al. 2021; Voolstra et al. 2020). The three main modules of the CBASS are thus the experimental aquaria, temperature controllers, and lighting arrays. This design allows for modification of each individual module to suit a variety of experimental needs (e.g., larger/fewer aquaria for different amounts/sizes of samples, varying number of temperature profiles, and modifications to lighting treatments), with a variety of slightly different setups currently being used successfully, although the primary design described herein was constructed to maximize portability and ease of deployment in remote field settings. The flow-through nature of the system allows for continuous water exchange from a water supply manifold. This means that additional water manipulations (e.g., pH, salinity, dissolved oxygen, or nutrients) are possible with addition/control of multiple pretreated water sources for each individual aquarium (Alderdice et al. 2022). Herein, we provide full design schematics of this system (Figs. 1 3), along with a detailed table of parts provided in the supplement (Supporting Information Table S1). As the system continues to be optimized and upgraded using the latest software and hardware, an up-to-date list of parts (including links to vendors/manufacturers), along with manuals and protocols are publicly available on GitHub: https://github.com/barshislab/CBASS Manual.

Experimental aquaria

The experimental aquaria consist of four hard plastic containers (56 cm × 34 cm × 21 cm Coleman Party Stacker 24-can portable coolers used in current design), each of which can be divided in half via an acrylic divider cut to size and affixed with silicone sealant (Fig. 1). The interior dimensions of each aquarium in the current design are 41.6 cm × 28.3 cm × 15.2 cm for an experimental volume of approximately 20 liters, or 10 liters if acrylic dividers are used to split aquaria into two separate units. Importantly, limited heat exchange occurs across the acrylic dividers that is sufficiently compensated for by the temperature control system to maintain differing temperatures between sides. When using alternative aquarium options (Supporting Information Table S1), it is important to account for changes to water volume and light reflection of the inside material of the aquarium, as both could affect coral physiology and the response to experimental heat stress. A small pump/powerhead (25 ml min^{-1}; Supporting Information Table S1) delivers water circulation within tanks and flow-through ($\sim$ 1.5 L h^{-1}) is achieved via an inlet line from a distribution manifold and separate water supply (e.g., large reservoir with pump or continuous seawater system), as well as an overflow bulkhead below the top of the aquarium for effluent. Two 50-W IceProbe thermoelectric chillers (Novatec; Supporting Information Table S1) are mounted through the back wall of each replicate aquaria (16 total) to provide cooling (Fig. 2). Alternatively, a single coil of $\sim$ 0.5-cm stainless steel tubing submerged in each aquarium (eight total; Supporting Information Fig. S2) can be used as a heat exchanger by pumping cold water through each coil via eight replicate pumps submerged in a single central reservoir. Heating in each tank is supplied by a single 200-W submersible titanium aquarium heater (eight total; Supporting Information Table S1) placed directly in each aquarium. A plastic grid (egg crate) is used to hold coral fragments in place above the bottom of the tank via rubber bands and waterproof paper stands or some other kind of attachment device (Fig. 2b).

Water inflow and outflow

Each tank is supplied with seawater individually through inflow lines, all connected to a single header tank connected through a manifold, or to individual header tanks. Flow rate into the tanks is powered by a marine pump, or simply gravity fed, and adjusted to a desired inflow rate by valves placed at the end of each inflow line. Inflow lines are held in place by small zip ties placed through small holes drilled into opposing sides of the tanks (Fig. 2c). Seawater outflows consist of two 34 mm bulkhead located in the front top corners of tanks, opposite the chillers (Fig. 2d). Outflow bulkheads can be equipped with hose barbs to secure outflow hoses and redirect outflow seawater to the desired location. As such, full 20-liter tanks contain two inflow lines and two outflow bulkheads, and tanks divided in half contain one inflow line and one outflow.

Lighting

Individual (1 per tank) or shared (1 per cooler/aquarium pair) dimmable, 165-W full-spectrum LED aquarium lights and light diffuser panels are suspended above each cooler via adjustable polyvinyl chloride (PVC) pipe frames (Fig. 1; Supporting Information Table S1), creating light treatments that are controlled and standardized across experiments held

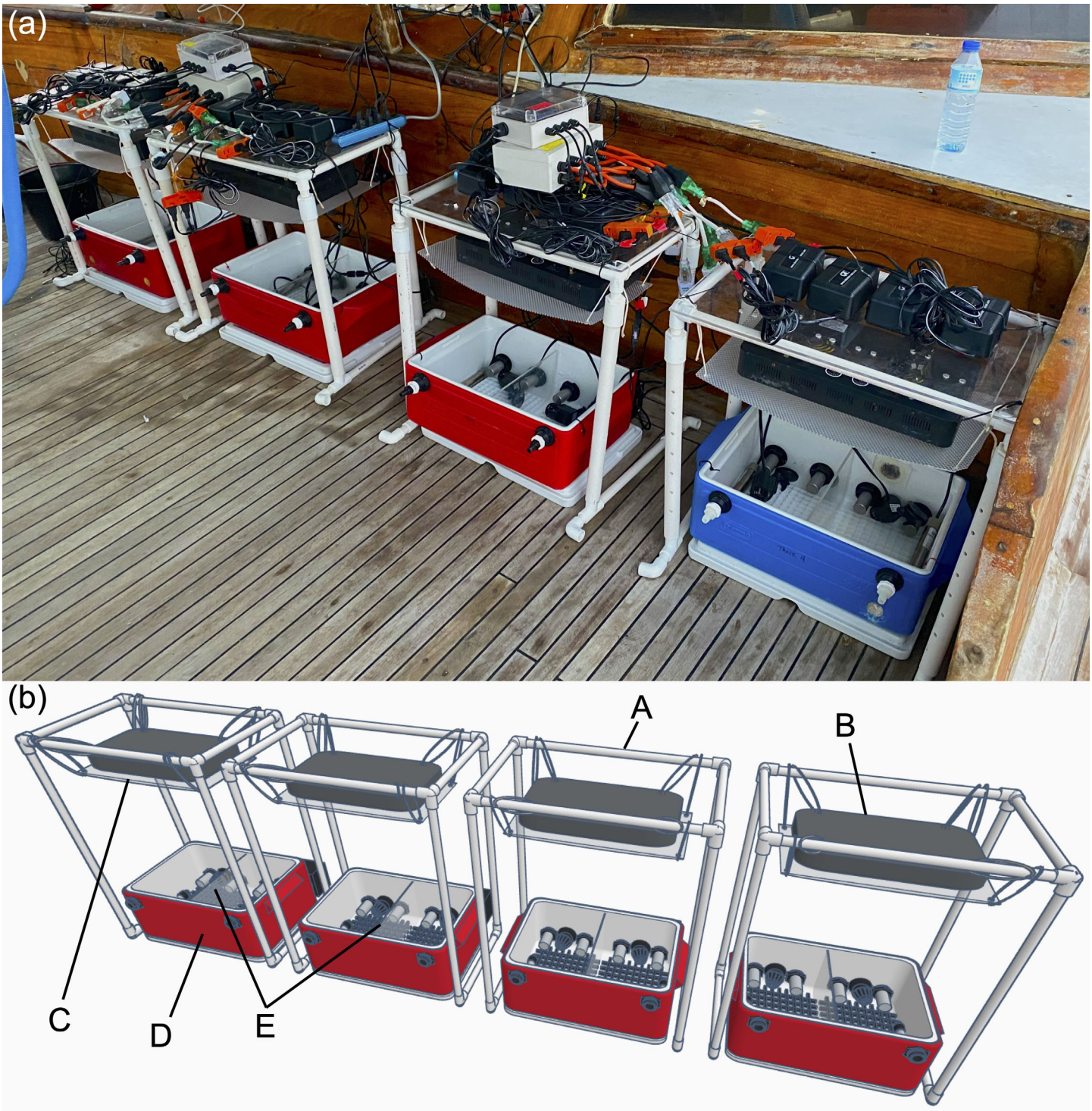


Fig. 1. (**a**) Photograph and (**b**) annotated CAD schematic of a full 8 tank (4 aquaria) CBASS. Annotations in (**b**) denote: A, light frames; B, 165 W full spectrum LED aquarium lights; C, light diffusers; D, experimental aquaria separated into two independent tanks using acrylic dividers; E, acrylic dividers. Details of the components and setup of each individual tank can be found in Fig. 2.

at different times. Target light exposures have ranged from ~ 150 to 600 μmol quanta $m^{-2}\,s^{-1}$, as measured using a LI-193 Spherical (LI-COR) or an Apogee MQ-510 Underwater Quantum Sensor and are adjusted manually using dimmers or using light filters (e.g., Lee Filters) based on native light conditions where corals were collected.

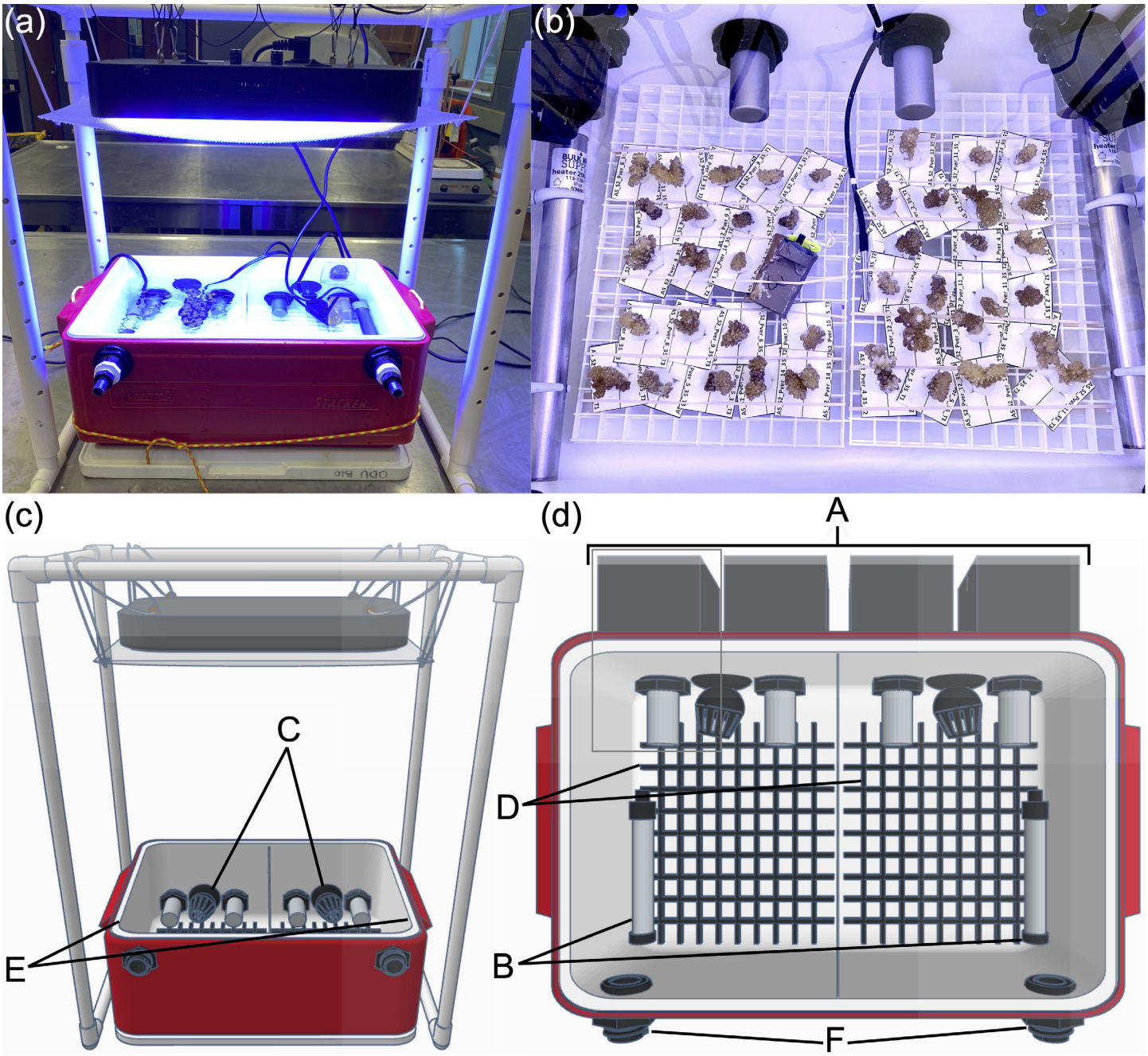


Fig. 2. (**a**) Front and (**b**) close up overhead photographs, along with annotated (**c**) front and (**d**) overhead CAD schematics of an experimental aquarium divided into two tanks. CAD schematics are annotated, indicating: A, four chillers (gray box indicates the exterior and interior components of the chillers); B, two heaters; C, two powerheads to enhance water circulation; D, egg crates (to which coral samples are secured using elastic bands, as shown in **b**); E, seawater inflow tubing; and F, seawater outflows. Full details denoting the exact location and size of the holes needing to be drilled for the chillers and outflows are available in the associated GitHub repository: https://github.com/barshislab/CBASS_Manual.

Temperature control

Temperature control is achieved via a custom-built controller based on the Arduino platform. Alternatively, tank temperatures can be controlled individually through the use of low-cost Inkbird (ITC-310T-B) temperature controllers at some cost to temperature accuracy. Each controller consists of a single low-voltage (5 V) controller enclosure and high-voltage (110-240 V) relay enclosure that can control four independent aquaria (Fig. 3). The controller enclosure is made up of an Arduino Mega 2560 unit (or suitable copy), a thin-film-transistor liquid-crystal (TFT LCD) display, and a custom-designed CBASS-R shield which includes an SD card slot, real-time clock

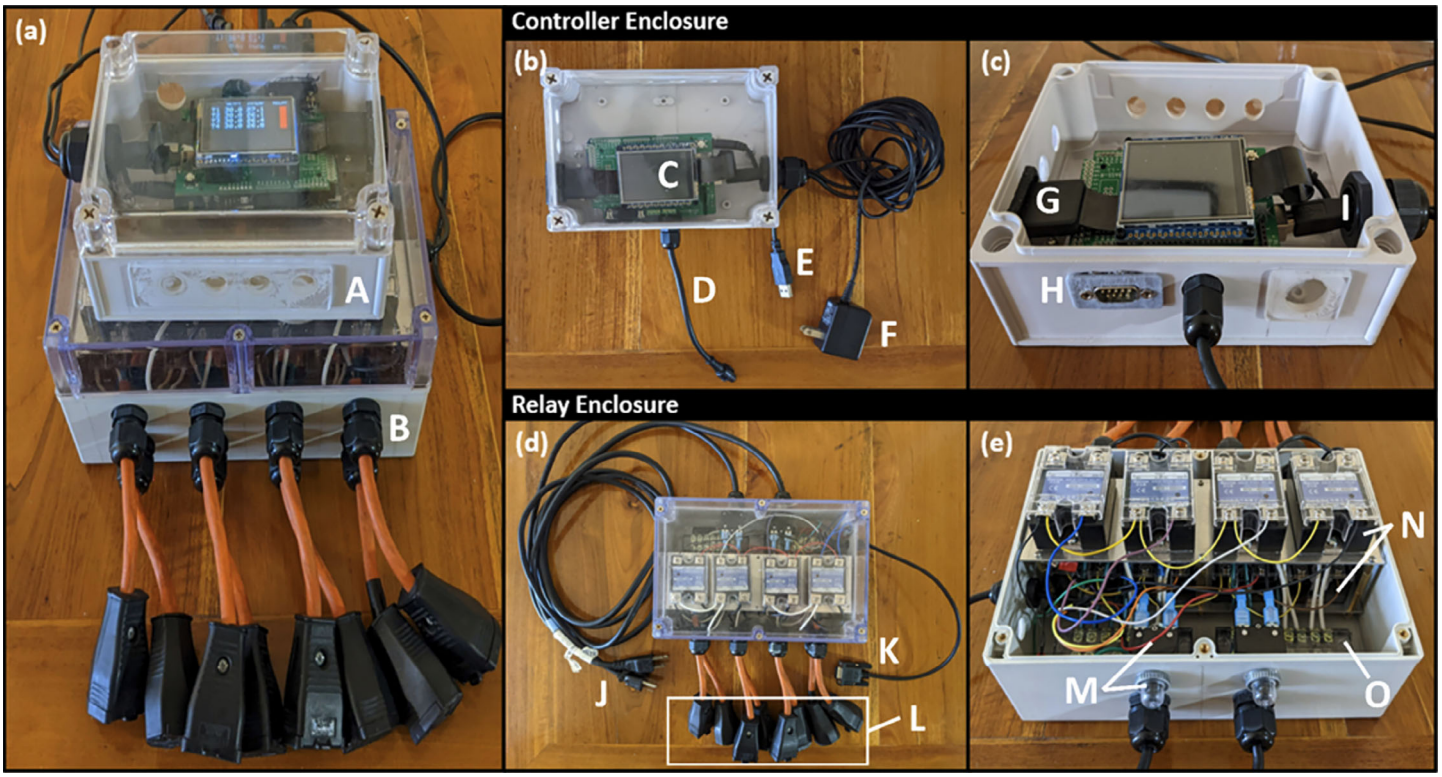


Fig. 3. Annotated photographs of an Arduino based CBASS controller enclosure and relay box, capable of controlling up to four individual temperature profiles. Photographs show an overview of the controller and relay boxes connected and set up for experiments (**a**), along with detailed photographs of the controller enclosure (**b**) and (**c**), and of the relay enclosure (**d**) and (**e**). Annotations represent: A, the controller enclosure; B, relay enclosure; and C, a digital TFT display, CBASS R shield, and Arduino Mega 2560 unit stacked together; D, the connection cable to four temperature probes; E, USB to con nect a computer to the controller; F, 9 V power supply for the controller; G, the SD card extender; H, DB 9 connector port; I, USB and 9 V connection to the Arduino Mega 2560; J, AC power cords; K, DB 9 cable to connect the controller and relay; L, AC output to the heaters and chillers; M, inline circuit breakers; N, two rows of SSRs; O, terminal blocks. A full parts list is available in Supporting Information Table S1.

module, and other small required resistors and components (Supporting Information Table S1). A full list of parts, assembly instructions, and user guide for the CBASS-R shield are publicly available on GitHub: https://github.com/VeloSteve/CBASS-R-Shield. Four DS18b20 waterproof temperature sensors are connected to the Arduino via the CBASS-R and a custom-made waterproof pigtail. Communication to the Arduino is through a USB cable and continuous power is supplied by a 9-V, 1000-mA AC/DC adapter (Supporting Information Table S1). All through-wall connections are made using waterproof cable glands or a splashproof DB-9 connector, with connections to the temperature probes using a waterproof wire connector (Supporting Information Table S1). Although the controller electronics are not expected to be fully waterproof, they are water resistant and can withstand most field conditions, such as occasional splashes and dust. The controller's basic functions are to read in temperature values from the individual temperature sensors (one per aquarium), read in temperature setpoints and timing from a "settings.ini" file (text based; *see* "SD Card Input Example" in the associated GitHub repository) stored on an SD card in the SD slot of the CBASS-R shield, and use a custom-designed pseudo proportional integral derivative (PID) controller loop to control a heating and chilling circuit, and maintain temperature in each experimental aquarium. These circuits are contained within the separate relay enclosure to reduce the overall footprint of the controllers, isolate high voltage from low voltage, and provide some redundancy in case of equipment damage/malfunction (i.e., if a relay component fails, only the relay enclosure needs to be replaced/repaired and vice versa for a controller component). Relay enclosures consist of eight solid-state relays (SSRs), eight output AC power cables and plugs, two input AC power cables, two power distribution blocks, and one DB-9 communication cable from the controller enclosure (Supporting Information Table S1). Alternatively, Robo-Tank fully assembled power bars can be used for a slightly lower cost and less assembly work, with the power bars equipped with Omron mechanical relays (Supporting Information Table S1). However, Robo-Tank power bars tested to date only have the capacity to power one 200-W heater per AC output, limiting the capacity of these power bars compared to SSRs.

In the field, we recommend bringing the following back-up components in case of equipment damage or malfunction: 1 2 spare chillers and heaters, 1 spare light, 1 2 spare powerheads, 1 backup pump, 1 spare controller, and 1 spare power bar or relay enclosure. With these fully redundant spare parts, no specialized tools are required for repairs in the field as all connections are solderless (either screw terminal or pin plug connections).

Electrical requirements

The total peak wattage per double-tank aquarium is ~ 766 W (50 W × 4 chillers, 200 W × 2 heaters, 165-W LED panel across two tanks, and ~ 1 W per aquarium for the Arduino power supply, with miniscule energy demand for circulation pumps) for a total peak wattage of the entire system of 3060 W. If these were all running simultaneously, the system would draw in excess of 22 A at 120 V across two 10 20 A 120-V lines: a high amount of current in many situations. However, all components are seldom on at the same time. In practice, we have successfully powered the eight-tank system using a 2200-W portable generator. In addition, the wiring of the SSR enclosures allows for dividing the main AC power supply (heaters and chillers) across as many as four circuits (two tanks/circuit) for flexibility in power delivery. Nonetheless, as with all custom electrical applications, an electrician should be consulted to determine if the available power supply is adequate and safe to run the system and desired experiments.

"Basic" CBASS for widespread use in remote settings

The CBASS concept is nicely designed for flexibility in the equipment used and how it is programmed. The setup described above is well suited to small moderate research installations, and even small mobile settings like research vessels, but there are simple modifications that drop the price significantly, make the operation more seamless, and may provide access to thermal response data for more remote coral reef locations, such as atolls and isolated villages. These modifications, which we denote "basic" CBASS, also allow local students and citizen scientists to participate fully in the collection, analysis, and storage of data, with very little training or technical expertise required. These modifications open up the standardization and replicability of CBASS to even more people across the reef world, increasing the diversity of settings where CBASS approaches could be deployed.

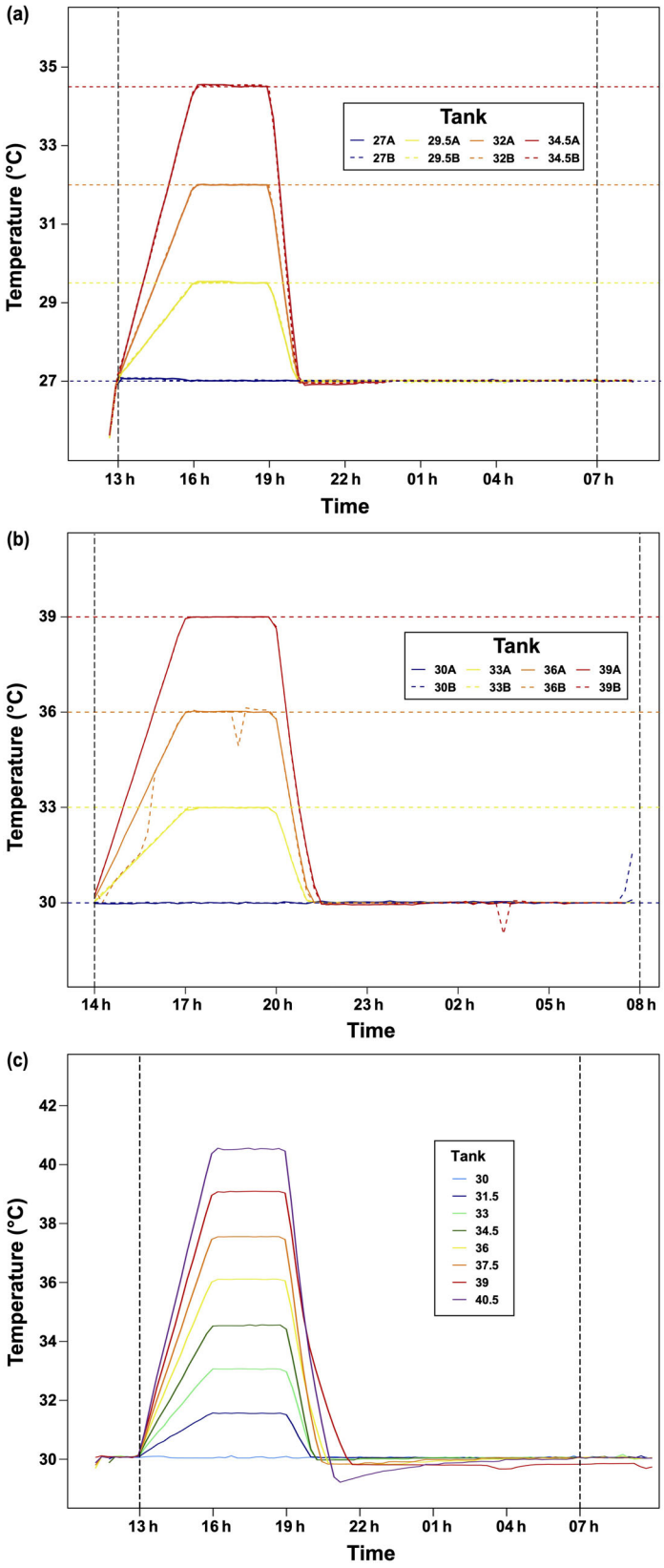
The major modification of the basic CBASS is the use of commercial Inkbird temperature controllers. With a built-in temperature probe, and outlets for chillers and heaters, one version of this low-cost (~ US$45) device can be programmed in 10 12 steps to deliver the bleaching stress conditions described for the full CBASS (programming example in Supporting Information Table S2; Fig. S3). The Inkbird controllers produce a more stepwise temperature profile and lack the finer-scale PID temperature control of the Arduino-based controllers. Without this stabilization, the temperature plateaus tend to have more variation (~ ± 0.5°C), since overshoots are less well controlled (Supporting Information Fig. S3). Critical monitoring of temperature profiles also important for regular CBASS assays can be accurately done with an inexpensive, cooking thermometer. A typical basic CBASS can be built for ~ $1700 (Supporting Information Table S1).

A second low-cost choice, that is also well suited to student involvement, is to assay corals following thermal stress exposure by photographing nubbins exposed to the different temperatures in a grid so that replicates of the same colony can be compared. By using a gray card in each photograph, exposures can be equalized. These photos can have 20 40 corals in them and be uploaded to cloud-based document storage, such as Google Drive™. We have assayed them with a five-point visual bleaching score (None, Visible, Moderate, Severe, Total; Walker et al. 2023), an approach that correlates well with more quantitative measures of bleaching (Cornwell et al. 2021; Evensen et al. 2022; Walker et al. 2022) and is suitable for scoring by machine learning algorithms. Alternatively, red (R), green (G), and blue (B) pixel intensities from an RGB photograph can be extracted to infer loss of chlorphyll density as inferred from an increase in pixel intensity of the red channel (Voolstra et al. 2020). Last, coral pigmentation/coloration can be scored against a color scale reference for broad implementation and consistency across scorers and studies (Alderdice et al. 2022).

Assessment

Technical assessment

The CBASS concept has successfully been deployed across sites in Saudi Arabia (4 sites), Djibouti (2), Israel (1), the Galapagos Islands (6), other Eastern Tropical Pacific sites (6), the Florida Keys (4), American Samoa (10), Brazil (1), French Polynesia (1), Monaco (1), Palau (39), and the U.S. Line Islands (2) in both remote and developed shore-based or lab settings, as well as on ship-board operations (Fig. 1; Supporting Information Fig. S1). Temperature has been controlled with both precision and accuracy from 12°C to 40.5°C (note that the lower temperatures utilized the chilling coil configuration shown in Supporting Information Fig. S2). Temperature precision has been assessed through high-frequency logging of temperatures during experiments (5-s intervals) using the built-in temperature probes and HOBO® Pendant loggers placed in the tanks. Accuracy of the temperature measurements is limited by the accuracy stated by manufacturers of the temperature probes (VKTech, Shenzhen, China) and HOBO® Pendant loggers (Onset). To calibrate the temperature probes, we recommend performing a trial CBASS run and logging temperatures using both a factory-calibrated logger (such as a HOBO logger) and the CBASS probes. The average temperature measured by the HOBO logger for the two temperature hold phases (hold and recovery) during



the CBASS run can then be compared to the average temperature measured by the probes for the same phases. If there is a difference in temperatures, custom offsets can be set for each individual temperature probe in the Arduino code (for the Inkbird controllers, temperature offsets can be adjusted through the user menu). The CBASS trial run can then be repeated to verify the new Arduino settings, with the temperature measurements of Arduino and HOBO loggers compared again. These steps should be repeated until temperatures recorded by the HOBO loggers and probes match.

Our approach allows users to collect coral fragments and conduct full sets of experimental assays within a 24 h period. For each experiment, replicate coral ramets are attached to labeled squares of underwater paper and secured to plastic grids at the base of the tanks via rubber bands, with fragments from the same coral colony assessed across all temperature treatments/tanks immediately upon return from collection on the reef. As such, experiments are typically started within 2 3 h of collection, minimizing variation in recovery rate between and within genotypes as part of collection and transport. Collection of "field" controls (ramets for which physiology was assessed immediately upon return from collection) and "T0" controls (ramets for which physiology was assessed after ~ 2 h in the tanks but prior to the start of the temperature profiles) showed little difference in physiological performance when compared to ramets in the control treatment (Evensen et al. 2021).

To date, treatments typically range from the local maximum monthly mean (MMM) seawater temperature of a given site (based on NOAA satellite data; Liu et al. 2014), or an average of multiple sites, as a control/baseline temperature treatment, along with three elevated temperature profiles increasing in 3 4°C increments, or seven profiles in 1 2°C increments. For example, control temperatures for experiments conducted in Eilat, Israel were 27°C, the local MMM, with three elevated temperature profiles of 29.5°C, 32°C, and 34.5°C (Fig. 4a; Evensen et al. 2021). In turn, temperature

Fig. 4. Examples of temperature profiles produced by the CBASS for experiments conducted (**a**) in a land based laboratory setting in Eilat, Israel (from Evensen et al. 2021), (**b**) aboard a small vessel off Al Wajh, Saudi Arabia (from Evensen et al. 2022), and (**c**) in an outdoor lab setting near Thuwal, Saudi Arabia. For (**a**) and (**b**), each experiment consisted of four duplicated temperature profiles lasting 18 hours, with target temper atures of 27°C, 29.5°C, 32°C, and 34.5°C in Eilat and 30°C, 33°C, 36°C, and 39°C in Al Wajh. Target temperatures in (**c**) were 30 41.5°C in 1.5°C increments. Temperatures were recorded every 5 s and averaged over 15 min for plotting clarity. Vertical dotted lines indicate the beginning and end of each experiment, and horizontal dotted lines in (**a**) and (**b**) indicate target temperatures for each tank. Note the difference in start and end times for each experiment (x axes depict time of the day in 24 hour format), which were adjusted to account for the difference in sunset times during each experiment.

treatments for a recent set of experiments across three sites in the central Red Sea, started at 30°C, the average MMM across the sites, with elevated temperature profiles reaching 33°C, 36°C, and 39°C (Fig. 4b; Voolstra et al. 2021; Evensen et al. 2022). These temperature profiles are designed to capture the full biological stress response of corals (from no response to total bleaching), representing the current sustained maximum temperatures (i.e., the MMM), temperatures that induce a bleaching response but do not kill most corals, and extreme temperatures that results in bleaching and subsequent mortality of many corals. Eight independent temperature profiles have also been used to increase the resolution at which the coral stress response can be assessed, allowing for genotype-level differentiation of thermal tolerance among corals from the same population (Cunning et al. 2021; data herein).

Response metrics and calculation of the coral thermal threshold

Multiple physiological and molecular aspects of the coral stress response have been assessed using the CBASS, including: standard bleaching metrics (after McLachlan et al. 2020) such as chlorophyll *a* and Symbiodiniaceae density, gene expression, and community composition of coral holobiont compartments (coral host, microbial symbiont, and prokaryotic microbiome; Voolstra et al. 2020, 2021; Naugle et al. 2021; Savary et al. 2021). In addition, measurements of photosynthetic efficiency (F_v/F_m) have been used to quantitatively compare thermal thresholds of various coral species, populations, and genotypes. F_v/F_m values have been used extensively to assess the response of algal symbionts to temperature changes (e.g., Jones et al. 2000; Fine et al. 2013; Schoepf et al. 2015; Jurriaans and Hoogenboom 2019), with changes in F_v/F_m strongly linked to coral bleaching severity (Warner et al. 1999) and differences in thermotolerance between Symbiodiniaceae species (Kemp et al. 2014). Across all these assays, thermal thresholds of corals can be compared by fitting log-logistic curves to the response data (using the R software package "drc"; Ritz et al. 2015) and using the model fits to determine the temperature at which the response metric (e.g., F_v/F_m, visual bleaching scores, Symbiodiniaceae densities) decreases by 50% relative to the control (baseline) value for each individual coral genotype within a population, termed the effective dose 50 (ED50). This approach yields a colony-based ED50 from which a mean (± SD) ED50 for each coral population studied is determined, allowing for empirical and statistical comparisons of thermal thresholds between coral genotypes and/or populations. F_v/F_m is an attractive measure as the response variable for this approach because it is non-destructive, repeatable, consistent, and relatable to bleaching (Voolstra et al. 2020; Evensen et al. 2021, 2022; Nielsen et al. 2022).

The aforementioned response variables have been measured both at the end of the heat-hold period (after 3 h of heat stress), 7 8 h into the assays (after 3 h of heat stress and 1 h of ramping down to baseline temperatures), and at the end of the 18 h assays, following an overnight recovery period at the control temperature (i.e., the MMM; Fig. 4). Overall, response patterns of the physiological metrics were consistent across the time points (Evensen et al. 2021), indicating that F_v/F_m measurements as soon as 7 8 h into the acute thermal assays may be used to resolve differences in thermal tolerance between coral populations (Voolstra et al. 2020, 2021; Evensen et al. 2021, 2022). By contrast, for many corals, physical expulsion of symbionts requires longer, and an overnight recovery period can be beneficial, allowing for elucidation of fine-scale differences in the response to heat stress (Savary et al. 2021). Longer periods of time incubating corals after heat stress tends not to change results measurably: up to 7 d after heat stress, Walker et al. (2022) showed strong stability of both bleaching and mortality patterns after CBASS-style heat pulse experiments. However, Nielsen et al. (2022) did find sampling time to be an important component of the experimental design for acute heat stress assays, reinforcing the need for standardized approaches to increase comparability among studies.

Flexibility and variations

A major advantage of CBASS assays is that they are inherently flexible they can be used in many settings, for many species, in many ways. For example, fast-bleaching corals such as Acroporids respond well to the single-day temperature pulse we describe here. However, some slower-bleaching Poritid corals may not bleach in a single day unless temperatures are high enough to kill them. In such cases, repeat CBASS cycles and/or multiday exposure to lowered treatment temperatures may be needed to separate more or less tolerant populations. Likewise, multiday exposures to lower temperature spikes can generate moderately bleached colonies for follow-on recovery experiments (Walker et al. 2023). An important aspect of CBASS experiments is that they need to be tuned to local conditions, species, and coral resilience: for example, the temperature range used in a specific setting often needs adjustment after one or two initial experiments in a new region ('pilot experiment'). A key element of these uses is that they be comparable across experiments within a region, and repeatable in other settings. The control systems used in CBASS allow adequate control of temperature profiles, light, and water motion to achieve both flexibility and repeatability.

Discussion

We present a portable experimental platform and standardized analytical framework to rapidly assess and compare the thermal tolerances of corals across a variety of reefs and regions. The system can be, and has successfully been, deployed aboard small and large vessels, in remote shore-based settings, and within developed research facilities. To date, experiments using the CBASS have focused on determining

the effects of thermal stress on corals, because increasing temperatures across the globe have rapidly emerged as the biggest threat to coral reefs (Hughes et al. 2018). The flexibility of the system allows it to be useful across coral species and reef habitats, while also allowing for future modifications to assess the response of a variety of organisms to multiple stressors, including nutrient enrichment, deoxygenation, and elevated pCO_2, through modifications to the in-flow seawater source. With the damage of ocean warming increasing in countless ecosystems, notably coral reefs, at an alarming rate, our approach addresses a need to rapidly evaluate thermal thresholds to compare mechanisms and drivers of thermal tolerance in corals, and to pinpoint thermally resilient coral populations and genotypes.

Longer studies, common garden experiments, and Symbiodiniaceae assessments are undoubtedly essential complements to the CBASS approach (Evensen et al. 2021; Voolstra et al. 2020; Grottoli et al. 2020). However, the low cost and portability of CBASS are crucial to evaluating relative differences in coral thermotolerances and predicting the impacts of ocean warming on local coral reefs, the vast majority of which are located on the shores of isolated, developing nations. In addition, the CBASS allows for running standardized experiments among disparate locations, enabling direct cross-comparability and meta-analyses of resulting data. When combined with even lower cost "basic" CBASS alternatives, citizen science and local student participation can broaden the comparative study of coral thermal tolerance globally in a way that empowers a high diversity of practitioners in coral science and contributes directly to local conservation and restoration decisions.

The use of short-term acute experiments to determine genotypic- or species-specific tolerances to thermal stress is not a new concept and has already proven to be a successful approach for corals (Barshis et al. 2013; Palumbi et al. 2014; Bay and Palumbi 2015; Klepac and Barshis 2020; Cornwell et al. 2021) and other organisms (Bedulina et al. 2010; Overgaard et al. 2012). Still, an important consideration of our experimental approach is the duration of the heat stress exposure relative to naturally occurring heat stress events on coral reefs. Indeed, acute thermal exposures over 18 h are likely to yield different physiological responses than ecological impacts of bleaching events that typically last weeks to months (Fordyce et al. 2019), though intertidal reef zones and recent marine heatwaves can produce similar acute thermal stress profiles (Barker 2018; Barshis et al. 2013; Hobday et al. 2016, Straub et al. 2022).

Concurrent experiments comparing coral physiology following acute (18 h) 32°C assays using the CBASS with those obtained from prolonged thermal exposures using a larger experimental system, the Red Sea Simulator (Bellworthy and Fine 2018), revealed strikingly similar relative response patterns of corals from the same population in Symbiodiniaceae density changes, host protein, and F_v/F_m (Evensen et al. 2021), though molecular mechanisms driving these responses differed (Savary et al. 2021). Likewise, multiday results of lower temperature stress exposure paralleled higher stress single-day experiments among corals in Palau (Walker et al. 2023). Overall, the similar results delivered by acute thermal assays using CBASS assays to longer experiments, conducted in more established experimental systems (Voolstra et al. 2020; Evensen et al. 2021) indicate considerable promise for this approach in determining differences in thermal tolerance among and within coral species to more prolonged thermal stress events. Nonetheless, more studies are required to determine if differences among populations in resistance to acute thermal stress equate to differences in bleaching resistance and resilience, and ultimately the long-term persistence of coral populations.

In addition to revealing similar responses of corals to acute and chronic thermal stress exposures, the results from Evensen et al. (2021) and Voolstra et al. (2020) highlighted F_v/F_m as a physiological response variable indicative of overall coral holobiont performance under thermal stress, and as a useful proxy for bleaching. In line with the portability and high-throughput approach of the CBASS, we have used F_v/F_m as a metric to determine ecologically relevant differences in thermal tolerance between corals. As an example, using the CBASS, we determined the thermal threshold at which corals exhibited a 50% decline in F_v/F_m relative to baseline/control temperatures, termed the F_v/F_m ED50, for four coral species across six sites spanning the entire length of the Red Sea to Djibouti in the Gulf of Aden in ca. 2 weeks of experiment time (~ 2100 km; Evensen et al. 2022). Through the combined CBASS and F_v/F_m ED50 approach, we resolved species-specific latitudinal patterns of thermal tolerance, with corals generally exhibiting higher thermal thresholds at warmer sites (results from multiple studies conducted in the Red Sea presented in Fig. 5; Voolstra et al. 2021; Evensen et al. 2022). Notably

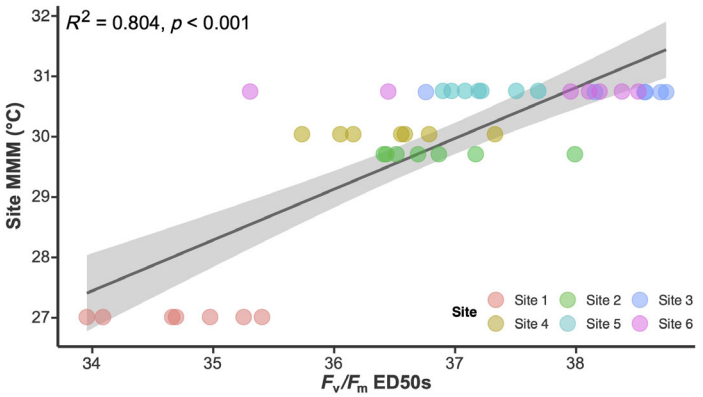


Fig. 5. Correlation between F_v/F_m based ED50 thermal thresholds and Maximum Monthly Mean (MMM) sea surface temperatures for *Stylophora pistillata* colonies collected and assayed across six Red Sea reef sites during the summer (6 7 colonies per site, n 41). The black line represents the linear regression, with gray shaded area indicating the 95% confidence interval.

though, we also found lower thermal thresholds for *Porites lobata* at the warmest site in the southern Red Sea, the only species found at this site, indicating that corals in this region may be struggling to recover from recent bleaching events and adapt to warming seawater temperatures in this region.

At the scale of individual genotypes, Cunning et al. (2021) assayed 229 colonies of *Acropora cervicornis* across six nurseries spanning the Florida's coral reefs and found higher variation in F_v/F_m ED50 within nurseries than across them, with the F_v/F_m ED50 approach yielding highly reproducible rankings of coral genotypes across replicate assays. In turn, Cornwell et al. (2021) used the CBASS approach but measured symbiont retention of 313 corals from 39 reefs in Palau: they found widespread occurrence of heat-tolerant corals even in forereef areas, with higher frequencies in warmer patch reef regions. These results showcase the flexibility and general value of having controlled, replicable heat stress experiments in a variety of coral settings.

Importantly, ED50 values calculated through our approach are not absolute values or representative of temperature thresholds reached during natural bleaching events. To what extent ED50 thermal limits or other metrics assessed during acute assays correspond to longer studies or natural bleaching proxies, such as visual bleaching scores or Symbiodiniaceae retention, is a matter of current investigation (*see* Evensen et al. 2022 and Fig. 5). Of particular importance is the link between the physiology of symbiosis and symbiont expulsion. A drop in F_v/F_m signals a decline in the efficiency of photochemistry (Butler 1978) and represents a decline in symbiont-host coordination. However, whether a particular drop in F_v/F_m results in bleaching and whether this threshold is consistent across species and individuals are key questions. Indeed, comparisons of acute thermal thresholds remains just a first step in identifying thermally tolerant corals and measurements of bleaching *sensu stricto* remain a valuable assessment for CBASS approaches.

Empowering coral science

More extensive experimental systems capable of maintaining long-term laboratory-based experimental treatment conditions, such as the Red Sea Simulator in Israel (Bellworthy and Fine 2018), are often used to compare coral responses in common conditions as a way to explore the mechanisms of coral heat tolerance, yet can cost millions of dollars. The low-cost, standardized, portable nature of CBASS allows for the assessment of a much larger number and diversity of reef sites. In addition, inexpensive, local common garden experiments can serve as complementary tool following CBASS assays to determine which heat resistant corals identified by CBASS experiments are durably heat resistant. This is important because the variation in field-derived heat tolerance measures we describe herein can be caused by adaptation or acclimation to different local conditions, as well as possible differences in Symbiodiniaceae, microbiomes, and coral genes (Barshis et al. 2013; Palumbi et al. 2014; Dixon et al. 2015; Ziegler et al. 2017; Fuller et al. 2020; Naugle et al. 2021). As a result, CBASS results are the beginning of an exploration of durable coral tolerance patterns that will be useful for identification of heat-tolerant colonies for conservation efforts of marine protected areas or in restoration projects (Voolstra et al. 2021).

These local goals are most productively driven by local researchers/stakeholders in view of conservation needs of their specific communities, and they benefit from a seamless integration of coral reef science and community deliberation. The CBASS, particularly the "basic" CBASS version, can be delivered and supported in a wide variety of communities and can be the focus of local students and community leaders. For example, the "basic" CBASS has been used by students at the Palau Community College to measure coral heat resistance in student theses and can be a local assay tool for a wide range of practical reef questions across other sessile marine invertebrates. Putting the capacity to assay and monitor coral heat resistance across seasons, species, and reefs into the hands of local managers across the reef world has the chance to increase community inclusion in reef science and scale up efforts to find and use heat-tolerant corals throughout the challenging century of warming ahead.

Conclusions

The motivation for the development of the CBASS standardized experimental framework is to rapidly assess the susceptibility of reef-building corals to thermal stress at a rate faster than that at which coral populations are dying to global warming, as well as allowing access to more remote reef locations across the globe. To date, the CBASS approach to determining thermal tolerance, which includes the use of the portable tank and temperature control system and acute thermal assays, has been used at scale across the Red Sea and Gulf of Aden, as well as at multiple sites in the Pacific Ocean and Caribbean. In addition to identifying potentially resilient corals for future research and conservation, these data can eventually provide a global, standardized assessment of coral physiological and genomic responses to thermal stress for use in predictive modeling efforts, along with data on survival and long-term population dynamics (e.g., recruitment) and to determine environmental drivers of thermal tolerance and future bright spots under ocean warming. Although a clearer understanding of the association between acute thermal tolerance and *in situ* bleaching susceptibility is crucial, the CBASS approach has begun to resolve historical, taxonomic, population-/individual-specific, and possibly recent environmental drivers of variation in coral thermal thresholds, highlighting the potential for a standardized, short-term thermal assay as a cost-effective, rapid approach to assessing ecological and evolutionary variation in the upper thermal limits of corals.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Conflict of Interest

None declared.

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