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Remarkably high and consistent tolerance of a Red Sea coral to acute and chronic thermal stress exposures

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Abstract

Global warming is resulting in unprecedented levels of coral mortality due to mass bleaching events and, more recently, marine heatwaves, where rapid increases in seawater temperature cause mortality within days. Here, we compare the response of a ubiquitous scleractinian coral, *Stylophora pistillata*, from the northern Red Sea to acute (7 h) and chronic (7–11 d) thermal stress events that include temperature treatments of 27°C (i.e., the local maximum monthly mean), 29.5°C, 32°C, and 34.5°C, and assess recovery of the corals following exposure. Overall, *S. pistillata* exhibited remarkably similar responses to acute and chronic thermal stress, responding primarily to the temperature treatment rather than duration or heating rate. Additionally, corals displayed an exceptionally high thermal tolerance, maintaining their physiological performance and suffering little to no loss of algal symbionts or chlorophyll *a* up to 32°C, before the host suffered from rapid tissue necrosis and mortality at 34.5°C. While there was some variability in physiological response metrics, photosynthetic efficiency measurements (i.e., maximum quantum yield *Fv*/*Fm*) accurately reflected the overall physiological response patterns, with these measurements used to produce the *Fv*/*Fm* effective dose (ED_{50}) metric as a proxy for the thermal tolerance of corals. This approach produced similar ED_{50} values for the acute and chronic experiments (34.47°C vs. 33.81°C), highlighting the potential for acute thermal assays with measurements of *Fv*/*Fm* as a systematic and standardized approach to quantitively compare the upper thermal limits of reef-building corals using a portable experimental system.

Reef-building corals are the foundation species for some of the most diverse marine communities in the world, yet the ecosystems they build are being threatened by a number of environmental stressors at both local and global scales (Kennedy et al. 2013). In recent years, warming seawater temperatures as a result of anthropogenic CO_2 emissions have emerged as the principal threat to reef-building corals, causing coral bleaching and mortality at unrivaled scales (Hughes et al. 2017*a*, 2018). Since 1998, rising seawater temperatures have resulted in three global mass bleaching events, with the most severe of these events, spanning 2015–2016, causing severe bleaching (> 30% of corals bleached) on more than half of 100 globally distributed reefs (Hughes et al. 2018). In turn, 99% of the world's coral reefs are predicted to experience severe bleaching events annually before the end of the century (van Hooidonk et al. 2016).

The threat of ocean warming to reef-building corals has led to considerable research into the effects of elevated seawater temperatures on the physiology of corals and their symbionts since the 1970s (Jokiel and Coles 1977; Glynn 1984; Hoegh-Guldberg and Smith 1989). Indeed, there is extensive evidence that the symbiotic relationship between corals and the unicellular alga (Symbiodiniaceae; LaJeunesse et al. 2018) inhabiting their tissue breaks down above certain temperature thresholds (reviewed in Jokiel 2004). However, thermal stress events can be highly variable in their intensity and duration. Recent extreme thermal events, termed marine heatwaves, have resulted in tissue necrosis and coral mortality within days of

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heating (Leggat et al. 2019), compared to previously described bleaching events that yielded more distinct succession of coral bleaching and eventual recovery or mortality over multiple weeks (Marshall and Baird 2000; Hughes et al. 2017*b*). Indeed, variation in duration, intensity, and rate of onset of thermal stress events are likely to elicit different responses in corals. While bleaching during slower and less intense heating often is attributed to the expulsion of defunct symbionts that are damaging the host through oxidative stress (Weis 2008), tissue and symbiont loss in response to acute stress is likely due to host tissue necrosis and/or host-cell disassociation (sensu Gates et al. 1992). Thus, developing a standardized experimental approach to assess coral thermal tolerances is important to ensure results are comparable across studies (McLachlan et al. 2020).

Current projections indicate that, even if global warming is limited to 1.5°C, up to 70%–90% of corals worldwide will be lost by mid-century (Hoegh-Guldberg et al. 2018). However, the severity of these impacts is projected to vary greatly among locations (van Hooidonk et al. 2016), with many coral populations already identified as having a superior thermal tolerance compared to most due to prior exposure to elevated temperatures (Oliver and Palumbi 2011; Howells et al. 2012; Schoepf et al. 2015). Thus, in addition to ensuring comparable results within and between studies, a standardized approach is important to determine the relative tolerance thresholds of corals across species, populations, and locations, and accurately identify corals that are more likely to survive in future, warmer oceans. Such corals/populations can then become study models for further research into the genetic and physiological mechanisms that dictate thermal tolerance, and improve efforts to genetically engineer stress tolerant corals (i.e., assisted evolution; van Oppen et al. 2015; Jin et al. 2016) and restore reefs through assisted migration (Dixon et al. 2015; van Oppen et al. 2017).

In the present study, we focused on a *Stylophora pistillata* population from the Gulf of Aqaba in the northern Red Sea that previously has exhibited an exceptional thermal tolerance (Bellworthy and Fine 2017; Grottoli et al. 2017; Krueger et al. 2017). While corals typically undergo bleaching when exposed to temperatures 1–2°C above their local maximum monthly mean (after Liu et al. 2014) for extended periods of time, corals in this region have been found to withstand temperatures up to 7°C above the local maximum monthly mean (34°C and 27°C, respectively), which is hypothesized to be due to exposure to a selective thermal barrier during their migration northwards from the southern Red Sea (Fine et al. 2013). In contrast to previous studies that have simulated thermal stress over weeks-to-months, we assess the ability of these corals to tolerate a high intensity marine heatwave-type event (sensu Hobday et al. 2016) lasting 7–11 d, as the frequency of these events has increased in recent years in the northern Red Sea (Genevier et al. 2019). We then compare the response of the same colonies to an 18 h acute thermal assay, consisting of a 7 h heating period, to assess the validity of using a standardized, rapid heat stress experiment that is capable of resolving historical differences in thermal tolerance between corals across microhabitat reef sites (Voolstra et al. 2020*a*). As the bleaching response involves both the coral host and algal symbionts (and possibly other microbial entities such as bacteria, archaea, and viruses), we conduct direct measurements of a suite of response variables representative of host (protein concentration, respiration rate) and symbiont physiology (photosynthesis, *Fv*/*Fm*, chlorophyll *a* (Chl *a*)), as well as their symbiosis (symbiont density). These measurements were conducted during and after heat stress across both experiments to assess if the use of acute thermal assays can be used to accurately determine holobiont thermal tolerance. From this, we present a novel approach to integrate the physiological performance across thermal exposures into a single measure and provide a quantitative proxy to determine the relative thermal thresholds of corals, termed the *Fv*/*Fm* ED_{50}, which represents the temperature at which 50% of the photosynthetic efficiency is lost relative to the baseline temperature.

Methods

Experimental overview

The study was conducted at the Interuniversity Institute for Marine Sciences (IUI) in Eilat, Israel (Fig. S1) in January–February 2019. Ramets from eight genets of *S. pistillata* were collected from the IUI coral nursery, located at ~ 8 m depth directly in front of the research station in the Gulf of Aqaba, Red Sea. While symbiont type was not assessed in the present study, previous studies have shown that shallow-water (< 17 m) *S. pistillata* in this location exclusively harbor *Symbiodinium* spp./Clade A (Lampert-Karako et al. 2008; Winters et al. 2009; Byler et al. 2013). Ramets from each genet were used in two experiments designed to assess the physiological response of the corals and their symbionts across varying temperature treatments of acute and chronic heat stress. In each experiment, corals were exposed to four temperature treatments (27°C (summer ambient), 29.5°C, 32°C, and 34.5°C), with the focus of the study to contrast the heating rate and duration of thermal stress exposure between the experiments (Fig. 1), though these also utilized different experimental systems (detailed below). While average sea surface temperature at the time of the experiment was ~ 22°C, the maximum monthly mean of 27°C was selected as the experimental baseline treatment as the purpose of the experiments was to determine the upper-most thermal limits of the corals under future scenarios of ocean warming.

For each experiment, ramets were brought back to the research station and placed into the experimental tanks immediately following collection to minimize the potential for any genet-specific rates of recovery from collection or acclimation to the tanks. Two additional ramets from each genet were collected to assess the physiological state of the corals

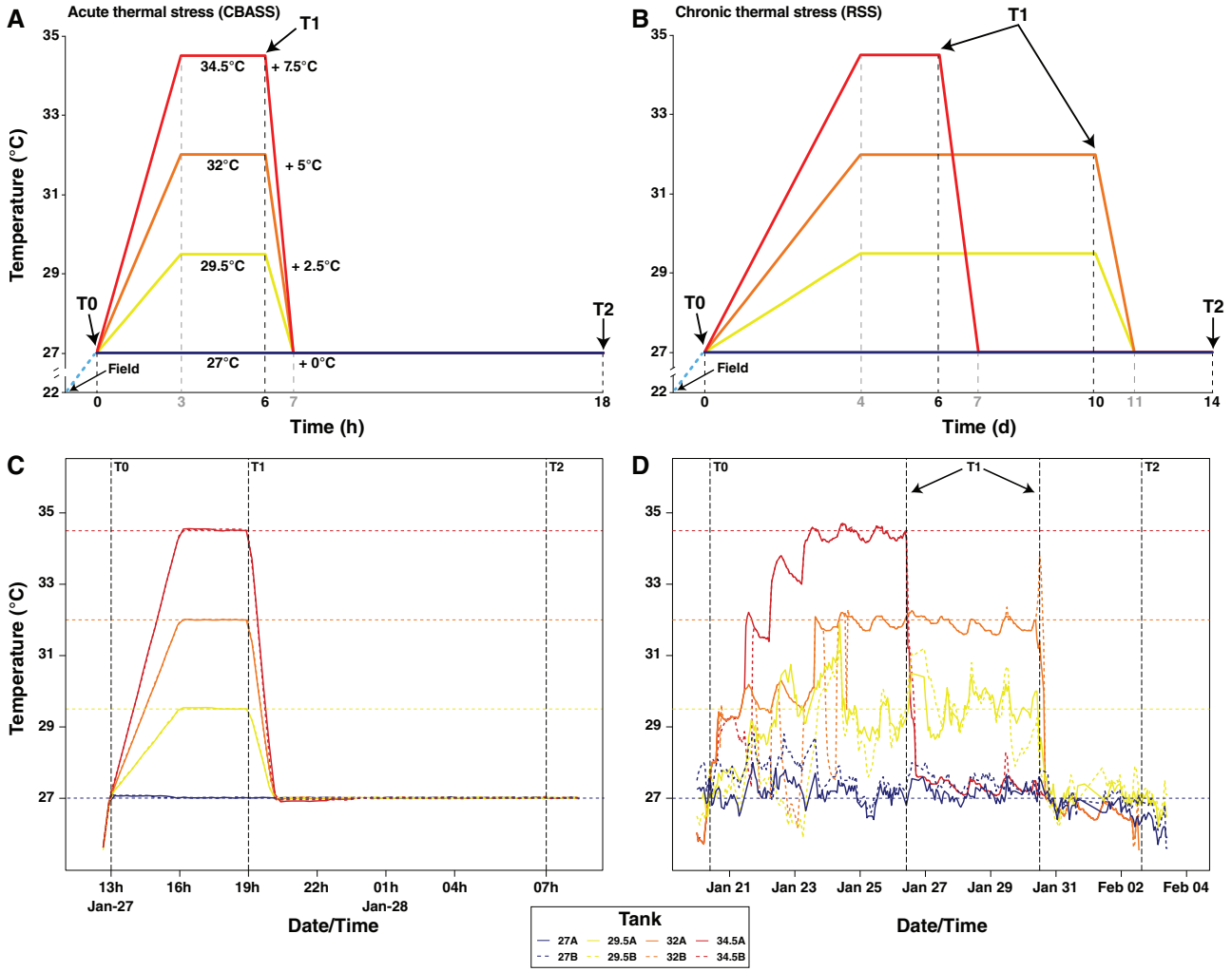

Fig. 1. (**a** and **b**) Targeted and (**c** and **d**) recorded temperature profiles for the acute (Coral Bleaching Automated Stress System) and chronic (Red Sea Simulator) heat stress experiments, demonstrating the ramp up from ambient winter temperatures (22°C) to 27°C (the maximum monthly mean), before exposing corals to target experimental temperatures 29.5°C, 32°C, and 34.5°C. Sampling time points are indicated in each panel with dashed vertical lines: "T1" representing the end of the heating hold and "T2" represents the end of the recovery period. Temperature data were collected at 5 s and 1 min intervals in the acute and chronic experiments, respectively, with dashed horizontal lines representing the targeted hold temperatures for the experiments.

(physiological measurements detailed below) prior to the experiments. Physiological state of the first ramet (termed "field control") was assessed directly upon collection from the field (at 22°C) and prior to placing corals into the experimental tanks. Physiological state of the second ramet (termed "T0") was assessed after ~ 2 h incubation in the experimental tanks (and immediately prior to the start of the experiments), during which time seawater temperatures in the tanks was steadily ramped up from 22°C to 27°C. Field control and T0 measurements were collected for all response variables for the acute experiment and all response variables excepting photosynthesis and respiration for the chronic experiment.

Acute heat stress experiment

The acute heat stress experiment was conducted over 18 h (January 27–28) using the Coral Bleaching Automated Stress System (Voolstra et al. 2020*a*). The stress system consists of four replicated 10-liter flow-through tanks, each capable of running independent temperature profiles (total of eight tanks). Temperature control in each tank is achieved using a combination of IceProbe Thermoelectric chillers (Nova Tec) and 150-200 W aquarium heaters linked to a custom-built controller (Arduino MEGA 2560). Due to space limitations, only five of the eight genets sampled for the chronic experiment were used in the acute heat stress experiment. Two ramets from each of the five genets were randomly arranged in each tank (80 ramets total), with two replicate tanks for each of the four temperature treatments. Temperatures in the tanks were gradually ramped up to the respective target temperatures over 3 h, followed by a 3 h hold at max temperature, a 1-h ramp down to 27°C, and 11-h recovery period at 27°C (Fig. 1a). The physiological state of the corals was assessed at

the end of the hold period ("T1") and following the recovery period ("T2"). Tanks were supplied with 500-μm filtered seawater at a rate $\sim$ 2 L h^{-1} ($\sim$ 5 h renewal rate), with each tank equipped with powerhead pumps (Sun JVP-110 series) to increase internal water flow. Tanks were located indoors and received $\sim$ 300 μmol quanta m^{-2} s^{-1} from LED aquarium lights (GalaxyHydro, Roleandro) for 12 h per day, as measured via a Li-Cor spherical quantum PAR (Photosynthetically Active Radiation) sensor (LI-192, Li-COR).

Chronic heat stress experiment

The chronic heat stress experiment was conducted in the Red Sea Simulator (Bellworthy and Fine 2018) over 2 weeks, from January 20th to February 3rd. Two ramets from each of the eight genets were randomly arranged in each of eight 40-liter tanks (16 ramets/tank, 128 ramets total), with two replicate tanks (i.e., technical/tank replicates) for each of the four temperature treatments. Temperatures in the tanks then were gradually ramped up to the respective target temperatures over the next 4 d. The temperature treatments were held for 6 d, except for the 34.5°C treatment, which was only held at maximum temperature for 2 d as corals started to exhibit considerable paling, with the physiological state of the corals assessed at the end of the hold period ("T1") for each tank. Treatments were then ramped back down to 27°C over 1 d and held at 27°C for a 3-day period of recovery (7 d for the 34.5°C treatment) from heat stress (Fig. 1b). Finally, the physiological state of the corals was assessed following the recovery period ("T2"). Throughout the experiment, tanks were supplied with 500-μm filtered seawater at a rate $\sim$ 10 L h^{-1} ($\sim$ 4 h renewal rate), with tanks equipped with powerhead pumps (Sun JVP-110 series) to increase water flow. Tanks were located outdoors, shaded by black mesh cloth to expose corals to maximum daily Photosynthetically Active Radiation (PAR) light intensities of $\sim$ 300 μmol quanta m^{-2} s^{-1}, as measured via a Li-Cor spherical quantum PAR sensor (LI-192, Li-COR), over a 10.5–11 h photoperiod.

Physiological response measurements

In total, six response variables were assessed at each timepoint, in each experiment. Live metabolic measurements included dark adapted maximum quantum yield of photosystem II of the algal symbionts (*Fv*/*Fm*), and respiratory and net photosynthetic rate. Destructive response variables included host tissue protein content, Chl *a* concentration, and algal symbiont density. Additionally, Chl *a* per cell was calculated based on measurements of Chl *a* concentration and symbiont densities (both per cm^2).

The maximum quantum yield of photosystem II (*Fv*/*Fm*, dimensionless) of the symbionts was measured on dark-acclimated corals ($\sim$ 45 min of dark acclimation) directly in the experimental tanks using a pulse amplitude-modulated (PAM) fluorometer (Junior PAM, WALZ). Following these measurements, a subset of ramets (n = 5) from the five genets that were common to both experiments were transferred to individual 80 mL chambers for measurements of respiration and net photosynthetic rates. Chambers were sealed and maintained at the respective treatment temperatures from which the ramets originated using a circulating water bath (MRC BL-30 liter) and water mixed in the chambers using magnetic stirrers. Corals first were incubated in the dark to measure respiration rates and then illuminated using a high intensity grow light at 100 μmol quanta m^{-2} s^{-1} to measure net photosynthetic output, with the dark and light incubations each lasting $\sim$ 20 min. Oxygen flux in the chambers was measured at 1-s intervals using oxygen probes (FireSting O2, Pyroscience), with a blank chamber (containing no coral) also assessed during each run to account for any change in oxygen caused by instrument drift or microbial metabolic activity.

Following the live measurements of physiological performance, fragments were snap-frozen in liquid nitrogen and stored at – 80°C until further processing. Frozen fragments were airbrushed using 0.22 μm filtered seawater, with tissue slurries homogenized (Diax 900, Heidolph Instruments) and aliquoted into individual 1 mL samples for measurements of host protein, Chl *a*, and symbiont density. Homogenized protein and Chl *a* samples were stored at – 80°C until processing, while samples for algal symbiont density were fixed immediately with 5 μL of 8% glutaraldehyde and stored at 4°C until measurements of cell counts.

Host protein homogenates were centrifuged at 5000*g* and 4°C for 10 min to separate the host supernatant from Symbiodiniaceae cells. The host supernatant was further centrifuged at 16,000*g* and 4°C for 10 min to remove any other particulate material. Total host protein concentration was determined using a Coomassie (Bradford) Protein Assay (Pierce, Thermo Scientific) and bovine serum albumin standard (Bradford 1976). Triplicate protein measurements were conducted for each sample using a multi-scan spectrum spectrophotometer (Biotek HT Synergy plate reader) at 450 and 595 nm.

For chlorophyll concentrations, homogenates were centrifuged at 5000*g* and 4°C for 10 min to separate the host supernatant from Symbiodiniaceae cells. The supernatant was discarded, and the symbiont pellet was resuspended in 1 mL of 100% acetone. Samples were stored at 4°C in the dark for 21 h, and vortexed at regular intervals to promote chlorophyll extraction. Once the chlorophyll was fully extracted, samples were centrifuged for 5 min at 2000*g* at 4°C to remove any particulates. Chl *a* concentrations were measured in duplicate for each sample based on absorbances at 630 nm, 664 nm, and 750 nm, using a multiscan spectrum spectrophotometer (Biotek HT Synergy plate reader; after Ritchie 2006).

The fixed symbiont samples were prepared for cell count measurements by adding SDS (0.01% final concentration) and passing the samples through 35 μm cell strainers. Cell counts were measured in duplicate for each sample using a flow cytometer (MACSQuant Analyzer 10, Miltenyi Biotec).

Surface area was determined using the single wax dipping method (Stimson and Kinzie 1991), with photosynthetic and

respiratory rates, symbiont densities, and protein and Chl *a* concentrations all normalized to the surface area of each ramet.

Statistical analysis

The effects of temperature and experiment duration (i.e., acute vs. chronic) on overall coral physiology (considering all physiological response variables) were analyzed using a permutational multivariate ANOVA (PERMANOVA) with the "vegan" package (Oksanen et al. 2018) in R (R Core Team 2018). Temperature and experiment duration were included as fixed effects. Time points were analyzed separately as the purpose of the experiment was to compare temperature treatments within each time point, with temperatures at each time point representing different treatment conditions (i.e., T1 treatments were at their respective treatment temperatures, while T2 treatments were all at 27°C). Data were standardized and PERMANOVA was conducted on Euclidian distances, with 999 permutations used to generate *p* values. Post hoc pairwise comparisons were conducted using "pairwiseAdonis" (Martinez Arbizu 2019) with Bonferroni adjusted *p*-values, comparing temperature treatments within the acute and chronic experiments separately due to the inability to compute pairwise comparisons for multiple interacting main effects in PERMANOVA.

Individual response variables were analyzed using linear mixed effects (LME) models, with temperature and experiment as fixed effects, and genet and tank replicate as random effects to account for non-independence of fragments from the same colonies and any potential tank effects. Time points again were analyzed separately. Additionally, LMEs were conducted for each response variable to compare the 27°C treatments to the field control and measurements at T0, using the same aforementioned model structure. Models were conducted in the "lmerTest" package (Kuznetsova et al. 2017) in R. Model simplification was conducted by backwards elimination of predictor variables (step function), with F-tests used to compare full and reduced models upon removal of the fixed effects. For significant main effects, Tukey's honestly significant difference (HSD) post hoc pairwise comparisons were conducted using the package "emmeans" (Lenth et al. 2020). For metabolic processes (respiration and net photosynthesis), rates of oxygen flux were determined using "RespR" (Harianto et al. 2019). Measurements of *Fv*/*Fm* at T2 ("recovery") in the chronic experiment were not collected, thus, the model for *Fv*/*Fm* measurements for the acute experiment at T2 was fitted with temperature as the fixed factor and tank replicate and genet as random factors. For all models, distributions of the residuals were plotted to ensure they fit a normal distribution and residuals were plotted against fitted values to confirm that the errors had constant variance. Data were log-transformed to help meet these assumptions when necessary. Figures were plotted in R using "ggplot2" (Wickham 2016) or in Prism (v. 8.0; GraphPad Software, Inc.).

Measurements of Chl *a* and metabolic rates conducted at T2 on ramets from the 34.5°C treatment in the chronic experiment were dropped from the analyses as they were mostly dead and overgrown by microbial biofilms and turf algae, providing unreliable values.

***Fv*/*Fm* 50% effective dose (ED_{50}) metric**

In order to produce a standardized and comparable proxy to quantitively determine the upper thermal limit of the corals in each experiment, we computed a critical temperature threshold using measurements of *Fv*/*Fm*. Measurements of *Fv*/*Fm* were fitted to log-logistic dose–response curves (DRCs) using the package "drc" (Ritz et al. 2015), with model selection based on Akaike's Information Criterion (AIC; Table S2) and individual curves fit for each experiment. From these, an "ED_{50}" parameter (effective dose 50) was obtained for each experiment, representing the *x*-value at the inflection point of the curve (in this case the temperature) where *Fv*/*Fm* values in the model fit were 50% lower in comparison to the starting values of the model. This provided a quantitative thermal threshold, designated as the *Fv*/*Fm* ED_{50}, for *S. pistillata* in each experiment.

All code used in the statistical analyses is available in the electronic notebook associated with this publication on the Barshis Lab GitHub: https://github.com/BarshisLab/CBASS-vs-RSS-Physiology.

Results

Treatment conditions

Temperature treatments were consistently maintained throughout the acute and chronic experiments to match the targeted temperature profiles (Fig. 1). In the acute experiment, all tank temperatures matched the targeted temperatures of 27, 29.5, 32, and 34.5°C during the hold (all ± 0.1 S.E.M; Fig. 1c). In the chronic experiment, temperatures exhibited some variability, though still closely matched the target temperatures, averaging 27.1 and 27.4, 29.5 and 29.5, 31.9 and 31.7, and 34.4 and 34.4°C in the targeted 27, 29.5, 32, and 34.5°C treatments, respectively (all ± 0.1 S.E.M; Fig. 1d). Based on the NOAA climatology for this particular site (extracted from coralreefwatch.noaa.gov), these treatments were equivalent to 0.6, 3.0, 5.6, 5.3 Degree Heating Weeks (DHW), respectively.

Holobiont response

The physiological traits of the holobiont varied depending on the temperature treatment and experiment. At T1 (end of the heating hold), there was an interactive effect of experiment duration × temperature on holobiont physiology ($p = 0.02$), with all response variables contributing to differences between treatments in the PERMANOVA ($p < 0.01$; Fig. 2). For the acute experiment, there was a difference in holobiont physiology between field and 27°C ramets (*adj*. $p = 0.03$), but no difference between the field and T0 ramets or T0 and 27°C ramets (*adj*. $p \geq 0.63$). Within both experiments, holobiont physiology of corals in the 34.5°C treatment differed from all other temperature treatments (*adj*. $p < 0.05$).

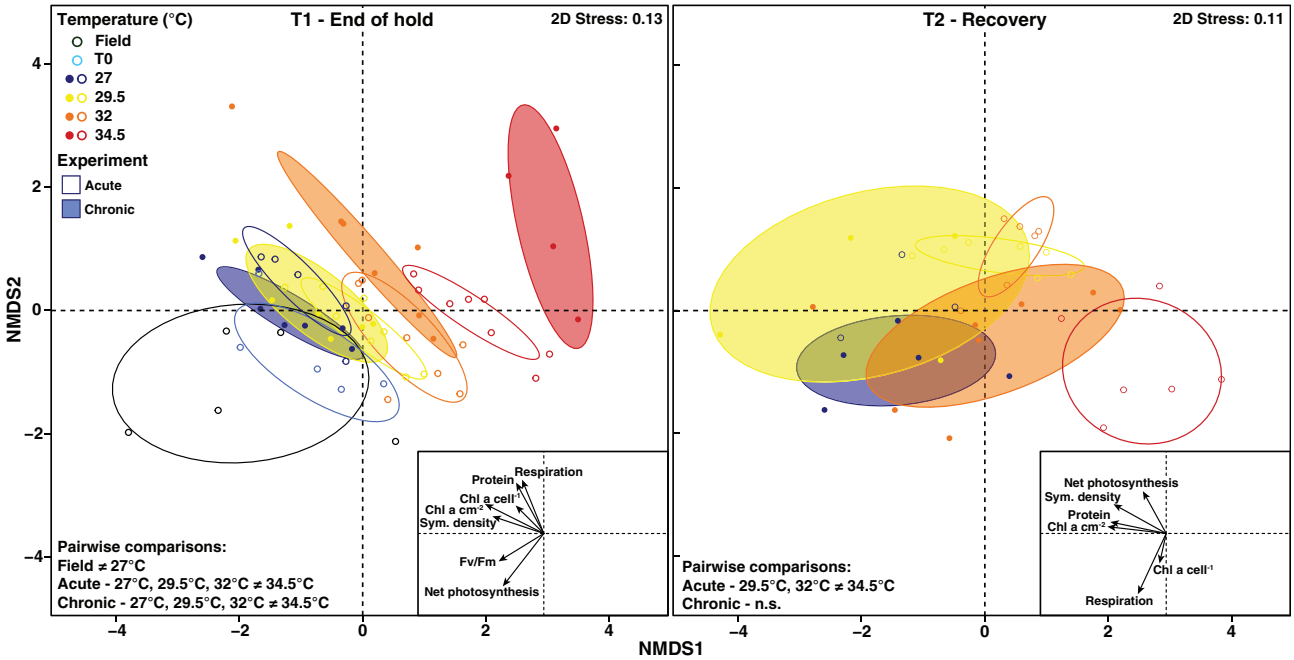

Fig. 2. Non-metric multidimensional scaling (nMDS) plot displaying similarities within temperature treatments in the acute (open ellipses) and chronic (shaded ellipses) experiments, at time point 1 (left) and 2 (right). Ellipses encompass 2/3 of the replicates in each treatment. For both time points, there was a temperature × experiment duration effect on holobiont physiology, with results from pairwise comparisons indicated in the bottom left of each panel. Vectors driving differences between treatments are located in the bottom-right corner of each panel, with the length of the vector representing the strength of the variable in driving differences. At T2, the chronic 34.5°C treatment was not included due to high coral mortality and *Fv/Fm* was not assessed.

Following the recovery period (T2), there again was an interactive effect of temperature × experiment duration on holobiont physiology ($p < 0.05$), with all response metrics contributing to differences between treatments. Pairwise comparisons revealed a difference between the 34.5°C treatment and the 29.5°C and 32°C treatments in the acute experiment (*adj.* $p \leq 0.02$), and no difference between 27°C, 29.5°C, and 32°C treatments in the chronic experiment (dead corals from the 34.5°C treatment were omitted from the analysis).

Host protein, Chl *a*, and symbiont density

There were no differences in host protein, Chl *a*, and symbiont densities between field, T0, and 27°C ramets ($p > 0.05$). At T1, temperature had an overall negative effect on host protein concentration ($p = 0.02$; Table S1) regardless of experiment duration. Protein concentrations remained stable up to 32°C, before declining by 37% at 34.5°C, compared to all other treatments (Fig. 3a). Similarly, only temperature had an effect on host protein at T2, driven by an 80% decrease in protein concentration at 34.5°C compared to the other treatments ($p < 0.01$; Fig. 3b).

For Chl *a* concentration ($\mu g\ cm^{-2}$), there was an overall effect of temperature at T1 ($p < 0.01$), with little change in Chl *a* up to 32°C, but declining by 62% at 34.5°C (Fig. 3c) and no difference between experiments. At T2, there was an experiment duration × temperature effect on Chl *a* concentration ($p < 0.01$), with Chl *a* 59% lower in the 34.5°C treatment compared to all other temperature treatments in the acute experiment, 42% lower in the 32°C vs. the 27°C and 29.5°C treatments in the chronic experiment (all $p < 0.01$), and 40% lower in the acute vs. chronic experiment at 29.5°C ($p < 0.05$; Fig. 3d).

At T1, there was an interactive effect of experiment duration × temperature on symbiont density (cells cm^{-2}) ($p < 0.01$; Table S1). In the acute experiment, symbiont densities were 40% lower at 34.5°C compared to the 27°C and 32°C treatments ($p \leq 0.04$), 75% lower than all other treatments in the chronic experiment ($p < 0.01$), and 58% lower in the chronic vs. acute experiments at 34.5°C ($p < 0.01$; Fig. 3e). At T2, there was also an experiment duration × temperature effect on symbiont density ($p < 0.01$; Fig. 3f). In the acute experiment, symbiont densities were 69% lower at 34.5°C compared to all other temperatures ($p < 0.01$). In the chronic experiment, symbiont densities exhibited a more gradual decrease with increasing temperature, with symbiont densities 26% lower at 32°C compared to 27°C and 29.5°C ($p \leq 0.03$) and 99% lower at 34.5°C compared to all other temperatures ($p < 0.01$). Symbiont densities were 26% lower in the acute vs. chronic experiment at 29.5°C ($p < 0.01$), but 99% lower in the chronic vs. acute experiment at 34.5°C ($p < 0.01$; Fig. 3f).

Concentration of Chl *a* (pg) per cell was unaffected by temperature at T1 ($p = 0.38$) but did differ between experiments

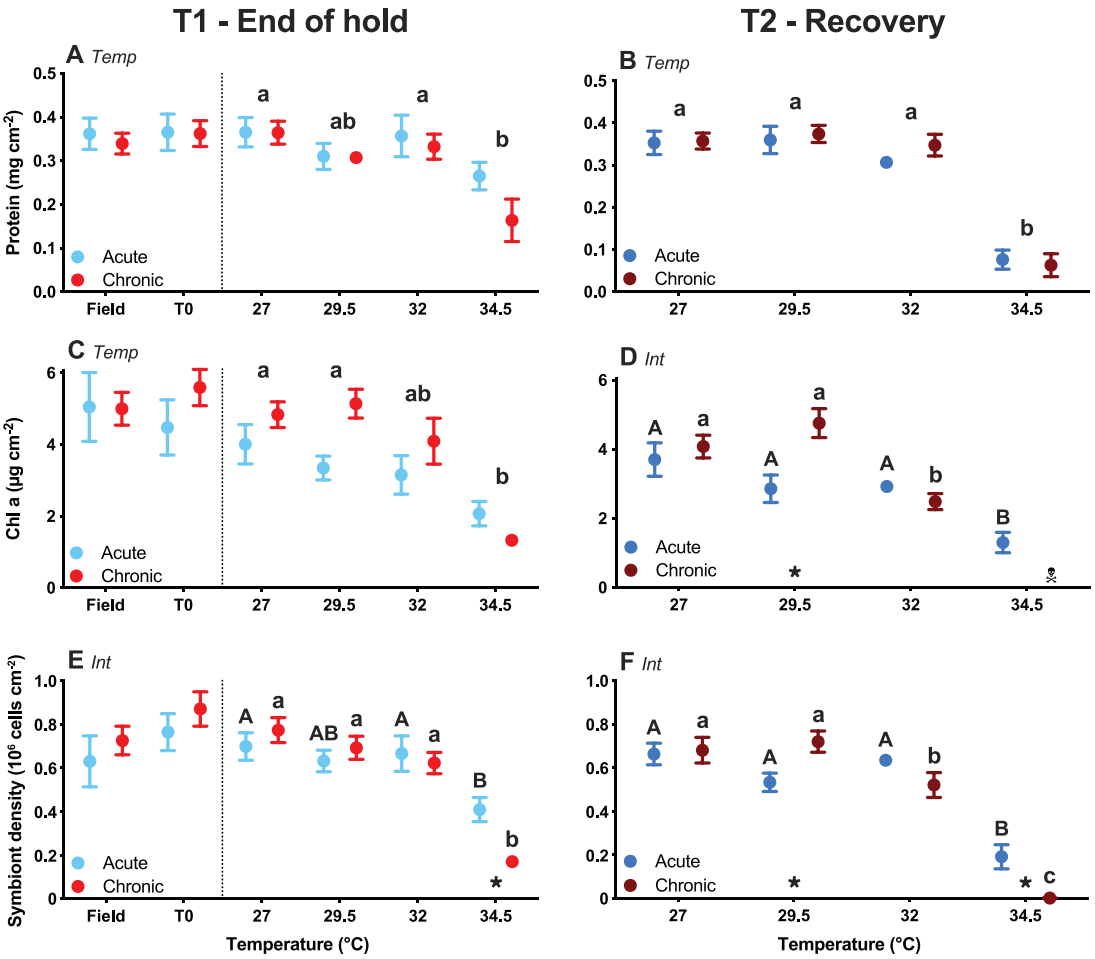

Fig. 3. Measurements of (**a**,**b**) host protein, (**c**,**d**) chlorophyll *a* (Chl *a*), and (**e**,**f**) symbiont density at each time point in the acute (blue) and chronic (red) experiments. Time point 1 panels (**a**,**c**,**e**) also include measurements conducted on field and "T0" samples. The designations "*Temp*," "*Exp*," and "*Int*" indicate significant effects of temperature, experiment, or their interaction. Letters denote differences among treatments across experiments when only temperature was significant, while asterisks denote differences between experiments within a temperature treatment when experiment was significant. For an interactive effect, uppercase and lowercase letters represent differences in the acute and chronic experiment, respectively. Points represent means ± SE (n = 5–16 per treatment). Error bars are missing for treatments with very little variance as these were smaller than the symbols denoting the mean. For Chl *a* measurements, the 34.5°C treatment at time point 2 in the chronic experiment was omitted from the analysis as results were affected by microbial films and turf algae overgrowing the dead coral skeletons.

($p < 0.01$; Table S1), with Chl *a* per cell 23% lower for corals in the acute experiment than the chronic experiment (Fig. S2a). Conversely, there was no difference in Chl *a* per cell between experiments at T2 ($p = 0.10$), but there was an effect of temperature ($p = 0.03$), with concentrations 21% lower at 32°C compared to all other temperatures (Fig. S2b).

Photosynthesis, respiration, and *Fv/Fm*

Metabolic rates differed between field and T0 ramets and ramets in the 27°C treatment in the acute experiment (no field or T0 ramets assessed for the chronic experiment). Respiration rates were 42% lower in field and T0 samples compared to the 27°C treatment ($p \leq 0.05$), while net photosynthetic rates were 63% lower at T0 and 27°C compared to the field corals ($p \leq 0.04$).

For respiration, there was an experiment duration × temperature effect at T1 ($p < 0.01$; Table S1). In the acute experiment, respiration was highest at 27°C and 29.5°C, and was on average 112% higher than at 32°C and 34.5°C ($p \leq 0.04$). Conversely, respiration was highest at 32°C in the chronic experiment, with respiration 75% higher at 32°C than 34.5°C ($p = 0.03$; Fig. S3a). At T2, respiration was affected by both temperature and experiment ($p < 0.01$). Overall, respiration rates were 55% lower in the acute experiment compared to the chronic experiment (Fig. S3b), with respiration rates at 27°C higher than at 29.5°C and 32°C ($p < 0.01$).

For net photosynthesis, there was an experiment duration × temperature effect at T1 ($p < 0.01$; Table S1), with differences between the experiments at all temperatures but the 29.5°C treatment ($p \leq 0.02$; Fig. S3c). In the acute experiment, net

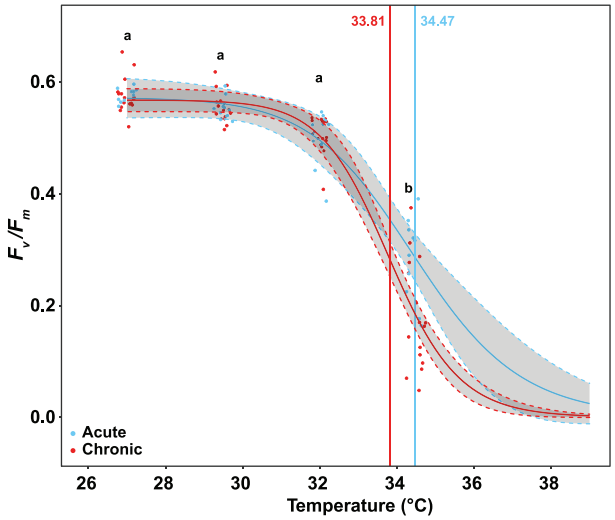

Fig. 4. Maximum quantum yield of photosystem II (*Fv/Fm*) at time point 1 fitted to log-logistic, dose response curves (dotted lines represent 95% confidence intervals). Curve fits were used to determine the *Fv/Fm* ED_{50} for each experiment (vertical lines), which represent the *x*-value at the inflection point of the curve (in this case the temperature) where *Fv/Fm* values in the model fit were 50% lower in comparison to the starting values of the model. This approach demonstrates the consistency of this non-destructive *Fv/Fm* measurements to determine the upper thermal thresholds of corals across acute and chronic heat stress (n = 10–16 per treatment), despite differences in temperature ramping, duration, and light conditions between experiments. Letters denote differences between temperature treatments across both the acute and chronic experiments.

photosynthesis was reduced from 0.05 ± 0.01 in the 27°C and 29.5°C treatments compared to – 0.09 ± 0.01 in the 32°C and 34.5°C treatments (mean ± S.E.M., n = 13–16). In the chronic experiment, there was a gradual decline in net photosynthesis, going from 0.17 ± 0.03 in the 27°C treatment, to – 0.32 ± 0.05 in the 34.5°C treatment. At T2, there remained an effect of temperature on net photosynthesis ($p < 0.01$; Fig. S3d), with net photosynthetic rates 71% lower for corals in the 32°C treatment compared to the 27°C treatment ($p = 0.01$).

Most notably, applying the *Fv/Fm* ED_{50} approach, we found consistent thermal threshold values across the chronic and acute experiments, with the 33.81°C *Fv/Fm* ED_{50} in the chronic experiment slightly lower than the 34.47°C threshold in the acute experiment due lower *Fv/Fm* values at 34.5°C in the chronic experiment. Results of the linear mixed effects models indicated that *Fv/Fm* was affected by temperature only at T1 ($p < 0.01$; Table S1), with *Fv/Fm* 60% lower at 34.5°C compared to all other temperatures (Fig. 4).

Discussion

The present study found a remarkably similar response of the coral *S. pistillata* and its associated algal symbionts to acute (7 h) and chronic (7–11 d) thermal stress, with a clear upper thermal threshold between 32°C and 34.5°C, beyond which corals suffered rapid tissue necrosis and mortality. While corals exposed to 32°C experienced some decline in physiological performance, with decreases in Chl *a* concentration (per cm^2) and net photosynthetic yield, corals began to recover from thermal stress once temperatures returned to 27°C. In turn, there was little evidence of bleaching up to 32°C, as demonstrated by a maintenance of symbiont densities and concentrations of Chl *a*. These results support past indications that corals in the Gulf of Aqaba tolerate temperatures much higher than those experienced locally, maintaining physiological performance up to 32°C (~ 5°C above local maximum monthly mean) regardless of the duration or rate of onset of thermal stress (Fine et al. 2013; Krueger et al. 2017). In turn, our results indicate that intense heat spikes above a critical threshold lasting just a few hours can result in considerable coral mortality. Although such acute changes in seawater temperature may be unlikely for fringing reefs such as those in the Gulf of Aqaba, these findings highlight the need to consider fine-scale variability in seawater temperature when characterizing and predicting the impacts of future thermal stress events on corals in more variable reef environments, such as back reefs.

Crucial to the mechanics of coral bleaching is that corals expel their symbionts but retain their tissue, and thus have the potential to recover by reacquiring symbionts once seawater temperatures return below a coral's thermal threshold (Thornhill et al. 2006). In such instances, corals can not only recover from thermal stress, but also have the potential to increase their thermal tolerance by selecting for more stress tolerant symbionts (Grottoli et al. 2014; Silverstein et al. 2015). In the current study, corals showed little indication of bleaching, as assessed through measurements of symbiont density, in response to both acute and chronic thermal stress up to 32°C. Similarly, host protein and respiration (the principal metrics assessed related to the host) remained unchanged up until 32°C. However, host protein and symbiont density then rapidly declined at 34.5°C due to extensive tissue necrosis, with the strong decrease in symbiont density at 34.5°C more likely a reflection of tissue loss than a loss of symbionts within the remnant tissue. While such a response may have allowed corals to maintain their physiological performance well above peak local temperatures (which rarely exceed 28°C; Krueger et al. 2017), it ultimately led to a point of no return as corals continued to lose tissue and die after temperatures were brought back down to 27°C, even in the acute experiment.

Similar patterns of rapid tissue necrosis and mortality at high temperatures have been documented previously in both field and laboratory settings. Following in situ observations of rapid tissue loss and mortality on the Great Barrier Reef, a study found that *Acropora aspera* and *Pocillopora damicornis* experienced tissue loss and biofilm formation over the entire coral skeleton within 1–3 d of exposure to severe heat stress (34°C; Leggat et al. 2019). Similarly, *A. aspera* colonies from the Kimberley region in northwest Australia, home to corals naturally adapted to high water temperatures due to strong

diurnal temperature variations, experienced tissue necrosis within 5–6 d of exposure to increased seawater temperatures (34°C), and died 24–48 h later (Schoepf et al. 2015). Although average daily temperatures in both studies were only ~ 2–3°C above the local maximum monthly mean, short temperature spikes upwards of 34°C in each appeared sufficient to result in tissue loss and mortality. Thus, while exposure to natural temperature variability might reduce the risk of bleaching in some corals (Safaie et al. 2018), there is evidence of an upper limit to the amount of variability corals can tolerate.

An important consideration of the present study, and how it might relate to previous thermal stress studies on this species, is the heating rate and duration of the experiments (Grottoli et al. 2020). There is evidence that short, yet sudden and severe, marine heatwaves can yield distinct physiological responses compared to longer and more progressive bleaching events (Fordyce et al. 2019; Leggat et al. 2019). Indeed, there are numerous reports of bleaching (followed by mortality or recovery) for this species in Eilat and other locations (Hoegh-Guldberg and Smith 1989; Baird and Marshall 2002; Sampayo et al. 2008; Grottoli et al. 2017). Notably, Grottoli et al. (2017) found that *S. pistillata* bleached severely (95% loss of symbionts) following a 37-day exposure to 32°C in the winter months (February–April), though these corals still experienced no mortality. We found a 23% decrease in symbiont density for corals that had been incubated in the 32°C treatment, relative to the 27°C treatment, suggesting that corals may have suffered further symbiont loss had the chronic experiment been extended. In turn, a study of *S. pistillata* on Heron Island, Australia, reported high rates of tissue sloughing and mortality in a "high stress" treatment (32°C and 1000 μmol quanta m^{-2} s^{-1} for 8 h), while the majority of corals in a "low stress" treatment (31°C and 550 μmol quanta m^{-2} s^{-1} for 5 d) bleached but survived (Franklin et al. 2004). Thus, different temperature and/or light regimes may produce distinct physiological outcomes, though it remains largely untested whether a high tolerance of corals to short-term thermal stress is congruent with tolerance to long-term stress.

In addition to the experimental conditions, seasonality may have a strong impact on the response patterns and thermal thresholds observed (Berkelmans and Willis 1999). In contrast to the present study and that of Grottoli et al. (2017), a study by Fine et al. (2013) reported no visual signs of bleaching and just a 25% decrease in symbiont density for the same coral population after a 4-week incubation at 34°C, though the study took place during the summer (August) and thus used corals that were already acclimated to 27°C in situ. With the present study conducted in the winter, corals were heated from 22°C to 27°C prior to the start of the experiment, which led to increased respiration rates, indicating some impact of this rapid temperature change on coral physiology. Still, and notably, the response pattern observed herein is similar to that of a short-term experiment conducted in August using the same coral population, where corals were exposed to temperatures up to 32°C for 8 d (4.4 DHWs compared to 5.6 DHWs in the present study) and exhibited little change in symbiont density and Chl *a* (Bellworthy and Fine 2017). This fits well with the notion that differences in thermal tolerance are at least partially adaptive (Palumbi et al. 2014), and seasonality would be expected to be a variable, but not major contributor. Beyond 32°C, we found a sharp decline in symbiont density and high rates of mortality at 34.5°C, just 0.5°C higher than the maximum temperature in the study by Fine et al. (2013). Thus, while it is possible that the thermal threshold for this coral population lies within a very narrow range between 34°C and 34.5°C, a more likely explanation is an upper thermal threshold around 34°C, which is partially influenced by seasonal phenotypic variation (Berkelmans and Willis 1999) and variation in experimental designs (e.g., heating rate and duration).

Regardless, these corals clearly can tolerate, and even thrive under, temperatures far beyond local summer conditions for extended periods. Working on *S. pistillata* from the same coral nursery, Krueger et al. (2017) found evidence for a beneficial effect of exposure to 29°C (~ 5°C above in situ conditions during the experiment) over 1.5 months, with coral exhibiting improved photochemistry (i.e., *Fv*/*Fm*) and higher Chl *a* pigmentation that led to a doubling in net photosynthetic output. Thus, while this coral population may bleach under certain scenarios, these corals also appear to exhibit atypical responses to certain short- or long-term heat stress regimes, with no clear bleaching response. Instead, these corals appear able to maintain physiological performance until reaching an upper thermal limit ~ 34°C at which point the host suffers from rapid cellular breakdown. While this threshold appears to be consistent with aforementioned studies for other species in different regions, it is nonetheless likely that the upper thermal limits will vary across regions and latitudes.

Most metrics displayed very similar patterns in response to increasing temperatures across both the acute vs. chronic experiments, though there were some consistent differences. Notably, values of symbiont density and net photosynthesis were lower at T1 in the 34.5°C treatment in the chronic compared to the acute experiment, though this is unsurprising as corals in the chronic experiment had endured considerably more heat loading (up to 5.6 DHWs) and sustained time at high temperature. Interestingly, these differences were much less pronounced at T2 suggesting consistency in the aftereffects of the exposures. There also was a difference in the Chl *a* per cell between the experiments, with Chl *a* per cell decreasing in the acute experiment compared to field values, while remaining constant in the chronic experiment. The loss of pigments in the acute experiment may be due to light stress, as corals received constant artificial light in these tanks, compared to the chronic experiment that was exposed to natural fluctuating light regimes that may have been more representative of the reef environment.

The Coral Bleaching Automated Stress System recently has been used to assess the thermal tolerance of two *S. pistillata* populations in the Central Red Sea (Voolstra et al. 2020*a*).

While the previous study conducted a similar chronic experiment, with 9 d of heat stress, it focused on the ability of each experiment to resolve disparities in thermal tolerances across the two populations based on historical differences in observed bleaching susceptibility. As such, the study conducted no direct comparison of the stress response profiles between the acute and chronic experiments. The present study provides an important comparison of the coral stress response to acute and chronic thermal stress, with a complete assessment of the holobiont physiology in both experiments at corresponding time points and matching temperature intensities; as summarized by the multivariate analysis (Fig. 2). From this, we found that *Fv*/*Fm* measurements were consistent with most physiological response metrics and representative of holobiont performance under acute and chronic heat stress, with a slight reduction in *Fv*/*Fm* at 32°C and a substantial decline at 34.5°C that was more pronounced in the chronic experiment. Prompted by the similarity in conclusions that can be drawn from the acute and chronic thermal stress experiments with regard to thermal tolerance, we sought to develop a metric representative of holobiont thermal tolerance that can serve as a proxy to quantitatively compare the upper thermal limit of corals across populations, regions, and species. We computed the temperature threshold at which photosynthetic performance is halved relative to values in the 27°C treatment (baseline temperatures), the *Fv*/*Fm* ED_{50}, which produced similar proxies of upper thermal limits for both experiments, 34.47°C vs. 33.81°C for the acute and chronic experiments, respectively.

While *Fv*/*Fm* has been used as a non-invasive measurement of coral stress during heat exposure for many years (Warner et al. 1996), we propose combining acute heat stress assays using the experimental stress system with the *Fv*/*Fm* ED_{50} value as a standardized approach to quantitatively determine upper thermal thresholds of corals. Notably, using four temperature treatments (the maximum monthly mean and three heat stress treatments) was essential to modeling the dose response curves, an approach that was not possible in previous studies using a control and single heat stress treatment (e.g., Krueger et al. 2017) or the three temperatures of the "classic" experiment of Voolstra et al. (2020*a*). The *Fv*/*Fm* ED_{50} is not an absolute measure of thermal tolerance, as the absolute thermal tolerance of a coral is likely to be affected by the prospective diel/seasonal heating and light regime among other factors. Still, our results, along with those from Voolstra et al. (2020*a*) and Morikawa and Palumbi (2019), indicate some consistency in the response of corals to acute thermal assays and more prolonged thermal exposures and serve as evidence that acute thermal assays could provide realistic assessments of natural variance in the thermal tolerance of coral populations (Voolstra et al. 2020*b*). As such, this approach could hold great promise as a standardized diagnostic to empirically assess environmental and evolutionary drivers of thermal tolerance across large scales and identify coral populations with the greatest chance of surviving future impacts of global warming.

Data availability statement

All raw data, analyses, and scripts are available as an electronic notebook on GitHub (https://github.com/BarshisLab/CBASS-vs-RSS-Physiology) prior to publication.

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Conflict of Interest

None declared.

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