Symbiosis
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Symbiotic dinoflagellates divert energy away from mutualism during coral bleaching recovery

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Abstract
The future of coral reefs in a warming world depends on corals' ability to recover from bleaching, the loss of their symbiotic dinoflagellate algae (Symbiodiniaceae) during marine heatwaves. Heat-tolerant symbiont species can remain in symbiosis during heat stress, but often provide less photosynthate to the host than heat-sensitive species under ambient conditions. Understanding how heat stress changes the dynamics of this tradeoff between stress tolerance and mutualism contribution is crucial for predicting coral success under climate change. To test how symbiont resource allocation affects coral recovery from heat stress, we exposed the coral *Montipora capitata* hosting either heat-sensitive *Cladocopium* C31 (C) or heat-tolerant *Durusdinium glynnii* (D) to heat stress. D regained symbiont density and photochemical efficiency faster after heat treatment than C, but symbiont recovery did not restore coral biomass or calcification rates to pre-bleaching levels in the initial recovery period. D populations also contributed less photosynthate to the host relative to C, even during heat stress. Further, higher-density symbiont populations of both species retained more photosynthate than lower-density populations, and corals receiving less photosynthate exhibited reduced calcification rates and lower intracellular pH. This is the first evidence that symbiont density and carbon translocation are negatively related, and the first to establish a link between Symbiodiniaceae carbon translocation and coral cellular homeostasis. Together, these results suggest the energy demand of symbiont regrowth after bleaching reduces their mutualism contribution and can thus delay host recovery. Reestablishing a beneficial endosymbiosis imposes additional costs as holobionts overcome stress, and may explain latent mortality among coral populations after alleviation of heat stress in the field.

Keywords Coral bleaching · Symbiodiniaceae · Context-dependency · Climate change · Isotopes · Acid–base homeostasis

1 Introduction

Microbial endosymbiosis is a ubiquitous way of life that supports many key ecosystems (Gilbert et al. 2012; McFall-Ngai et al. 2013). However, the benefits of many symbioses are context-dependent, with both environmental conditions and partner identity determining whether a particular association improves or impairs host fitness (Bronstein 1994; Chamberlain et al. 2014; Hoeksema and Bruna 2015). For example, while some endosymbionts have a neutral or negative effect on host fitness during stress, other symbiont species can confer stress tolerance on their hosts (e.g. (Russell and Moran 2006; Rodriguez et al. 2008; Bénard et al. 2020)). Yet these hardy symbionts can also cost host fitness in the absence of that specific stress, such as bacteria that improve aphid heat tolerance but also reduce host fecundity relative to other bacterial strains (Dunbar et al. 2007), or fungal endophytes that enhance drought tolerance at the expense of host growth (Cheplick 2007). These patterns suggest that stress tolerance (defined here as symbiont and host survival) may come at the expense of mutualistic function. This can produce a "hardiness-benefit" tradeoff, where symbionts that improve host fitness during stress provide less fitness benefit than other stress-intolerant symbionts in the absence of that particular stressor. As global climate change outpaces evolutionary adaptation for many species (e.g. (Kelly et al. 2012; Diniz-Filho and Bini 2019)), it is crucial to better understand how environmental disturbances impact the persistence and function of mutualisms.

Reef-building corals rely on endosymbiotic dinoflagellate algae (Symbiodiniaceae) to survive, and variation in the

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environmental sensitivity of this association may produce such a hardiness-benefit tradeoff. Under normal conditions, coral animals use energy gained from photosynthate released by their dinoflagellate symbionts (Muscatine and Porter 1977) to secrete the massive calcium carbonate skeletons that sustain reefs' iconic biodiversity, including a staggering 25% of all marine species (Fisher et al. 2015). However, at just 1-2ºC above mean summer temperatures, the coral-dinoflagellate mutualism can break down in a process known as coral bleaching (Glynn 1996; Hoegh-Guldberg et al. 2007; Hughes et al. 2017). Some corals host Symbiodiniaceae species that remain *in hospite* despite high temperatures, possibly because these algae can maintain higher photochemical efficiency under heat stress than more thermosensitive symbionts ((Rowan 2004; Berkelmans and van Oppen 2006; Cunning et al. 2018; Carballo-Bolaños et al. 2019); but see (Silverstein et al. 2017)). Corals with these thermotolerant algae are more likely to survive heat stress events (e.g. (Matsuda et al. 2020; Claar et al. 2020; Palacio-Castro et al. 2021)), likely as a result of receiving similar (Matthews et al. 2020) or more photosynthate-derived metabolites (Hoadley et al. 2021) than corals with heat-sensitive algae during stress. However, these thermotolerant algae often provide less photosynthate to their hosts under ambient conditions, limiting host growth (e.g. (Little et al. 2004; Stat et al. 2008; Jones and Berkelmans 2010; Lesser et al. 2013; Cunning et al. 2015a; Pettay et al. 2015; Wall et al. 2020)). Coral symbionts may therefore exhibit a functional tradeoff between thermotolerance and nutritional benefit. It is critical to understand the consequences of this tradeoff for coral persistence as severe marine heatwaves increase in frequency and severity (Frölicher et al. 2018).

The energetic costs of recurrent bleaching may shift both the costs and benefits of symbiosis, potentially granting a crucial survival advantage to corals hosting thermotolerant symbionts despite their tendency to provide less fixed carbon to the host (Cunning et al. 2015a, 2018). Reef futures depend on corals' ability to withstand increasingly frequent bleaching events (Hughes et al. 2017; Matsuda et al. 2020)), as bleached corals can starve within weeks if they do not regain their symbionts (e.g. (Thornhill et al. 2011; Hughes et al. 2017; Matsuda et al. 2020)), leaving reefs vulnerable to erosion (Perry and Morgan 2017; Leggat et al. 2019) and consequent biodiversity loss (Pratchett et al. 2011). But while bleaching recovery requires the symbiont population to regrow (Jones and Yellowlees 1997; Putnam et al. 2017), host costs of symbiosis maintenance increase with both temperature and symbiont density (Hoogenboom et al. 2010; Cunning et al. 2015b) thus destabilizing symbiont cooperation (Tremblay et al. 2016; Baker et al. 2018; Rädecker et al. 2021). Moreover, though colonization studies in coral juveniles suggest that thermotolerant symbionts grow faster than thermosensitive symbionts *in hospite* (Yuyama and Higuchi 2014), we do not know whether this rapid growth supports symbionts' ability to sustain host nutrition (Wooldridge 2013) or how these dynamics play out in adult colonies recovering from bleaching. Therefore, there is an urgent need to better understand Symbiodiniaceae photosynthate provisioning and use during bleaching recovery in adult corals.

In order to examine how population recovery of different Symbiodiniaceae species affects host physiology after bleaching, we used a 'living library' of bleaching-susceptible and bleaching-resistant colonies of the reef-building coral *Montipora capitata* in Kāne'ohe Bay, Hawai'i (Matsuda et al. 2020). These colonies have exhibited consistent bleaching phenotypes across multiple heatwaves (Matsuda et al. 2020; Innis et al. 2021), and bleaching resistance has been attributed to the proportion of the thermotolerant symbiont *Durusdinium glynnii* (LaJeunesse et al. 2004; LaJeunesse and Thornhill 2011; Wham et al. 2017) present: bleaching-resistant *M. capitata* tend to host primarily *D. glynnii* (D-colonies), while bleaching-susceptible individuals host primarily *Cladocopium* C31 (C-colonies) (Cunning et al. 2016; Dilworth et al. 2021). Isotopic signatures indicate that C-colonies assimilate more autotrophic carbon than D-colonies at ambient temperatures (Wall et al. 2020), suggesting that *Cladocopium* C31 are more generous symbionts than *D. glynnii*, which matches trends observed for these two genera in other host species (e.g. (Little et al. 2004; Cunning et al. 2015a; Pettay et al. 2015)). In addition, a recent heatwave (2019) led to acidification of the intracellular pH (pH_i) of C-colonies but not D-colonies, suggesting greater physiological stress in C-colonies during heat stress (Innis et al. 2021). We hypothesize that these changes in cellular homeostasis may be due to reductions in coral carbon assimilation in C-colonies during heat stress, which could be driven by disruptions in carbon translocation from the symbiont (Tremblay et al. 2016; Baker et al. 2018; Rädecker et al. 2021), and/or the loss of symbionts from the colony (Dilworth et al. 2021; Innis et al. 2021). However, what benefits corals hosting different symbiont species receive during and after heat stress remains unknown. Here we investigated how heat stress and recovery influence the energetic contribution of *Cladocopium* C31 vs. *D. glynnii*-dominated symbiont communities to the coral host (*M. capitata*), and whether variability in this contribution due to species-specific symbiont heat stress responses affects coral cellular homeostasis. This study is a novel and important step in understanding energy dynamics of different coral holobionts during bleaching recovery, and advances our understanding of how symbiotic partners allocate benefits in nutritional symbioses under stress.

2 Materials and methods

2.1 Field collections

Between June 7–10, 2019, six fragments per genet (N = 60 fragments total) were sampled from previously identified bleaching-susceptible and bleaching-resistant depth-matched neighboring pairs of *M. capitata* colonies on Patch Reef 13, Kāne'ohe Bay, HI (Matsuda et al. 2020), confirmed July 9, 2019 to host either predominantly *Cladocopium* C31 (mean = 100% ± 0 SE) or *Durusdinium glynnii* (mean = 91.8% ± 3.33 SE) (Table S1; (Dilworth et al. 2021)). Corals were then transported to the Hawai'i Institute of Marine Biology (HIMB) in coolers containing ambient seawater. Immediately on arrival at HIMB, corals were placed in outdoor flow-through seawater tables. Water tables were semi-shaded to allow light levels similar to those on the reef (diel maximum of ~ 450 µmol m^{-2} s^{-1} at 12:00). Each coral fragment was glued to a plastic plug using non-toxic ethyl cyanoacrylate (CorAffix, Two Little Fishies Inc., Miami Gardens FL, USA). After two days of acclimation, corals were distributed among 6 indoor flow-through seawater tanks (25 gal) (Table S2). Specifically, fragments from each genet were randomly allocated to either the ambient or high-temperature treatment, then randomly assigned to 1 of 3 tanks within the 2 temperature treatments and rotated weekly within treatments to minimize tank effects. All fragments were illuminated to levels of photosynthetically active radiation similar to those on the reef (~ 400 µmol/m^2/s) (EcoTech Radion XR30w Pro, EcoTech Marine, Bethlehem PA, USA). Tanks were cleaned every two days to prevent algal overgrowth.

2.2 Incubation

All tank temperatures were monitored and adjusted automatically at 15 min intervals for the duration of the experiment (APEX Controllers, Neptune Systems, Morgan Hill CA, USA) (Fig. 1A). All tanks were programmed to include a diel fluctuation of 1ºC to mimic in situ conditions (Table S2). Ambient tanks ranged from 27º at night to 28º at midday so that daily averages remained well below the region's coral bleaching threshold (28 + 1ºC) throughout the experiment, incurring no experimental degree heating weeks (eDHW; sensu (Leggat et al. 2022)); this temperature range was also similar to the diel range in Kāne'ohe Bay from the preceding month (Innis et al. 2021). For the heat stress treatment, the maximum daily tank temperature was increased by 1ºC per day to a maximum of 31ºC. After one week at a daily maximum of 31ºC, an additional degree was added to the heat stress treatment so that the daily maximum was 32ºC for one more week, for a total of 5.45 eDHW. After this two-week acute heat stress period, half the corals in each treatment were sampled and the heat treatment tanks were returned to ambient temperatures (Fig. 1A). The experiment continued for an additional 4.5 weeks, during which all remaining corals were kept at ambient temperatures (~ 28ºC). Corals were

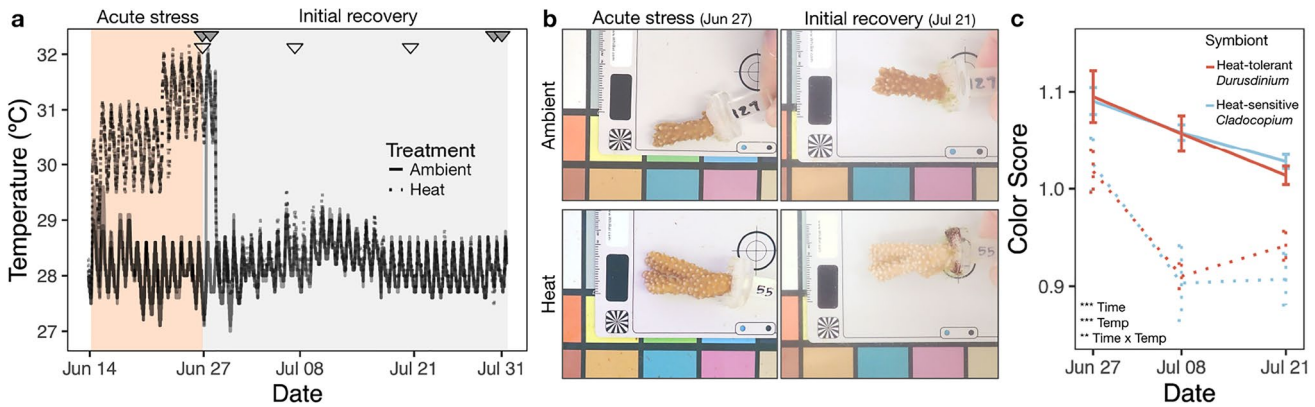

Fig. 1 Experimental heating induced early bleaching signs in *Montipora capitata*. **(A)** Water temperatures in heated (N = 3 tanks, dotted lines) and ambient (N = 3 tanks, dashed lines) mesocosms, recorded in each tank every 15 min. Pink shading, acute stress period; gray shading, initial recovery period. Gray filled triangles (Jun 27, Jun 28, Jul 30, and Jul 31) indicate timepoints when fragments were weighed and samples were frozen for physiology, while open triangles (Jun 27, Jul 8, and Jul 21) indicate timepoints when fragments were photographed. **(B)** Images of representative ambient (top) and heat-treated (bottom) coral fragments after the acute stress (left) and initial recovery (right) periods. Fragments both hosted *D. glynni* and had representative red intensity values relative to the dark red standard (lower left square of the standard in each image) (ambient Fragment 127: 1.05 on Jun 27 and 1.01 on Jul 21; heat-treated Fragment 55: 1.01 on Jun 27 and 0.85 on Jul 21). **(C)** Color scores calculated from red channel intensity relative to dark red standard (B) for heat-treated (dashed line) and ambient-temperature (solid line) corals. Insets show results of linear model with time point (Time) and temperature (Temp) (** = $p < 0.01$, *** = $p < 0.001$); a separate model including symbiont species as a factor did not find it to be significant ($p = 0.848$) and was outperformed by this model

sampled at the end of this time period, the recovery time point (Fig. 1A).

2.3 *In vivo* coral performance

Dark-adapted photochemical yield (F_v/F_m) was measured for each coral daily throughout the heat treatment period, and weekly throughout the initial recovery period. F_v/F_m measurements were taken ~ 1 h after sunset using a Diving-PAM (Walz GmbH, Effeltrich, Germany) 5-mm-diameter fiber-optic probe as described (Innis et al. 2021). Corals were photographed after the heat treatment period, 10 days into initial recovery, and at the end of the initial recovery period (Olympus Tough TG-4, Tokyo, Japan), and coral color was quantified as the measured red channel intensity relative to a color standard (DKC-Pro White Balance & Color Calibration Chart, DGK Color Tools, Boston MA, USA; Genuine WhiBal, Michael Tapes Design, USA). At each sampling point (acute stress and initial recovery), oxygen evolution of 20 fragments (N = 10 from each temperature history) was measured to calculate photosynthetic rate and light-enhanced dark respiration rate (LEDR) as previously described (Innis et al. 2021) (Fig. S2). Fragments were chosen haphazardly so that each coral genet under both temperature regimes was represented.

2.4 Stable isotope tracer experiment

After each respirometry experiment, those 10 fragments per temperature per time point (N = 40 fragments total) were placed upright on a rack in clear 10-gallon tubs of filtered seawater with pumps for circulation. 99% $NaH^{13}CO_3$ (Cambridge Isotope Labs, Tewksbury MA, USA) was added to non-limiting concentrations (31.2 µM; atom % enrichment $NaH^{13}CO_3 = 2.678\%$). During this pulse, tubs were covered with plastic wrap to minimize $^{13}CO_2$ offgassing and maintained at their experimental temperatures using an external water bath. After 7 h in the light to incorporate the tracer, fragments were rinsed in a bucket of clean seawater and transferred to a darkened flow-through tank for a 12-h chase period. Corals were then snap-frozen at -80ºC until further processing.

2.5 Physiological analyses

The 40 pulse-chase fragments were thawed on ice and airbrushed using filtered seawater (FSW) to remove all tissue. The resulting slurry was homogenized at 25,000 rpm for 10 s using a tissue homogenizer (Fisherbrand 850 Homogenizer, Fisher Scientific, Waltham MA, USA) and aliquoted for subsequent assays. Symbiodiniaceae concentrations were determined in triplicate within 6 h of airbrushing using a flow-cytometer (Guava easyCyte 5HT, Luminex, Austin TX, USA) as described (Krediet et al. 2015; Innis et al. 2021). If symbiont density could not be measured on the day of airbrushing, it was measured using a hemocytometer to avoid undercounting after chlorophyll deterioration. Chlorophyll *a* and c_2 were extracted in acetone and measured on a spectrophotometer (BioTek PowerWave XS2, Agilent, Santa Clara CA, USA), and total concentrations were calculated (Jeffrey and Humphrey 1975) all as described (Innis et al. 2021). Protein concentration in the host fraction was measured on a spectrophotometer (BioTek ELx808, Agilent) using the Bradford method. Total host lipids were measured colorimetrically on a spectrophotometer (BioTek PowerWave XS2, Agilent) as described (Baumann et al. 2021). Surface area was determined by the single wax dipping method (Veal et al. 2010).

For ^{13}C analysis, sample homogenates were separated by differential centrifugation into coral and dinoflagellate fractions at 7000 × g for 5 min. The supernatant was removed as the host fraction, after which the pellet was washed in FSW and spun down again. The supernatant was again added to the host fraction. The symbiont fraction was then resuspended in FSW. Separated samples were then dried to stable weight (48 h at 50ºC), enclosed tightly in tin capsules (EA Consumables, Marlton, NJ), and sent for ^{13}C enrichment analysis by continuous-flow Isotope-Ratio Mass Spectrometry (UC-Davis Stable Isotope Facility, Davis CA, USA). Two technical replicates were included for eight symbiont and host fractions. ^{13}C atom percent excess (APE) was calculated by subtracting published baseline non-enriched Kane'ohe Bay *M. capitata* summer mean ^{13}C atom-% (host mean at-% = 1.0888, SD = 0.0012; symbiont mean at-% = 1.0887, SD = 0.0012; Wall et al. 2020) from our enriched ^{13}C atom-% values (host mean at-% = 1.0989, SD = 0.0036; symbiont mean at-% = 1.121866, SD = 0.0106) (Slater et al. 2001).

2.6 Coral intracellular pH measurements

For the initial recovery time point, one fragment per genet within each treatment was used to measure intracellular pH (pH_i). Coral cells were isolated from each fragment and loaded with the pH-sensitive dye SNARF1-AM (Thermo Fisher Scientific) as described (Barott et al. 2017; Innis et al. 2021; Brown et al. 2022). Cells were pelleted, resuspended in filtered seawater, and imaged at 25ºC in a glass-bottomed dish using an inverted confocal microscope (LSM 710, Zeiss, Oberkochen, Germany) with image acquisition settings identical to ref. (Innis et al. 2021). At least eight gastrodermal cells containing dinoflagellate symbionts (symbiocytes) were

imaged from each coral. Corals lacking sufficient stained symbiocytes due to bleaching were excluded from the dataset. SNARF-1 fluorescence ratios within coral cytoplasm were quantified in ImageJ, normalized to background fluorescence, and converted to pH using a species-specific calibration curve generated on the same microscope, as described ((Innis et al. 2021); Fig. S2).

2.7 Statistical analysis

To test whether heat treatment caused bleaching, we modeled coral color with time point and treatment temperature history as fixed effects and genet as a random intercept (Fig. 1C) using a linear model followed by pairwise comparison of estimated marginal means using the *emmeans* package (Lenth et al. 2017) with Tukey HSD adjust p-values. This model was selected by comparison with several other models using corrected Akaike Information Criterion weights (*MuMIn* package (Barton 2015)). To test how corals responded to heat treatment, we generated linear mixed effects models (*lme4* package (Bates et al. 2014)) for each physiological variable as a function of symbiont species (C and D) and treatment temperature history (28, 31), again using genet as a random intercept. To avoid overfitting models given our population size, we generated separate models for both the acute and initial recovery experimental timepoints (Fig. 1, Table S3). To assess coral health over time, we modeled each physiological variable as a function of symbiont species and timepoint (acute stress and initial recovery), with genet as a random effect, generating separate models for both temperature histories (Fig. 1, Table S4). We examined each model's Q-Q and residual plots to check that model residuals met assumptions of normality and constant variance. Where data were heavy-tailed, we used the *LambertW* package to Gaussianize the distribution. Significance of fixed effects and their interactions were determined using Satterthwaite's Type III ANOVA. To explore whether coral accumulation of symbiotic photosynthate affects host physiological function across treatments, we ran linear regressions (Pearson's product-moment correlation) comparing host photosynthate accumulation with intracellular pH and calcification. To explore how symbiont population dynamics and energy provisioning affected coral physiology, we modeled relationships between symbiont population traits and host physiology across treatments using either linear regressions, generalized additive models, or general linear models with quasibinomial distribution (where host response variables followed a non-linear distribution). All analyses were run in RStudio version 4.0.2 (R Core Team 2012) and graphs were produced using the package ggplot2 (Wickham 2016).

3 Results and discussion

3.1 Coral symbiont thermotolerance confers no nutritional advantage to hosts during heat treatment

Experimental heat treatment caused coral bleaching symptoms and colony stress regardless of symbiont species (Figs. 1B-C, 2). Coral color decreased significantly in heat-treated corals relative to ambient corals at all time points following heat treatment (Tukey HSD, $p < 0.01$) (Fig. 1C). Symbiont photochemical efficiency ($p < 0.0001$) and chlorophyll *a* concentration ($p = 0.002$) also decreased for heat-treated corals relative to ambient, confirming that bleaching occurred, and heat-treated corals also displayed lower light sensitivity of photosynthesis (α) (Fig. 2A, C, E; $p = 0.011$). Although heat treatment did not significantly decrease symbiont cell density ($p = 0.096$, Fig. 2B), gross photosynthesis ($p = 0.178$, Fig. 2D), or fixed carbon incorporated into the symbiont fraction (^{13}C atom % excess, APE) ($p = 0.095$, Fig. 2F), heated corals assimilated less photosynthetically fixed carbon ($p < 0.001$; Fig. 2K). This deficiency signals lower photosynthate translocation from the symbiont population to the coral host after heat stress, consistent with previous empirical (Tremblay et al. 2016; Baker et al. 2018; Rädecker et al. 2021) and theoretical (Detmer et al. 2022) studies. Possibly as a result, heat-treated coral colonies also calcified less than ambient corals ($p = 0.044$, Fig. 2J) (Fig. 2B). However, regardless of treatment, corals hosting *Cladocopium* C31 (hereafter C-colonies) incorporated more fixed carbon overall than corals hosting *Durusdinium glynnii* (hereafter D-colonies) ($p = 0.018$; Fig. 2K). This was surprising given that carbon benefits to corals hosting *Cladocopium* spp. can be lost at higher temperatures in other host species (Cunning et al. 2015a). Our results suggest that during the early stages of heat stress, *M. capitata* hosting *Cladocopium* C31 retain a photosynthate incorporation advantage over D-colonies.

3.2 Health of host and symbiont population are uncoupled during initial bleaching recovery

Cladocopium C31 continued to decline in photochemical efficiency and cell density one month after cessation of heat stress (F_v/F_m ~ Timepoint * Symbiont, $p = 0.046$; Symbiont Density ~ Timepoint * Symbiont, $p = 0.034$; Fig. 3A-B), and heat-stressed C-colonies still had fewer symbionts (Temperature * Symbiont, $p = 0.014$) and less chlorophyll (Temperature * Symbiont, $p = 0.008$) than ambient C-colonies (Fig. S3B-C). However, while *D. glynnii* maintained steady photochemical efficiency and

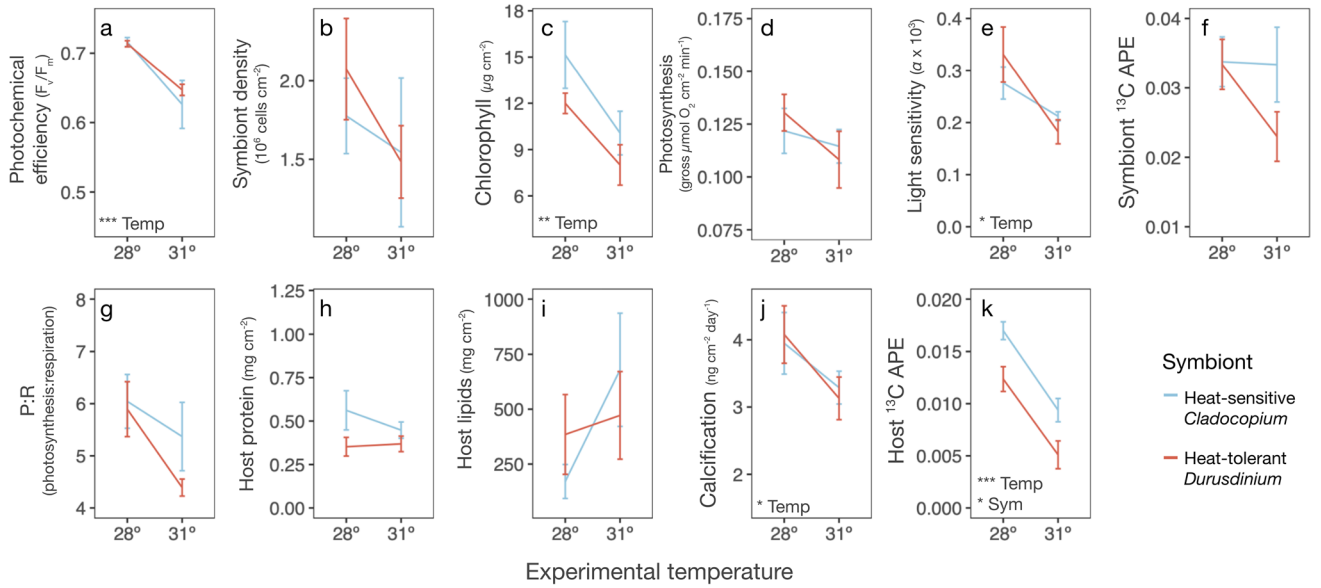

Fig. 2 Experimental heating caused bleaching and physiological stress in *Montipora capitata* regardless of symbiont association. **A-F)** Heat decreased symbiont photochemical efficiency (F_v/F_m) **(A)**, chlorophyll **(C)**, and light sensitivity (*α*) **(E)** though it did not affect symbiont areal density **(B)**, gross photosynthesis **(D)**, or net carbon fixation **(F)**. **G-K)** Heat decreased coral calcification **(J)** and net fixed carbon assimilation (^{13}C atom %) **(K)** although it did not affect photosynthesis:respiration **(G)**, or total host protein **(H)** or lipids **(I)**. Coral photosynthate assimilation (host.^{13}C atom-% excess, APE) also was higher for C-corals than for D-corals regardless of temperature **(K)**. Error bars = SEM. N = 5 genets per group. Insets show results of linear mixed effects models with temperature (Temp) and symbiont (Sym), with coral genet as a random intercept (* = p < 0.05, ** = p < 0.01, *** = p < 0.001). Full model results are reported in Table S3

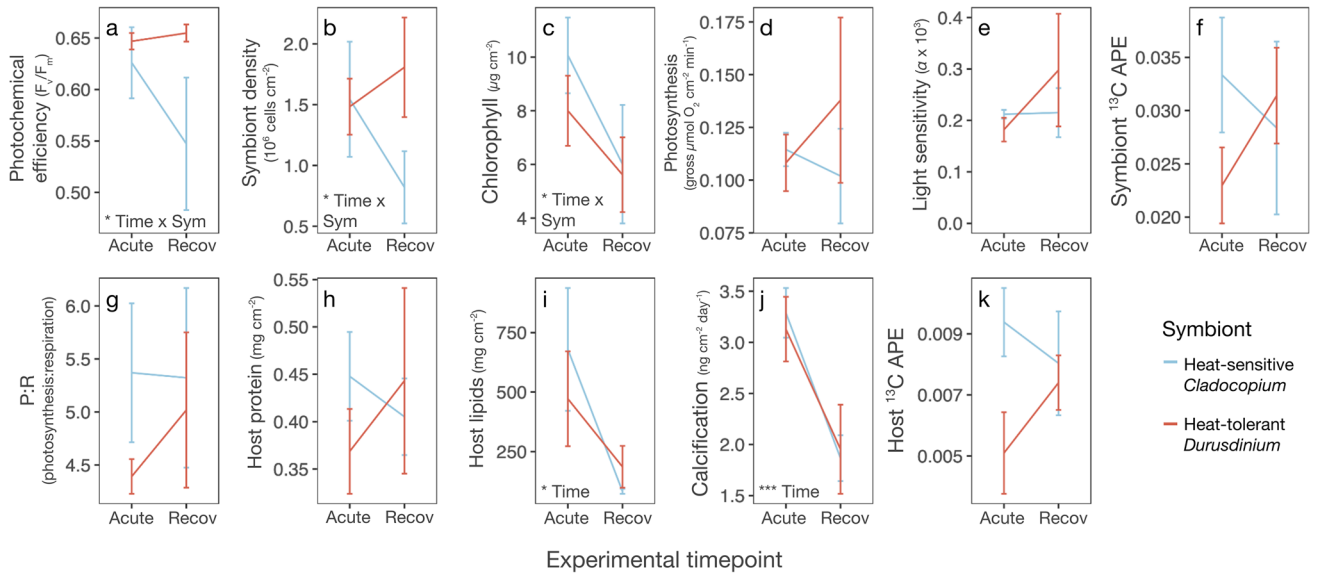

Fig. 3 Symbiont and host physiology trajectories are uncoupled during the month following heat stress. **A-F)** Thermotolerant and thermosensitive symbionts responded differently after heat stress: thermotolerant *Durusdinium* retained or improved photochemical efficiency (F_v/F_m) **(A)** and symbiont density **(B)** over initial recovery ('Recov') after acute stress ('Stress'), whereas thermosensitive *Cladocopium* continued to lose both. *Cladocopium* also lost more chlorophyll **(C)** than *Durusdinium* did. Gross photosynthesis **(D)**, light sensitivity of photosynthesis **(E)**, and symbiont carbon fixation **(F)** were unaffected by timepoint or symbiont species. **G-K)** *Durusdinium* thermotolerance did not improve host physiology following heat stress: symbiont species did not affect photosynthesis:respiration **(G)**, total host protein **(H)** or lipids **(I)**, or calcification **(J)**, or photosynthate assimilation **(K)**. Error bars = SEM. N = 5 genets per group. Insets show results of linear mixed effects models with time point (Time) and symbiont (Symb), with coral genet as a random intercept (* = p < 0.05, ** = p < 0.01, *** = p < 0.001). Full model results reported in Table S4

began returning to normal cell densities within the host at this same time point, this early *D. glynnii* recovery did not confer D-colonies greater P:R, protein, lipids, calcification, or host carbon assimilation relative to C-colonies ($p \geq 0.210$; Figs. 3G-K, S3G-K). Instead, neither group of corals had recovered to their initial growth or carbon assimilation rates at this time, suggesting that 4 weeks is still early in recovery. These results also contradict our hypothesis that the faster-recovering *D. glynnii* offers corals an advantage after heat stress relative to those hosting *Cladocopium* C31.

Delayed host recovery following the end of heat stress is consistent with observations that Symbiodiniadeae populations recover faster than the host during the initial stages of bleaching recovery (Fitt et al. 1993, 2000; Levas et al. 2018). In our study, host lipids continued to deplete after heat stress abated ($p = 0.027$, Fig. 3I), which is consistent with prior evidence that corals remain energetically limited during bleaching recovery (Leinbach et al. 2021), and in particular that *M. capitata* catabolize lipids to survive bleaching (Grottoli and Rodrigues 2011) and require at least 6 weeks to replenish energy reserves (Rodrigues and Grottoli 2007). Given that *M. capitata* can also compensate for lost symbionts by increasing heterotrophy (Grottoli et al. 2006; Hughes and Grottoli 2013), the lack of feeding during the experiment may have contributed to their slow early recovery. Furthermore, even ambient-treated corals experienced physiological stress in our mesocosms, displaying declines in photochemical efficiency ($p < 0.0001$, Fig. S4A), chlorophyll concentration ($p = 0.002$, Fig. S4C), calcification rates ($p < 0.0001$, Fig. S4J), and host carbon assimilation ($p < 0.001$, Fig. S4K), possibly due to sub-bleaching heat stress in situ prior to sampling and/or during the experiment: Kāne'ohe Bay was already anomalously warm by the time of collection in June 2019 (Dilworth et al. 2021; Innis et al. 2021), causing ambient tank temperatures to peak above 29ºC (these corals' projected bleaching threshold) several times during the experiment (Fig. 1A). However, as all corals were exposed to these conditions, they do not alter our result that faster-recovering *D. glynnii* symbionts were not sufficient to restore *M. capitata* carbon assimilation, biomass, or skeletal growth rates after early bleaching.

3.3 Coral intracellular pH and calcification depend on symbiotic benefits

The effect of heat stress on coral intracellular pH (pH_i) depended on symbiont species. Specifically, D-colonies recovering from heat stress exhibited decreased pH_i relative to ambient corals ($p = 0.018$; Fig. 4A). This is consistent with evidence from other marine invertebrates that metabolic depression following thermal stress decreases H^+ extrusion, leading to intracellular acidification (Pörtner and Bock 2000; Pörtner 2008). However, C-colonies did not exhibit acidification, and instead maintained normal pH_i during early bleaching recovery (Temperature * Symbiont, $p = 0.036$; Fig. 4A). Since D-colonies tend to be more bleaching-resistant (Cunning et al. 2016; Silverstein et al. 2017; Matsuda et al. 2020), and coral species' bleaching susceptibility predicts the degree of pH_i dysregulation after heat

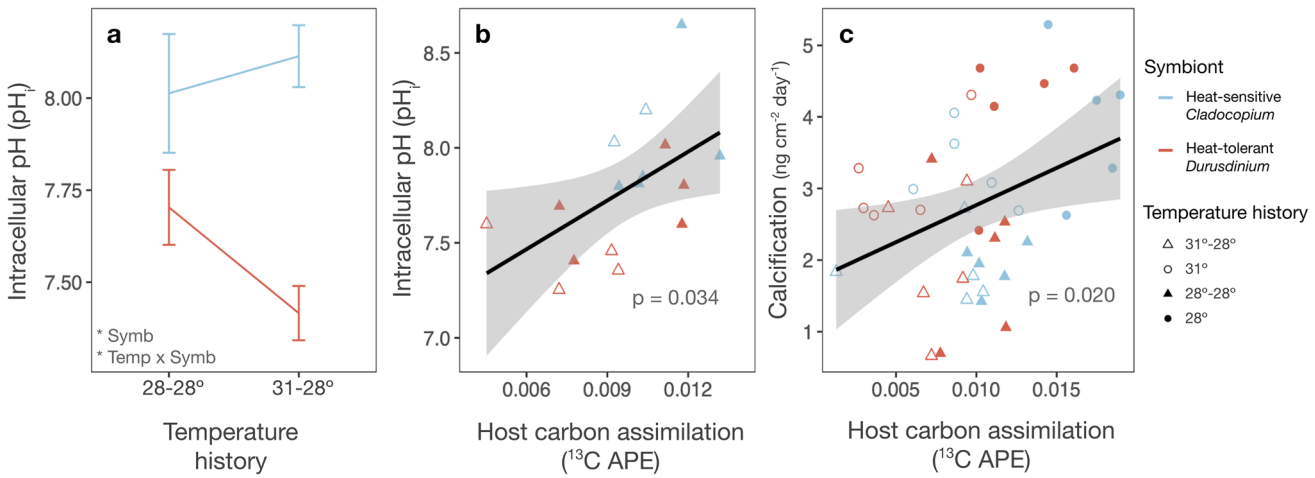

Fig. 4 Assimilated carbon from symbiosis predicts coral calcification and intracellular pH after heat stress. **A)** Symbiont thermotolerance affects how temperature alters coral symbiocyte intracellular pH (pH_i) during bleaching recovery. Inset shows result of linear mixed effects model with symbiont species (Symb) and temperature treatment (Temp), with coral genet as a random intercept (*=$p < 0.05$). Error bars = SEM. Full model results reported in Table S3. **B)** Host fixed carbon assimilation is positively correlated with symbiocyte pH_i. Gray line, linear correlation; inset, linear regression p-value ($t = 2.3506$, $df = 14$, $R^2 = 0.2830$). **C)** Host fixed carbon assimilation predicts colony calcification rates. inset, linear correlation p-value ($t = 2.438$, $df = 35$, $R^2 = 0.1452$). Black lines show linear regressions; gray area = 95% confidence interval

stress (Gibbin et al. 2015), we had expected D-colonies' pH_i to be more resilient to heat treatment. Instead, C-colonies in this study may have had a pH_i regulatory advantage over D-colonies because *Cladocopium* C31 continued translocating more fixed carbon than *D. glynni* to their hosts despite acute heat stress (Fig. 2K), providing the host with the energy needed to maintain acid–base homeostasis. Indeed, we found that host carbon assimilation from the symbionts was positively correlated with intracellular pH (pH_i) across experimental groups ($R^2 = 0.283$, $p = 0.034$; Fig. 4B), suggesting corals deriving more energy from symbiosis are better able to maintain acid–base homeostasis. While it is also possible that endosymbiont metabolism led to pH_i differences, as dinoflagellate photosynthesis can increase host cell pH_i (Laurent et al. 2013; Gibbin et al. 2014; Gibbin and Davy 2014; Barott et al. 2017), corals in this experiment were dark-adapted prior to measuring pH_i, minimizing any residual differences in pCO_2 and pH_i due to symbiont photosynthesis. In addition, there was no correlation between per-alga photosynthetic rate and host pH_i (Fig. S5). Therefore, D-colony cellular acidification following heat stress was more likely a result of organismal-level deficiencies in energy provisioning.

Interestingly, the greater acidification we observed here in D-colonies is inverse to the pattern observed for these same corals during an in situ marine heatwave, when C-colonies had more acidic pH_i than D-colonies (Innis et al. 2021). We attribute this discrepancy to the greater severity of bleaching during the heatwave than in the present study: C-colonies had 54.3% fewer symbionts (374,806 ± 85,644 cells cm^{-2}) at peak bleaching in situ than did heat-treated C-corals at the end of this experiment (820,710 ± 296,699 cells cm^{-2}), likely due to the greater duration of heat stress (~ 3 months in the field vs. 14 days in our mesocosms). In contrast, in situ D-colonies still had 864,905 ± 98,103 cells cm^{-2} at the peak of bleaching (Innis et al. 2021). The greater severity of symbiont loss in C-colonies in situ thus likely erased any energetic advantage they had during early heat stress over D-colonies. Therefore, the relative benefits of hosting certain symbionts clearly depends on both the duration and severity of heat stress. Additional studies directly relating photosynthate assimilation to coral pH_i regulation are necessary to determine how this fundamental cellular process will respond to increasingly frequent and severe marine heatwaves.

Similar to pH_i, coral calcification rate was directly related to host photosynthate assimilation ($p = 0.020$, $R^2 = 0.134$, Fig. 4C), suggesting that corals receiving more carbon from their symbionts can maintain higher skeletal growth rates. However, despite the parallel correlation between symbiocyte pH_i and host photosynthate assimilation (Fig. 4B), coral calcification was not related to pH_i ($p = 0.792$, $R^2 = 0.005$, Fig. S6). This was surprising, because corals use similar mechanisms to control pH_i (Barott et al. 2017) and pH of the extracellular calcifying medium (pH_{ECM}) (Barott et al. 2020), and we had expected corals with acidified pH_i to be less able to maintain alkaline pH_{ECM} and thus calcification. One possibility is that because thermal stress decreases marine invertebrate cellular H^+ efflux (Pörtner and Bock 2000), heat treatment might have led to accumulation of protons within host cells specifically, decreasing pH_i without affecting extracellular pH. Alternatively, since these corals were early in recovery (Rodrigues and Grottoli 2007; Leinbach et al. 2021) and still showing signs of accumulated metabolic stress (Fig. 3I-J), they likely had limited available ATP for pH regulation to simultaneously maintain homeostasis (pH_i) and elevated pH_{ECM} for calcification. Corals might thus prioritize maintaining pH_i at the expense of pH_{ECM} during bleaching recovery, perhaps by altering ion transporter activity, expression or localization (Barott et al. 2015a), complicating between-colony comparisons of total pH regulatory capacity. This 'preferential pH regulation' has been observed in air-breathing fishes that rapidly regulate pH_i in response to CO_2 stress while allowing extracellular pH to remain acidic (Harter et al. 2014; Shartau et al. 2020), although further research is needed to explore the possibility that cnidarians also use preferential pH_i regulation to balance the multiple acid–base challenges they face, including dinoflagellate photosynthesis, cellular respiration, environmental acidification, and calcification (Putnam & Barott et al. 2017).

3.4 Symbiont growth-mutualism tradeoff

Surprisingly, dinoflagellate density within host tissue failed to predict host physiology across Symbiodiniaceae species and temperature treatments. We originally hypothesized that corals retaining more symbionts during and after heat stress would assimilate more photosynthate (Hughes and Grottoli 2013). Because symbiont photosynthesis provides most of these corals' energy (Wall et al. 2020), we tested for correlations between symbiont cell density and host performance. Higher symbiont density did not correlate with greater host carbon assimilation ($R^2 = 0.030$, $p = 0.289$; Fig. 5A), lipid content ($p = 0.292$; Fig. S8A) or calcification rate ($R^2 = 0.088$, $p = 0.064$; Fig. S8B). Instead, Symbiodiniaceae cell density was directly correlated with the amount of labeled carbon that symbionts retained instead of translocating to the host ($p = 0.017$, $R^2 = 0.141$; Fig. 5B), a measure of symbiont photosynthate 'selfishness' (Baker et al. 2018). We obtained a similar result calculating symbiont cell density areally ($p = 0.012$, Fig. S7A), confirming that the effect was not an artifact of differences in host protein content (Cunning and Baker 2012, 2014) and that the amount of photosynthate retained by a symbiont population was directly related to its density. It is also possible that that higher-density symbiont populations translocated less

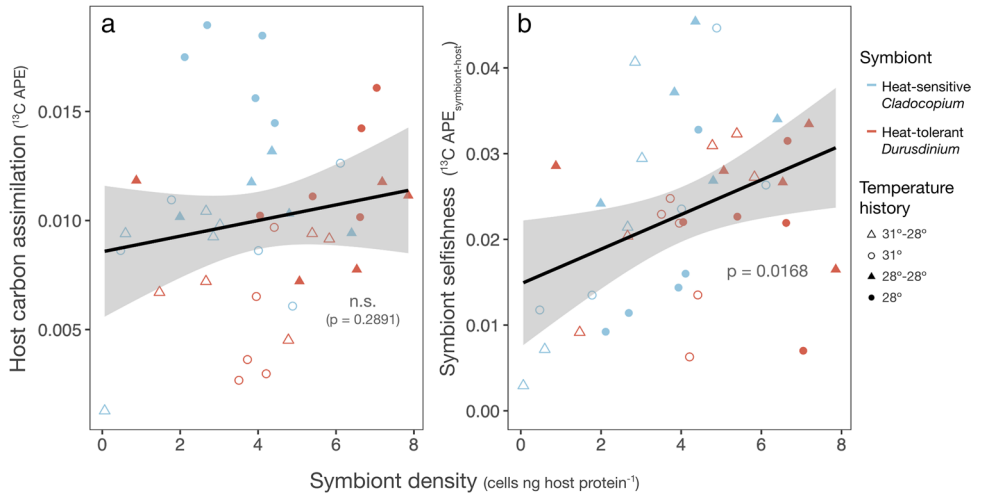

Fig. 5 During and after heat stress, symbiont population growth increases selfishness. **A)** Symbiont density in host tissue does not predict host fixed carbon accumulation. Inset shows result of linear regression ($t = 1.075$, $df = 38$, $R^2 = 0.0295$). **B)** Symbiont density is positively correlated with selfishness. Selfishness was calculated by subtracting ^{13}C enrichment (^{13}C APE) in the host fraction from ^{13}C APE in the symbiont. Inset shows result of linear regression ($t = 2.5018$, $df = 38$, $R^2 = 0.1414$). Black lines show linear regressions; gray areas = 95% confidence intervals

fixed carbon in response to symbiont self-shading (Hoogenboom et al. 2010; Cunning et al. 2015b, 2017). However, light-limited symbionts typically upregulate chlorophyll to compensate (Falkowski and Dubinsky 1981; Iglesias-Prieto and Trench 1997), and we instead found that per-symbiont chlorophyll was lowest at high symbiont densities ($p < 0.0001$; Fig. S8C). Furthermore, colony gross photosynthesis was not sufficient to predict coral carbon assimilation ($R^2 = 0.003$, $p = 0.743$, Fig. S9), so differences in total symbiont productivity cannot account for symbiont cell density increasing photosynthate retention. Another alternative is that corals hosting more symbionts respired more during the pulse-chase experiment (e.g. (Hoogenboom et al. 2010; Cunning and Baker 2014; Cunning et al. 2015b, 2017); but see (Krueger et al. 2020)), resulting in lower host carbon assimilation. But while total colony respiration increased with symbiont density ($R^2 = 0.107$, $p = 0.042$; Fig. S8D), we found no correlation between total colony respiration and photosynthate retained by dinoflagellates ($R^2 = 0.012$, $p = 0.517$, Fig. S7B), indicating the density-photosynthate retention relationship is not simply because increased respiratory burden decreases host carbon assimilation. Finally, symbionts at higher densities kept a larger proportion of the total assimilated photosynthate than those at lower densities ($p = 0.039$, Fig. S10). Therefore, symbionts at higher densities sequestered a greater share of the carbon they fixed.

Our finding that multiple species of Symbiodiniaceae retain more (Fig. 5B) and a greater proportion (Fig. S10) of photosynthate at high cell densities (Fig. 5B) is consistent with theory suggesting that symbionts remaining *in hospite* after coral bleaching can adjust their energy allocation in response to changes in density (Wooldridge 2013). If Symbiodiniaceae can divert photosynthate from the mutualism to their own cell division, recovering to normal dinoflagellate density may prompt a corresponding decrease in mutualistic behavior (Fig. 6). At ambient conditions, corals invest ATP in regulating inorganic nitrogen and carbon inside the symbiosome to promote algal photosynthesis (Wooldridge 2009; Barott et al. 2015b; Thies et al. 2022), while the symbiont translocates most resulting photosynthate back to the coral (Muscatine and Porter 1977) (Fig. 6A). After bleaching, however, algae appear to prioritize their own recovery by translocating less fixed carbon to the coral host (Figs. 2K, 5B, 6B). Such a change in resource allocation coming at the expense of the coral could explain why energetic losses from bleaching can persist for months (e.g. (Leinbach et al. 2021)), and why sexual reproduction (a costly process) can suffer even years after colonies regain normal symbiont populations (e.g. (Levitan et al. 2014; Johnston et al. 2020)).

While some symbionts are known to provide less mutualistic benefit at high densities (e.g. in the Branchiobdellid-crayfish cleaning symbiosis (Brown et al. 2012)), this study is to our knowledge the first evidence suggesting a mutualistic nutritional endosymbiont could maintain or achieve higher densities during stress by diverting resources away from cooperation. It may also help explain observations that corals benefit most from intermediate symbiont densities (Hoogenboom et al. 2010; Cunning and Baker 2014; Cunning et al. 2015b). Existing symbiont-density-dependent coral bleaching frameworks focus mainly on symbiont light- and CO_2-limitation and increased respiratory costs at high densities; they assume each symbiont population translocates a constant proportion of the photosynthate they fix.

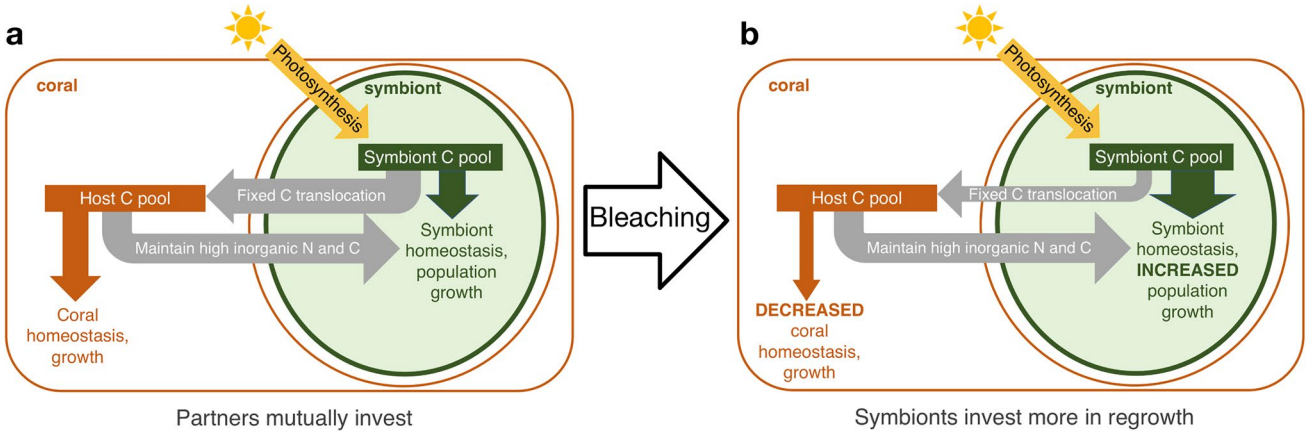

Fig. 6 Model of symbiont resource reallocation. **A)** Without stress, coral investment in symbiont photosynthesis and growth (lower gray arrow) produces returns in photosynthetically fixed carbon translocated to the coral (top gray arrow). **B)** After a bleaching event reduces symbiont population density, symbionts may invest in their own cell division and regrowth (larger green arrow) at the expense of translocating fixed carbon to the coral (smaller gray arrow). The coral is left with fewer resources (smaller orange box) for its own growth and recovery (smaller orange arrow) while maintaining a smaller but growing symbiont population (gray arrow)

Our results instead add to the evidence that corals' optimal symbiont abundance also depends on how the algae allocate their fixed carbon (e.g. (Rädecker et al. 2021; Detmer et al. 2022)). Therefore, symbiont nutrient exchange strategy may itself be a dynamic regulator of bleaching susceptibility. High symbiont cell densities can increase bleaching risk (e.g. (Cunning and Baker 2012; Cornwell et al. 2021); but see (Hoadley et al. 2021)), a phenomenon thought to result from increased reactive oxygen species accumulation following symbiont photostress (e.g. (Nesa and Hidaka 2009; Wooldridge 2009; Silverstein et al. 2015)). However, heat can cause bleaching in cnidarians without increases in reactive oxygen (Dungan et al. 2022), oxidative damage (Nielsen et al. 2018), or even photostress (Tolleter et al. 2013). The positive relationship we found between symbiont density and photosynthate retention provides an alternative mechanism by which large symbiont populations could increase bleaching likelihood when bleaching is frequent: diminished but fast-regrowing symbiont populations may leave recovering corals carbon-deprived, poorly equipped to face the energetic demands of heat stress, and thus even more vulnerable to the carbon limitation theorized to cause bleaching (Wooldridge 2009, 2013; Cunning et al. 2017; Rädecker et al. 2021; Detmer et al. 2022). Future bleaching recovery research should correlate symbiont in vivo regrowth rates with carbon translocation to better validate this framework.

3.5 Implications for holobiont survival under climate change

Though immediately costly to the coral, subsidizing dinoflagellate regrowth could be a viable strategy for long-term fitness: the faster a host regains equilibrium symbiont density, the sooner it will restore its full autotrophic potential (e.g. (Hughes and Grottoli 2013)). However, with some reefs predicted to bleach annually by 2043 under the current emissions scenario (van Hooidonk et al. 2016), even corals that recover all their symbionts quickly after bleaching (e.g. (Rodrigues and Grottoli 2007; Matsuda et al. 2020)) may no longer be able to sustain stressful cycles of symbiont regrowth, complicating attempts to predict which corals could be 'climate-proof' (Grottoli et al. 2014; Putnam et al. 2017; Morikawa and Palumbi 2019; Drury et al. 2022; Brown and Barott 2022). It is therefore crucial to prioritize resilient and sustainable mutualism function in research aiming to support coral conservation. While assisted evolution of Symbiodiniaceae in vitro to increase thermotolerance (e.g. (Chakravarti and van Oppen 2018)) or colonization (e.g. (Buerger et al. 2020)) is a promising new approach, future work should test that the experimentally evolved algae still provide enough nutritional benefits to the coral host. In fact, artificial selection for endosymbiont mutualistic traits can endanger mutualism stability when selection is performed outside of the host (Morran et al. 2016). As the most thermotolerant coral holobionts appear to rely on extensive coral-dinoflagellate coevolution rather than symbiont shuffling (Howells et al. 2020), future coral bleaching recovery studies must go beyond measuring dinoflagellate growth and instead measure partner traits that favor the functional stability of this critical mutualism (van Woesik et al. 2022).

More broadly, predicting mutualisms' resilience to climate change requires understanding the actual function of the interaction under stress. We found that deficiencies in symbiotic nutrient exchange uncouple partner recovery trajectories during mutualism reestablishment. Researchers must therefore distinguish between the separate processes of symbiont regrowth and mutualism recovery when one

or more partners can withhold resources to favor reproduction over cooperation. Finally, reestablishing a beneficial endosymbiosis can itself be costly for holobionts surviving stress, highlighting the need for more mechanistic study of how mutualisms recover from stress in our current era of unprecedented environmental change.

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Author contributions L.A.-W. and K.L.B. conceived and designed the study. L.A.-W. collected samples, prepared materials, collected and analyzed data, and wrote the first draft of the manuscript. K.L.B. collected and analyzed data, and edited and approved the final manuscript.

Data availability Data and R code are available on GitHub at github.com/allenwaller/MontiporaSymbStrategy.

Declarations

Conflicts of interest The authors declare that we have no conflicts of interest.

References

Baker DM, Freeman CJ, Wong JCY et al (2018) Climate change promotes parasitism in a coral symbiosis. ISME J 12:921–930

Barott KL, Barron ME, Tresguerres M (2017) Identification of a molecular pH sensor in coral. Proc Biol Sci 284. https://doi.org/10.1098/rspb.2017.1769

Barott KL, Perez SO, Linsmayer LB, Tresguerres M (2015a) Differential localization of ion transporters suggests distinct cellular mechanisms for calcification and photosynthesis between two coral species. Am J Physiol Regul Integr Comp Physiol 309:R235–R246

Barott KL, Venn AA, Perez SO et al (2015b) Coral host cells acidify symbiotic algal microenvironment to promote photosynthesis. Proc Natl Acad Sci U S A 112:607–612

Barott KL, Venn AA, Thies AB et al (2020) Regulation of coral calcification by the acid-base sensing enzyme soluble adenylyl cyclase. Biochem Biophys Res Commun 525:576–580

Barton K (2015) MuMIn: Multi-Model Inference. R package version 1.47.1

Bates D, Mächler M, Bolker B, Walker S (2014) Fitting linear mixed-Effects models using lme4. J Stat Softw 67:1–48

Baumann JH, Bove CB, Carne L et al (2021) Two offshore coral species show greater acclimatization capacity to environmental variation than nearshore counterparts in southern Belize. Coral Reefs 40:1181–1194

Bénard A, Vavre F, Kremer N (2020) Stress & symbiosis: heads or tails? Front Ecol Evol 8. https://doi.org/10.3389/fevo.2020.00167

Berkelmans R, van Oppen MJH (2006) The role of zooxanthellae in the thermal tolerance of corals: a "nugget of hope" for coral reefs in an era of climate change. Proc Biol Sci 273:2305–2312

Bronstein JL (1994) Conditional outcomes in mutualistic interactions. Trends Ecol Evol 9:214–217

Brown BL, Creed RP, Skelton J et al (2012) The fine line between mutualism and parasitism: complex effects in a cleaning symbiosis demonstrated by multiple field experiments. Oecologia 170:199–207

Brown KT, Barott KL (2022) The costs and benefits of environmental memory for reef-building corals coping with recurring marine heatwaves. Integr Comp Biol. https://doi.org/10.1093/icb/icac074

Brown KT, Mello-Athayde MA, Sampayo EM et al (2022) Environmental memory gained from exposure to extreme pCO_2 variability promotes coral cellular acid–base homeostasis. Proc R Soc B Biol Sci 289:0220941

Buerger P, Alvarez-Roa C, Coppin CW et al (2020) Heat-evolved microalgal symbionts increase coral bleaching tolerance. Sci Adv 6:2498

Carballo-Bolaños R, Denis V, Huang Y-Y et al (2019) Temporal variation and photochemical efficiency of species in Symbiodinaceae associated with coral Leptoria phrygia (Scleractinia; Merulinidae) exposed to contrasting temperature regimes. PLoS One 14:e0218801

Chakravarti LJ, van Oppen MJH (2018) Experimental evolution in coral photosymbionts as a tool to increase thermal tolerance. Front Mar Sci 5. https://doi.org/10.3389/fmars.2018.00227

Chamberlain SA, Bronstein JL, Rudgers JA (2014) How context dependent are species interactions? Ecol Lett 17:881–890

Cheplick GP (2007) Costs of fungal endophyte infection in Lolium perenne genotypes from Eurasia and North Africa under extreme resource limitation. Environ Exp Bot 60:202–210

Claar DC, Starko S, Tietjen KL et al (2020) Dynamic symbioses reveal pathways to coral survival through prolonged heatwaves. Nat Commun 11:6097

Cornwell B, Armstrong K, Walker NS et al (2021) Widespread variation in heat tolerance and symbiont load are associated with growth tradeoffs in the coral Acropora hyacinthus in Palau. Elife 10. https://doi.org/10.7554/eLife.64790

Cunning R, Baker AC (2012) Excess algal symbionts increase the susceptibility of reef corals to bleaching. Nat Clim Chang 3:259–262

Cunning R, Baker AC (2014) Not just who, but how many: the importance of partner abundance in reef coral symbioses. Front Microbiol 5:400

Cunning R, Gillette P, Capo T et al (2015a) Growth tradeoffs associated with thermotolerant symbionts in the coral Pocillopora damicornis are lost in warmer oceans. Coral Reefs 34:155–160

Cunning R, Ritson-Williams R, Gates RD (2016) Patterns of bleaching and recovery of Montipora capitata in Kāne 'ohe Bay, Hawai 'i, USA. Mar Ecol Prog Ser 551:131–139

Cunning R, Muller EB, Gates RD, Nisbet RM (2017) A dynamic bioenergetic model for coral-Symbiodinium symbioses and coral bleaching as an alternate stable state. J Theor Biol 431:49–62

Cunning R, Silverstein RN, Baker AC (2018) Symbiont shuffling linked to differential photochemical dynamics of Symbiodinium in three Caribbean reef corals. Coral Reefs 37:145–152

Cunning R, Vaughan N, Gillette P et al (2015b) Dynamic regulation of partner abundance mediates response of reef coral symbioses to environmental change. Ecology 96:1411–1420

Detmer AR, Cunning R, Pfab F et al (2022) Fertilization by coral-dwelling fish promotes coral growth but can exacerbate bleaching response. J Theor Biol 541:111087
Dilworth J, Caruso C, Kahkejian VA et al (2021) Host genotype and stable differences in algal symbiont communities explain patterns of thermal stress response of Montipora capitata following thermal pre-exposure and across multiple bleaching events. Coral Reefs 40:151–163
Diniz-Filho JAF, Bini LM (2019) Will life find a way out? Evolutionary rescue and Darwinian adaptation to climate change. Perspect Ecol Conserv 17:117–121
Drury C, Martin RE, Knapp DE et al (2022) Ecosystem-scale mapping of coral species and thermal tolerance. Front Ecol Environ 20:285–291
Dunbar HE, Wilson ACC, Ferguson NR, Moran NA (2007) Aphid thermal tolerance is governed by a point mutation in bacterial symbionts. PLoS Biol 5:96
Dungan AM, Maire J, Perez-Gonzalez A et al (2022) Lack of evidence for the oxidative stress theory of bleaching in the sea anemone, Exaiptasia diaphana, under elevated temperature. Coral Reefs. https://doi.org/10.1007/s00338-022-02251-w
Falkowski PG, Dubinsky Z (1981) Light-shade adaptation of Stylophora pistillata, a hermatypic coral from the Gulf of Eilat. Nature 289:172–174
Fisher R, O'Leary RA, Low-Choy S et al (2015) Species richness on coral reefs and the pursuit of convergent global estimates. Curr Biol 25:500–505
Fitt WK, McFarland FK, Warner ME, Chilcoat GC (2000) Seasonal patterns of tissue biomass and densities of symbiotic dinoflagellates in reef corals and relation to coral bleaching. Limnol Oceanogr 45:677–685
Fitt WK, Spero HJ, Halas J et al (1993) Recovery of the coral Montastrea annularis in the Florida Keys after the 1987 Caribbean ?bleaching event? Coral Reefs 12:57–64
Frölicher TL, Fischer EM, Gruber N (2018) Marine heatwaves under global warming. Nature 560:360–364
Gibbin EM, Davy SK (2014) The photo-physiological response of a model cnidarian–dinoflagellate symbiosis to CO2-induced acidification at the cellular level. J Exp Mar Bio Ecol 457:1–7
Gibbin EM, Putnam HM, Davy SK, Gates RD (2014) Intracellular pH and its response to CO2-driven seawater acidification in symbiotic versus non-symbiotic coral cells. J Exp Biol 217:1963–1969
Gibbin EM, Putnam HM, Gates RD et al (2015) Species-specific differences in thermal tolerance may define susceptibility to intracellular acidosis in reef corals. Mar Biol 162:717–723
Gilbert SF, Sapp J, Tauber AI (2012) A symbiotic view of life: we have never been individuals. Q Rev Biol 87:325–341
Glynn PW (1996) Coral reef bleaching: facts, hypotheses and implications. Glob Chang Biol 2:495–509
Grottoli AG, Rodrigues LJ (2011) Bleached Porites compressa and Montipora capitata corals catabolize δ13C-enriched lipids. Coral Reefs 30. https://doi.org/10.1007/s00338-011-0756-0
Grottoli AG, Rodrigues LJ, Palardy JE (2006) Heterotrophic plasticity and resilience in bleached corals. Nature 440:1186–1189
Grottoli AG, Warner ME, Levas SJ (2014) The cumulative impact of annual coral bleaching can turn some coral species winners into losers. Glob Chang Biol 20:3823–3833
Harter TS, Shartau RB, Baker DW et al (2014) Preferential intracellular pH regulation represents a general pattern of pH homeostasis during acid–base disturbances in the armoured catfish, Pterygoplichthys pardalis. J Comp Physiol B 184:709–718
Hoadley KD, Pettay DT, Lewis A, Wham D (2021) Different functional traits among closely related algal symbionts dictate stress endurance for vital Indo-Pacific reef-building corals. Glob Chang Biol 27:5295–5309
Hoegh-Guldberg O, Mumby PJ, Hooten AJ et al (2007) Coral reefs under rapid climate change and ocean acidification. Science 318:1737–1742
Hoeksema JD, Bruna EM (2015) Context-dependent outcomes of mutualistic interactions. In: Mutualism. Oxford Academic, pp 181–202
Hoogenboom M, Beraud E, Ferrier-Pagès C (2010) Relationship between symbiont density and photosynthetic carbon acquisition in the temperate coral Cladocora caespitosa. Coral Reefs 29:21–29
Howells EJ, Bauman AG, Vaughan GO et al (2020) Corals in the hottest reefs in the world exhibit symbiont fidelity not flexibility. Mol Ecol 29:899–911
Hughes AD, Grottoli AG (2013) Heterotrophic compensation: a possible mechanism for resilience of coral reefs to global warming or a sign of prolonged stress? PLoS One 8:e81172
Hughes TP, Kerry JT, Álvarez-Noriega M et al (2017) Global warming and recurrent mass bleaching of corals. Nature 543:373–377
Iglesias-Prieto R, Trench RK (1997) Acclimation and adaptation to irradiance in symbiotic dinoflagellates. II. Response of chlorophyll-protein complexes to different photon-flux densities. Mar Biol 130:23–33
Innis T, Allen-Waller L, Brown KT et al (2021) Marine heatwaves depress metabolic activity and impair cellular acid-base homeostasis in reef-building corals regardless of bleaching susceptibility. Glob Chang Biol 27:2728–2743
Jeffrey SW, Humphrey GF (1975) New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. Biochem Physiol Pflanz 167:191–194
Johnston EC, Counsell CWW, Sale TL et al (2020) The legacy of stress: Coral bleaching impacts reproduction years later. Funct Ecol 34:2315–2325
Jones A, Berkelmans R (2010) Potential costs of acclimatization to a warmer climate: growth of a reef coral with heat tolerant vs. sensitive symbiont types. PLoS One 5:e10437
Jones RJ, Yellowlees D (1997) Regulation and control of intracellular algae (= zooxanthellae) in hard corals. Philos Trans R Soc Lond B Biol Sci 352:457–468
Kelly MW, Sanford E, Grosberg RK (2012) Limited potential for adaptation to climate change in a broadly distributed marine crustacean. Proc Biol Sci 279:349–356
Krediet CJ, DeNofrio JC, Caruso C et al (2015) Rapid, precise, and accurate counts of symbiodinium cells using the Guava Flow Cytometer, and a comparison to other methods. PLoS One 10:e0135725
Krueger T, Horwitz N, Bodin J et al (2020) Intracellular competition for nitrogen controls dinoflagellate population density in corals. Proc Biol Sci 287:20200049
LaJeunesse TC, Thornhill DJ (2011) Improved resolution of reef-coral endosymbiont (Symbiodinium) species diversity, ecology, and evolution through psbA non-coding region genotyping. PLoS One 6:e29013
LaJeunesse T, Thornhill D, Cox E et al (2004) High diversity and host specificity observed among symbiotic dinoflagellates in reef coral communities from Hawaii. Coral Reefs. https://doi.org/10.1007/s00338-004-0428-4
Laurent J, Tambutté S, Tambutté É et al (2013) The influence of photosynthesis on host intracellular pH in scleractinian corals. J Exp Biol 216:1398–1404
Leggat W, Heron SF, Fordyce A et al (2022) Experiment Degree Heating Week (eDHW) as a novel metric to reconcile and

validate past and future global coral bleaching studies. J Environ Manag 301:113919
Leggat WP, Camp EF, Suggett DJ et al (2019) Rapid coral decay is associated with marine heatwave mortality events on reefs. Curr Biol 29:2723-2730.e4
Leinbach SE, Speare KE, Rossin AM et al (2021) Energetic and reproductive costs of coral recovery in divergent bleaching responses. Sci Rep 11:23546
Lenth R, Singmann H, Love J et al (2017) Emmeans: Estimated marginal means, aka least-squares means. R package version 1.8.1-1
Lesser MP, Stat M, Gates RD (2013) The endosymbiotic dinoflagellates (Symbiodinium sp.) of corals are parasites and mutualists. Coral Reefs 32:603–611
Levas S, Schoepf V, Warner ME et al (2018) Long-term recovery of Caribbean corals from bleaching. J Exp Mar Biol Ecol 506:124–134
Levitan DR, Boudreau W, Jara J, Knowlton N (2014) Long-term reduced spawning in Orbicella coral species due to temperature stress. Mar Ecol Prog Ser 515:1–10
Little AF, van Oppen MJH, Willis BL (2004) Flexibility in algal endosymbioses shapes growth in reef corals. Science 304:1492–1494
Matsuda SB, Huffmyer AS, Lenz EA et al (2020) Coral bleaching susceptibility is predictive of subsequent mortality within but not between coral species. Front Ecol Evol 8. https://doi.org/10.3389/fevo.2020.00178
Matthews JL, Cunning R, Ritson-Williams R et al (2020) Metabolite pools of the reef building coral Montipora capitata are unaffected by Symbiodiniaceae community composition. Coral Reefs 39:1727–1737
McFall-Ngai M, Hadfield MG, Bosch TCG et al (2013) Animals in a bacterial world, a new imperative for the life sciences. Proc Natl Acad Sci USA 110:3229–3236
Morikawa MK, Palumbi SR (2019) Using naturally occurring climate resilient corals to construct bleaching-resistant nurseries. Proc Natl Acad Sci USA 116:10586–10591
Morran LT, Penley MJ, Byrd VS et al (2016) Nematode-bacteria mutualism: Selection within the mutualism supersedes selection outside of the mutualism. Evolution 70:687–695
Muscatine L, Porter JW (1977) Reef corals: Mutualistic symbioses adapted to nutrient-poor environments. Bioscience 27:454–460
Nesa B, Hidaka M (2009) High zooxanthella density shortens the survival time of coral cell aggregates under thermal stress. J Exp Mar Bio Ecol 368:81–87
Nielsen DA, Petrou K, Gates RD (2018) Coral bleaching from a single cell perspective. ISME J 12:1558–1567
Palacio-Castro AM, Dennison CE, Rosales SM, Baker AC (2021) Variation in susceptibility among three Caribbean coral species and their algal symbionts indicates the threatened staghorn coral, Acropora cervicornis, is particularly susceptible to elevated nutrients and heat stress. Coral Reefs 40:1601–1613
Perry CT, Morgan KM (2017) Bleaching drives collapse in reef carbonate budgets and reef growth potential on southern Maldives reefs. Sci Rep 7:40581
Pettay DT, Wham DC, Smith RT et al (2015) Microbial invasion of the Caribbean by an Indo-Pacific coral zooxanthella. Proc Natl Acad Sci U S A 112:7513–7518
Pörtner HO (2008) Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view. Mar Ecol Prog Ser 373:203–217
Pörtner HO, Bock C (2000) A contribution of acid-base regulation to metabolic depression in marine ectotherms. In: Life in the Cold. Springer Berlin Heidelberg, pp 443–458
Pratchett MS, Hoey AS, Wilson SK et al (2011) Changes in biodiversity and functioning of reef fish assemblages following coral bleaching and boral loss. Diversity 3:424–452
Putnam HM, Barott KL, Ainsworth TD, Gates RD (2017) The vulnerability and resilience of reef-building corals. Curr Biol 27:R528–R540
Rädecker N, Pogoreutz C, Gegner HM et al (2021) Heat stress destabilizes symbiotic nutrient cycling in corals. Proc Natl Acad Sci USA 118. https://doi.org/10.1073/pnas.2022653118
R Core Team (2012) A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-projectorg. Accessed May 2021
Rodrigues LJ, Grottoli AG (2007) Energy reserves and metabolism as indicators of coral recovery from bleaching. Limnol Oceanogr 52:1874–1882
Rodriguez RJ, Henson J, Van Volkenburgh E et al (2008) Stress tolerance in plants via habitat-adapted symbiosis. ISME J 2:404–416
Rowan R (2004) Thermal adaptation in reef coral symbionts. Nature 430:742–742
Russell JA, Moran NA (2006) Costs and benefits of symbiont infection in aphids: variation among symbionts and across temperatures. Proc Biol Sci 273:603–610
Shartau RB, Baker DW, Harter TS et al (2020) Preferential intracellular pH regulation is a common trait amongst fishes exposed to high environmental CO2. J Exp Biol 223. https://doi.org/10.1242/jeb.208868
Silverstein RN, Cunning R, Baker AC (2017) Tenacious D: Symbiodinium in clade D remain in reef corals at both high and low temperature extremes despite impairment. J Exp Biol 220:1192–1196
Silverstein RN, Cunning R, Baker AC (2015) Change in algal symbiont communities after bleaching, not prior heat exposure, increases heat tolerance of reef corals. Glob Chang Biol 21:236–249
Slater C, Preston T, Weaver LT (2001) Stable isotopes and the international system of units. Rapid Commun Mass Spectrom 15:1270–1273
Stat M, Morris E, Gates RD (2008) Functional diversity in coral–dinoflagellate symbiosis. Proc Natl Acad Sci U S A 105:9256–9261
Thies AB, Quijada-Rodriguez AR, Zhouyao H et al (2022) A Rhesus channel in the coral symbiosome membrane suggests a novel mechanism to regulate NH_3 and CO_2 delivery to algal symbionts. Sci Adv 8:0303
Thornhill DJ, Rotjan RD, Todd BD et al (2011) A connection between colony biomass and death in Caribbean reef-building corals. PLoS One 6:e29535
Tolleter D, Seneca FO, DeNofrio JC et al (2013) Coral bleaching independent of photosynthetic activity. Curr Biol 23:1782–1786
Tremblay P, Gori A, Maguer JF et al (2016) Heterotrophy promotes the re-establishment of photosynthate translocation in a symbiotic coral after heat stress. Sci Rep 6:38112
van Hooidonk R, Maynard J, Tamelander J et al (2016) Local-scale projections of coral reef futures and implications of the Paris Agreement. Sci Rep 6:39666
van Woesik R, Shlesinger T, Grottoli AG et al (2022) Coral-bleaching responses to climate change across biological scales. Glob Chang Biol. https://doi.org/10.1111/gcb.16192
Veal CJ, Carmi M, Fine M, Hoegh-Guldberg O (2010) Increasing the accuracy of surface area estimation using single wax dipping of coral fragments. Coral Reefs 29:893–897

Wall CB, Kaluhiokalani M, Popp BN et al (2020) Divergent symbiont communities determine the physiology and nutrition of a reef coral across a light-availability gradient. ISME J 14:945–958

Wham DC, Ning G, LaJeunesse TC (2017) Symbiodinium glynnii sp. nov., a species of stress-tolerant symbiotic dinoflagellates from pocilloporid and montiporid corals in the Pacific Ocean. Phycologia 56:396–409

Wickham H (2016) Programming with ggplot2. In: Wickham H (ed) ggplot2: elegant graphics for data analysis. Springer International Publishing, Cham, pp 241–253

Wooldridge SA (2013) Breakdown of the coral-algae symbiosis: towards formalising a linkage between warm-water bleaching thresholds and the growth rate of the intracellular zooxanthellae. Biogeosciences 10:1647–1658

Wooldridge SA (2009) A new conceptual model for the warm-water breakdown of the coral–algae endosymbiosis. Mar Freshw Res 60:483–496

Yuyama I, Higuchi T (2014) Comparing the effects of symbiotic algae (Symbiodinium) clades C1 and D on early growth stages of Acropora tenuis. PLoS One 9:e98999