Vol. 701: 41–65, 2022
https://doi.org/10.3354/meps14192

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published November 24

Acclimation to elevated temperatures in *Acropora cervicornis*: effects of host genotype and symbiont shuffling

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ABSTRACT: Climate change is increasing the average surface temperatures of tropical waters, creating unfavorable conditions for corals. Some species of coral can physiologically acclimate to elevated temperatures, but the degree to which genetic variation underlies differences in this ability is currently unknown. Acclimation to elevated temperatures in coral has been hypothesized to be due to either alterations in the symbiont community or to changes in gene expression. The present study investigated the ability of *Acropora cervicornis* to acclimate to elevated temperatures, estimated the heritability of plasticity in upper thermal tolerance, and tested whether observed acclimation patterns could be explained by symbiont shuffling. Coral fragments from a nursery in the Florida Keys (USA) were acclimated at either ambient (27 ± 1°C) or elevated (30 ± 1°C) temperatures and then exposed to a second heat stress (32 ± 1°C) and monitored for mortality. Fragments acclimated to elevated temperatures showed significantly longer lifespans in the subsequent heat stress than did those acclimated at ambient temperature. The ability to acclimate to elevated temperatures differed significantly among coral genets, yielding low, but significant, estimates of broad-sense heritability. A subsequent experiment revealed no changes in either bacterial or dinoflagellate communities of symbionts as a result of acclimation, suggesting that symbiont shuffling did not account for the differences in lifespan between treatments. While estimates of heritability were low, the results suggest that plasticity in upper thermal tolerance significantly differs among coral genets, and that acclimation is likely a result of alterations in gene expression as opposed to symbiont shuffling.

KEY WORDS: Acclimation · Thermal tolerance · Phenotypic plasticity · Heritability

1. INTRODUCTION

Anthropogenic activities have significantly increased concentrations of CO_2 in the atmosphere, creating a greenhouse effect that is projected to increase global surface temperatures by 1 to 4°C within the century (Hoegh-Guldberg et al. 2007, Intergovernmental Panel on Climate Change [IPCC] 2014). The predicted increase in ocean temperatures pose a significant threat to coral reefs, in part because many species tend to live at or near their upper thermal threshold (Jokiel & Coles 1990, Berkelmans & Willis 1999, Hoegh-Guldberg 1999). Changes in temperature also threaten the symbiotic relationship between corals and their algal symbionts. This symbiotic relationship is crucial for survival, as the photosynthetic

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symbionts provide the majority of the nutritional requirements of the coral (Jones et al. 2008, Muscatine 1990), making survival in a bleached state for prolonged periods of time impossible.

There are several potential strategies corals may utilize to combat rising surface temperatures. First, corals may disperse to higher latitudes to escape high average temperatures and irradiance. Geological evidence shows that coral reefs were once found at slightly higher latitudes relative to contemporary sites (Precht & Aronson 2004, Greenstein & Pandolfi 2008) and recent surveys have identified modern species in latitudes beyond their normal range (Precht & Aronson 2004, Yamano et al. 2011). Although these findings suggest gradual relocation is possible, relocation requires motility and the presence of suitable habitat within a reachable distance, and is thus constrained by the temporal lifecycle of corals and the limited time they exist as mobile larvae (Camp et al. 2018).

Another possible response to long-term changes in the thermal environment is for the population to adapt due to the death of colonies with low thermal tolerance, and the survival of tolerant colonies. Evolution by means of natural selection requires that genetic variation exists within a population in traits related to fitness (Fisher 1930). Previous studies have already determined that host genotype significantly influences thermal tolerance in some coral populations (Fitt et al. 2009, Császár et al. 2010, Weis 2010, Kenkel et al. 2015, Yetsko et al. 2020). To understand the potential evolution of thermal tolerance, several studies have investigated the amount of genetic variance (heritability) attributed to thermal tolerance (Meyer et al. 2009, Császár et al. 2010, Dixon et al. 2015, Kenkel et al. 2015, Dziedzic et al. 2019, Yetsko et al. 2020). These studies have indicated that many coral populations have relatively high heritability of a variety of thermal tolerance traits, making evolution of greater thermal tolerance a possibility (Bairos-Novak et al. 2021). Still, with one-third of all corals facing extinction (Carpenter et al. 2008), many of which have long generation times (ranging from 5 to 100 yr depending on species) (Babcock 1991), it is unlikely most corals will evolve fast enough to match the accelerated rate of anthropogenic climate change.

Acclimation or acclimatization is a third mechanism by which corals may respond to higher water temperatures. Acclimation is a physiological change within an individual in response to a specific environmental stressor (Fregley 1996), while acclimatization involves a coordinated physiological response to several simultaneous stressors (Bligh et al. 1976). While not necessarily a permanent solution, acclimation may function as an initial step that buys time for adaptation to occur (Chevin et al. 2010, Snell-Rood et al. 2018). Previous studies have shown prior exposure to elevated temperature improves the survivability of some coral species to future heat stress (Maynard et al. 2008, Middlebrook et al. 2008, Oliver & Palumbi 2011, Hackerott et al. 2021, DeMerlis et al. 2022). The ability of corals to expand thermal tolerance under stressful conditions is indicative of potential phenotypic plasticity, or the ability of an individual to express different phenotypes to tolerate new environments (Ghalambor et al. 2010). Phenotypic plasticity is a form of acclimation which improves the probability of survival in populations under stressful conditions without undergoing adaptation via directional selection (Pigliucci 2005). In this way, plasticity can be viewed as adaptive, so long as the cost of plasticity does not outweigh the contribution to survival (DeWitt et al. 1998). This ability is crucial for sessile organisms since it can increase survivability in species incapable of relocating to better conditions (Sultan 1995).

Two mechanisms have been hypothesized to explain phenotypic plasticity in coral. First, coral may alter gene expression patterns that result in physiological changes in response to environmental shifts. Previous work has shown that coral in highly variable thermal environments often show a great degree of plasticity in their patterns of gene expression (Barshis et al. 2013, Kenkel & Matz 2017). This sometimes manifests as greater variation in transcription patterns when exposed to a distinct environment (Kenkel & Matz 2017), or potentially as 'frontloading', where stress response genes are consistently expressed at elevated rates even during non-stressful conditions (Barshis et al. 2013). Notable examples of transcriptional changes include increased expression of genes that transcribe antioxidant, anti-apoptotic, and symbiont-recognition proteins (Császár et al. 2009, Bellantuono et al. 2012, Yetsko et al. 2020). Increased production of antioxidants may reduce damage from free radicals activated by photosystem II, while anti-apoptotic proteins may prolong cell life while under stress. Furthermore, symbiont recognition proteins may serve to reinforce the bonding of symbionts in the endoderm, reducing the likelihood of bleaching. Other species have been observed increasing expression of heat shock proteins and chromoproteins (Seneca et al. 2010) to maintain conformation among structural proteins and important enzymes, and assist in absorbing excess solar radiation. At the same time, downregulation of enzymes such as adenosine kinase and carbonic anhydrase,

2 enzymes important for growth and calcification respectively, suggest there may be a tradeoff in growth and maintenance of cell integrity while under thermal stress (Kenkel et al. 2013).

The second potential mechanism of plasticity involves changing the amount, type, or combination of symbionts that influence the response to temperature stress. It is well documented that coral dinoflagellate (Berkelmans & Van Oppen 2006, Oliver & Palumbi 2011, Howells et al. 2012) and bacterial (Ziegler et al. 2017) communities can influence thermal tolerance in the host. Furthermore, coral populations that reside in areas with high average temperatures typically contain thermally tolerant zooxanthellae and bacteria communities (Baker et al. 2004, Voolstra et al. 2021). Some coral species are capable of altering their symbiont communities during bleaching in a process known as 'symbiont shuffling' (Baker 2003, Mieog et al. 2007), where background populations of thermally resilient symbionts are retained while less tolerant clades are expelled (Silverstein et al. 2017, Cunning et al. 2018). Some corals have even been suggested to be able to incorporate new symbionts after bleaching by acquiring tolerant symbionts from the surrounding reef (symbiont switching) (Byler et al. 2013, Boulotte et al. 2016), although shuffling of rare taxa cannot be ruled out.

While both the alteration of host gene expression patterns and the shuffling of symbionts have been shown in some instances of acclimation, the results have not been consistent (Goulet 2006, Thornhill et al. 2009). Furthermore, little is known about the heritability of phenotypically plastic traits like thermal acclimation. It is known that plasticity can evolve, since it differs in predictable ways among species. For example, organisms that occupy highly variable habitats and climatic conditions typically exhibit highly plastic responses whereas those that live in relatively stable conditions show little plasticity (Via & Lande 1985, Via 1993). These patterns suggest that natural selection has likely shaped these traits, which can only occur if heritable variation exists among individuals. While the heritability of thermal tolerance in coral has been investigated in several recent studies (Császár et al. 2010, Dixon et al. 2015, Dziedzic et al. 2019, Yetsko et al. 2020), the heritability of thermal plasticity has not.

While corals tend to live in relatively stable thermal environments, shallow reefs exhibit relatively high variability in seasonal water temperature compared to other ocean zones (Leichter et al. 2006). This pattern suggests that corals residing near the surface may benefit from a significant amount of plasticity in thermal tolerance, especially when compared to species found in deeper zones. One species typically found within 10 m of the surface is the branching coral *Acropora cervicornis* (Tunnicliffe 1981, Rützler & Macintyre 1982). *A. cervicornis* is considered a threatened species that tends to be sensitive to environmental conditions, and it has declined throughout much of its range over the last several decades (Aronson & Precht 2001). These characteristics have made *A. cervicornis* a species of great concern throughout the Caribbean and of high interest for coral restoration programs. Its easily fragmented skeleton and proclivity for shallow water make it a suitable candidate for investigating the heritability of phenotypic plasticity of thermal tolerance, and for testing the mechanism of symbiont shuffling.

The present study addressed 3 questions regarding the ability of *A. cervicornis* to acclimate to thermal stress: (1) Does short-term exposure to heat stress result in greater survival during a second heat stress? (2) To what extent are differences in the ability to acclimate to thermal stress (phenotypic plasticity of thermal tolerance) due to genetic differences among coral genotypes? (3) Is acclimation to elevated temperature accompanied by symbiont shuffling? Answering these questions has the potential to aid conservationists by optimizing the effectiveness of coral reef recovery projects and advance the understanding of coral capacity to survive in a changing climate.

2. MATERIALS AND METHODS

2.1. Acclimation experimental design

A total of 12 approximately 15 cm long fragments from each of 20 genotypes (240 total fragments) of *Acropora cervicornis* were collected at random in March of 2018 from surplus stock in the Coral Restoration Foundation's (CRF) Tavernier Coral Tree Nursery. The genotypes were fragmented offshore and left in the nursery to recover for approximately 2 mo. After the recovery period, the fragments were transported to the Keys Marine Laboratory (KML) and allowed to recover for 3 d in nearshore-supplied water kept at ambient temperature (~27°C) in a pair of 492 l raceways with shading set to mimic light at 5 m, approximately the same depth as the Tavernier nursery. All tanks were cycled on a flow-through system with an approximate flow rate of 1.00 to 2.00 l min^{-1}. Fragments were arranged in a randomized grid design and placed on eggcrate ceiling panels at the bottom of each tank to elevate the fragments

from the raceway floor and to facilitate raceway maintenance when necessary. Genets were identified through a unique color-coded zip tie attached to each fragment.

After the initial recovery period, the 12 fragments of each genotype were evenly allocated among 4 different treatment groups. These treatments included: (1) ambient temperature acclimation followed by ambient moderate-term temperatures (AA), (2) ambient temperature acclimation followed by elevated moderate-term temperature (AE), (3) elevated temperature acclimation followed by ambient moderate-term temperature (EA), and (4) elevated acclimation followed by elevated moderate-term temperature (EE). For each of the 4 treatments groups, fragments were placed into 3 replicate treatment raceways (1 ramet per genotype in each raceway), yielding 20 genotypes in each of the 12 raceways. Each of the 12 raceways were 151 l in volume and cycled with a separate flow-through system with a flow rate of 1.00 to 2.00 l min^{-1}. Each of the fragments then underwent a 4 d acclimation period at 1 of 2 temperatures depending on the experimental treatment (Fig. 1). Both the AA and AE treatments were exposed to ambient temperatures for the acclimation period (27 ± 1°C), while the EA and EE treatments were exposed to the elevated temperature of 30 ± 1°C. For the elevated temperature acclimation, water was heated gradually over the course of 24 h, maintained at 30°C for 48 h, and then brought back to ambient conditions over an additional 24 h.

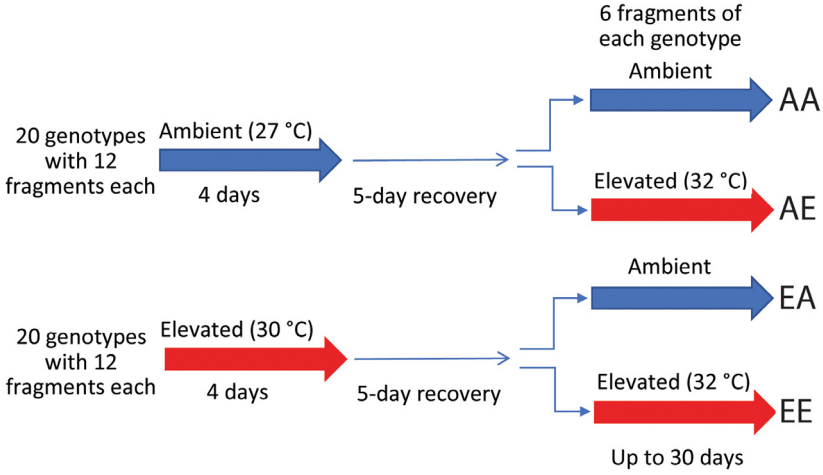

Fig. 1. The experimental design. Fragments of *Acropora cervicornis* underwent exposure to 1 of 2 acclimation temperatures for 4 d, followed by a 5 d recovery period and then an up to 30 d 'moderate-term' treatment at 1 of 2 temperatures. Two letter codes describing each treatment and utilized throughout the text are shown on the far-right of the figure. AA: ambient acclimation, ambient moderate-term; AE: ambient acclimation, elevated moderate-term; EA: elevated acclimation, ambient moderate-term; EE: elevated acclimation, elevated moderate-term

The acclimation period was followed by a 5 d recovery period at ambient temperature for all treatments, after which the fragments spent up to 30 d in a moderate-duration (sensu Grottoli et al. 2021) exposure at either ambient (AA and EA) or elevated temperatures of 32°C (AE and EE) (Fig. 1). The moderate-duration elevated temperature treatments underwent a 24 h ramp up to 32°C and were then maintained at that temperature for the duration of the experiment.

2.2. Monitoring

To monitor coral survival and health, the effective photosynthetic efficiency (PSII quantum yield) was measured in each fragment using a Diving PAM II pulse amplitude modulated (PAM) underwater fluorometer (Walz) with intensity and gain set to 3. Measurements were taken every 12 h to light-adapted corals at dawn and dusk (~07:30 and ~19:30 h) during the short-term acclimation and moderate-duration heat stress. Previous work by Rasher & Hay (2010) suggests that variable fluorescence/maximal fluorescence (F_v/F_m) scores of <0.2 correspond to severe bleaching and mortality. Any fragment that scored <0.2 F_v/F_m for 2 consecutive time periods was investigated under a microscope to verify that coral tissue had sloughed off the skeleton. Time of death was considered the time period at which all tissue had sloughed off the skeleton. Water temperature was measured using a YSI Pro Plus Water Quality Instrument (Fondreist Environmental), with 7 temperature readings taken each day between 07:30 and 17:30 h. Water in experimental tanks was heated through 2 mechanisms. First, the KML seawater system was used to elevate temperatures up to 30°C but a 500 W Titanium Aquarium Heater (Hygger) was required to increase water temperature above 30°C for the moderate-duration exposure in AE and EE treatments. Heaters were suspended near the surface in the center of the raceway in order to reach temperatures above 30°C. Additional information on specific details of the experimental design as suggested by Grottoli et al. (2021) and a log of temperatures experienced throughout the experiment can be found in Tables S1 & S2 in the Supplement at www.int-res.com/articles/suppl/m701p041_supp.pdf.

To determine if the short-term heat acclimation resulted in stress to the coral fragments, a linear mixed-effects model with treatment, time of day (TOD), and date as fixed factors with genet and tank as random factors (F_v/F_m ~ Treatment + TOD + Date + (1|Genet) + (1|Tank)) was used to detect differences in F_v/F_m among the 4 treatments before and after short-term heat acclimation. This model also allowed us to assess the amount of variability in F_v/F_m among genotypes and the other factors tested. A second linear mixed-effects model was utilized to determine if F_v/F_m during the acclimation period significantly affected lifespan under elevated temperatures. In this analysis, only data from the AE and EE treatments were explored since they were the only treatments in which mortality was observed (see Section 3). In this model, lifespan was compared to fixed factors of treatment, F_v/F_m during the acclimation period, TOD, and date, with random factors of genet and tank (Lifespan ~ Treatment + F_v/F_m + Date + TOD + (1|Genet) + (1|Tank)). Tukey's pairwise post-hoc tests were conducted on these models where appropriate. All linear mixed-effects models were performed using R version 4.1.0 (R Core Team 2021).

Differences in F_v/F_m among treatments were also analyzed after the acclimation period to determine if the decline in photosynthetic efficiency in the AE and EE treatments was relatively constant. First, a linear regression of F_v/F_m over time was conducted for the AE and EE treatments. Due to the mediocre fit of the linear regression, a second model was produced using the tvem package R version 4.1.0 (Tan et al. 2012, Dziak et al. 2021). This software was used to fit a time-varying longitudinal model with the penalized B-spline method, to determine if it had a better fit than a linear model.

Two methodologies were utilized to explore differences in lifespan among treatments. First, a Kruskal-Wallace test (PAST version 4.03) (Hammer et al. 2001) was used to determine significant differences in mean lifespan among AE, EE, EA, and AA treatments. Second, we considered the mixed-effects Cox proportional hazards model to study the average treatment effects on the survival performance of corals under elevated moderate-duration temperatures (AE and EE groups only, since all fragments survived in the AA and EA groups). Tank and genotype were treated as random effects in the model. To evaluate per-genet differences in survival, both genotype and the genotype × treatment interaction term were included in the model as covariates.

2.3. Measuring plasticity

The average lifespan (LS) of fragments in each treatment (LS_{AA}, LS_{AE}, LS_{EA}, and LS_{EE}) was then utilized to determine the thermal tolerance of each genotype at both acclimation temperatures. For the purposes of this study, we defined thermal tolerance as the difference in lifespan of fragments exposed to elevated temperatures and those that remained at ambient temperatures. This led to a pair of thermal tolerance estimates, including thermal tolerance when acclimated at ambient temperatures ($LS_{AA} - LS_{AE}$; hereafter 'ambient tolerance') and thermal tolerance when acclimated at elevated temperatures ($LS_{EA} - LS_{EE}$; hereafter 'elevated tolerance'). Since all control fragments (AA and EA) lived the entire 30 d (see Section 3), the lifespans of each of the 3 fragments of a genotype in either the AE or EE groups could be subtracted from 30 for the lifespan differential of a fragment. These differences were then averaged for each genotype. By this definition, genotypes with high thermal tolerance will be those with the smallest average difference between the lifespan of treatment fragments and control fragments.

We then quantified the overall plasticity of each genotype as the difference in thermal tolerance under different acclimation temperatures as per the equation:

$$\text{Amount of Plasticity} = (LS_{AA} - LS_{AE}) - (LS_{EA} - LS_{EE}) \quad (1)$$

This is simply the difference between the ambient tolerance and the elevated tolerance of a genotype. All pairwise comparisons of lifespan differentials of the fragments acclimated at ambient temperature and those acclimated at elevated temperature were made to estimate the average plasticity of a genotype (n = 3 ambient tolerance estimates × 3 elevated tolerance estimates = 9 pairwise comparisons per genotype). In this way, positive values of plasticity represent better performance when acclimated at elevated temperatures, which would suggest acclimation, while negative values represent better performance when acclimated at ambient temperatures, which would suggest the acclimation period induced stress that was not overcome during the rest period. Since the Cox proportional hazards model utilized above has low power to detect survival differences among genotypes given the small sample size (3 observations for each genotype per treatment), variation in thermal tolerance (both ambient and elevated) among genotypes was estimated using a 1-way ANOVA with post-hoc pairwise comparisons per-

formed utilizing a Tukey's post-hoc test. Variation in plasticity among genotypes was estimated with a Kruskal-Wallis analysis and compared pairwise using a Dunn's post-hoc test. To determine if the amount of thermal plasticity is predicted by thermal tolerance at either ambient or elevated temperature acclimation, plasticity estimates were then regressed against both ambient tolerance and elevated tolerance. All statistical tests were performed using SPSS version 25.

2.4. Heritability estimates

The heritability of thermal plasticity, ambient tolerance, and elevated tolerance were then estimated in both the broad and the narrow sense. Broad-sense heritability (H^2) is a more general measurement of the degree to which variance in phenotype (V_P) is determined by overall variance in genotype (V_G). Narrow-sense heritability (h^2), on the other hand, focuses on the proportion of phenotypic variance that is due specifically to additive genetic variance (V_A). Since V_A is the only portion of V_G that can predictably respond to selection, h^2 provides a measurement of the degree to which a trait can be altered by selection (Lande & Shannon 1996).

Broad-sense heritability was estimated using the clonal method described by Császár et al. (2010). A 1-way ANOVA (a general linear mixed-effects model fitted by restricted maximum likelihood) with thermal plasticity as the dependent variable and coral genotype as a random effect was utilized to estimate the various variance components in the data. Variance in thermal plasticity among fragments of the same genotype provides an estimate of environmental variance (V_E), while variance in thermal plasticity among genotypes provides an estimate of genetic variance (V_G). The sum of $V_G + V_E$ yields an estimate of phenotypic variance (V_P) and H^2 can then be estimated according to the equation (Falconer & MacKay 1996):

$$H^2 = V_G / V_P \quad (2)$$

SE was evaluated by the parametric bootstrapping method using the R statistical environment (v3.4.4, R Foundation for Statistical Computing), where bootstrap samples were simulated from a Gaussian distribution whose parameters are estimated based on the real sample (Nakagawa & Schielzeth 2010, Stoffel et al. 2017).

Narrow-sense heritability was estimated by regressing the phenotypic difference in thermal plasticity among fragments with pairwise relatedness (r) between fragments in a linear model as outlined by Ritland (1996a). These regressions provide an R^2 value that approximates the narrow-sense heritability of phenotypic plasticity of thermal tolerance. A number of models exist to estimate r between samples based on multi-locus genotype data. Each of these models is based on a different set of assumptions and performs best under different circumstances (Van De Casteele et al. 2001, Russello & Amato 2004, Csilléry et al. 2006). Since it is unknown which estimator best fits *A. cervicornis*, we estimated r utilizing a suite of models to determine how consistent the estimates of h^2 were. One method involved the marker-based program MARK (Ritland 2004), which utilizes a mathematical regression model developed by Lynch & Ritland (1999). Additional estimates of relatedness, including Trioml, Wang, Lynchli, Ritland, Lynchrd, Quellergt, and Dyadml, were also calculated using COANCESTRY v1.0.1.10 (Wang 2011) by bootstrapping multilocus genotype frequency data acquired from microsatellite loci described by Baums et al. (2005, 2009). The SE of h^2 was estimated as the SE of each linear regression between relatedness and phenotypic difference calculated in SigmaPlot (Systat Software).

Pairwise relatedness estimates were based on genotyping each of the coral genets at 15 microsatellite markers. One fragment from each genotype was initially preserved in 70% ethanol for genetic analysis. Subsamples of tissue and skeleton (~1 g) were immersed in 'Chaos' extraction buffer (4.5 M guanidine thiocyanate; 2% N-lauroylsarcosine; 50 mM ethylenediaminetetraacetic acid [EDTA], pH 8; 0.1 M 2-mercaptoethanol) and incubated at room temperature for 5 d. Total DNA was extracted using a magnetic bead protocol per the manufacturer's instructions (AMPure XP, Agencourt). Corals were then genotyped at the 15 microsatellite loci described by Baums et al. (2005, 2009) utilizing the published methodology. After amplification, samples were sent to the University of Florida Interdisciplinary Center for Biotechnology and Research where fragments were processed using a ThermoFisher 3730XL DNA Analyzer. Genotypes were visualized and using GeneMarker (SoftGenetics, V 3.0.1).

While heritability estimates of thermal plasticity, ambient tolerance, and elevated tolerance provide information on the degree to which genetic differences among genets result in different thermal tolerance attributes, it is also important to understand whether these traits correspond in any way. To determine if the amount of thermal plasticity is predicted by thermal

tolerance at either ambient or elevated temperature acclimation, a pair of linear regressions were performed on thermal plasticity estimates vs. ambient tolerance and elevated tolerance in SPSS version 25.

2.5. Detecting changes among symbionts

In the year following the experiments outlined above, a second experiment was performed to test whether changes in bacterial and dinoflagellate composition occurred during the acclimation period. For this experiment, 3 fragments from each of the same 20 genotypes utilized previously were collected from CRF's surplus stock in March of 2019, allowed to recover in the nursery for 2 mo, and transported to KML from CRF's offshore nursery using the same methodology outlined above. After a 3 d recovery, the fragments were separated into 1 of 3 groups. One fragment of each genotype served to represent the initial symbiotic community of that genotype. These fragments were removed from the raceways immediately following the recovery period and placed into individualized containers containing 70% ethanol to store for later genetic analysis. The other 2 fragments underwent the same 4 d acclimation period as described above, with one undergoing the ambient acclimation and the other the elevated acclimation treatment. After a 24 h recovery period following the acclimation treatment, the final 2 fragments of each genotype were removed from the raceways and stored in 70% ethanol in preparation for sequencing of the symbionts of each fragment.

Subsamples of tissue and skeleton (~1 g) were immersed in 'Chaos' extraction buffer. DNA was extracted and quantified using the bead protocol outlined above and suspended in 1× TE buffer. To detect changes in bacterial and dinoflagellate composition, PCR amplification of ribosomal DNA was conducted using Illumina primers for the 16S and ITS2 regions, respectively. ITS2 rDNA (eukaryotes) was amplified with the following primers from Hume et al. (2013, 2015), with ITS2 overhangs delineated in italics:

Forward: *TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG* GAA TTG CAG AAC TCC GTG AAC C

Reverse: *GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G*CG GGT TCW CTT GTY TGA CTT CAT GC

The V3 and V4 region of 16S rDNA (341F/785R prokaryotes) was amplified using the primers shown below (Klindworth et al. 2013), with overhangs delineated in italics:

Forward: *TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG* CCT ACG GGN GGC WGC AG

Reverse: *GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G*GA CTA CHV GGG TAT CTA ATC C

Both ITS2 and 16S genes were amplified via PCR (95°C for 3 min, followed by 30 cycles of: 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, then 72°C for 5 min, hold at 4°C) and indexed with an Illumina Nextera V2 Indexing Kit. The primary PCR amplification was completed using 12.5 µl of Kapa HiFi HotStart ReadyMix (Kapa Biosystems), 5 µl of each primer (1 µM), and 2.5 µl of extracted coral DNA solution. After primary amplification, samples were size selected using 20 µl Agencourt AmPure XP beads per sample and quantified using a Qubit Fluorometer 3.0. Equimolar amounts of the 16S and ITS2 reactions were separately pooled from an individual replicate according to their respective target gene, indexed using Nextera Index 1 and 2 primers (to generate uniquely identified samples), and enriched with the Kapa HiFi HotStart 2× PCR Master Mix. Libraries for both genes were quantified using a Qubit Fluorometer 3.0 and pooled in equimolar ratios. The 16S library was denatured using 0.2 N NaOH, diluted to 18 pM, and loaded with 10% PhiX clustering control on the Illumina MiSeq with the 600 cycle (2 × 300 bp paired end) Sequencing Reagents V3. The ITS2 library was denatured using 0.2 N NaOH, diluted to 18 pM, and loaded with 10% PhiX clustering control on the Illumina MiSeq at 500 cycles (2 × 250 bp paired end) with Sequencing Reagents V3.

2.6. Bioinformatics

Two software packages were used to process the sequence data. Qiime2 (Caporaso et al. 2010) was used to analyze raw sequencing data for 16S rDNA, while ITS2 sequence data were analyzed via SymPortal (Hume et al. 2018). To analyze changes in the bacterial community, demultiplexed and paired 16S forward and reverse fastq.gz files were acquired from the Illumina sequencing. The primers were removed and the data were trimmed (5 bp forward and reverse) to remove low quality reads while maintaining at least 50 bp overlap of forward and reverse reads using Qiime2 (Caporaso et al. 2010, version 2017.12, https://docs.qiime2.org/2017.12/). Trimmed and filtered reads were denoised and dereplicated to produce Amplicon Sequence Variants (ASVs), using DADA2 (Callahan et al. 2016). ASVs are defined as

single DNA sequence variants of the 16S rDNA marker gene with a 97% similarity to the targeted gene. ASVs exhibiting only 1 or 2 reads were removed from the dataset. The SILVA database (release 128, Quast et al. 2013) was utilized to assign taxonomy to 16S ASVs. A naïve Bayes classifier was trained and used to assign taxonomy (confidence = 0.7, classify-22 sklearn: Qiime2).

Demultiplexed and paired ITS2 forward and reverse fastq.gz files acquired from the Illumina sequencing were submitted directly to SymPortal (Hume et al. 2018). SymPortal is a public access software that uses next generation sequencing to detect unique sets of intragenomic ITS2 sequence variants, or Defining Intragenomic Variants (DIVs) indicative of genetically differentiated Symbiodiniaceae taxa. Samples were analyzed under default parameters in the SymPortal pipeline. To ensure sufficient quality, default analysis incorporates a suite of quality controls including Mothur 1.39.5 (Schloss et al. 2009), BLAST+ (Camacho et al. 2009), and minimum entropy decomposition (MED) (Eren et al. 2015).

To estimate differences in bacteria and dinoflagellates among treatment groups, the data were analyzed using the software PAST (V4.03, Hammer et al. 2001). Alpha diversity was calculated using the Shannon diversity index for each treatment group; initial, ambient acclimation, and elevated acclimation. A 1-way ANOVA was used to statistically compare among the Shannon index values of the 3 treatments. Differences in relative abundance of ASVs (Qiime2) and DIVs (SymPortal) among the 3 treatment groups were calculated using ANOSIM. To investigate the similarity in community composition between and within categories, the ASV and DIV tables were Hellinger transformed (Legendre & Gallagher 2001) and a Bray Curtis distance matrix was calculated and visualized using a non-metric multidimensional scaling (NMDS) ordination. Rarefaction curves were calculated for each marker to visualize the rate at which additional fragments increased the number of ASVs and DIVs and to ensure adequate sequence depth for 16S and ITS2 sequencing.

3. RESULTS

The linear mixed-effects model showed that during the acclimation period, F_v/F_m yields differed significantly among dates and treatments (Table 1). The short-term heat stress used to acclimate fragments to elevated temperatures resulted in a decrease in F_v/F_m yields in both the EA and EE groups ($p < 0.001$ for all pairwise comparisons with AA and AE) at approximately 108 h, and then returned to ambient levels by the fourth day of the recovery period (180 h) (Fig. 2). Time of day did not significantly affect F_v/F_m measurements. Of the random factors in the model, variation in F_v/F_m among genets was extensive,

Table 1. Results of a linear mixed-effects model testing variation in photosynthetic efficiency (F_v/F_m) during a thermal acclimation period. Fixed factors in the model included treatment, time of day, date, and genotype, with tank being treated as a random factor. TOD: time of day; see Fig. 1 for definition of treatment groups

Fixed effects	Estimate	SE	df	*t*	p
Intercept	751.898	2.447	70.277	307.234	$<2 \times 10^{-16}$
Treatment AE	−11.426	1.399	8.001	−8.165	3.77×10^{-5}
Treatment EE	−14.745	1.399	8.001	−10.536	5.73×10^{-6}
Treatment EA	−2.755	1.399	8.001	−1.969	0.085
TOD	0.177	0.879	3807.002	0.201	0.841
Date	−2.614	0.192	3807.015	−13.624	$<2 \times 10^{-16}$
Random effects	**Variance**	**SD**			
Genet	50.513	7.107			
Tank	0.619	0.787			
Residual	742.053	27.241			

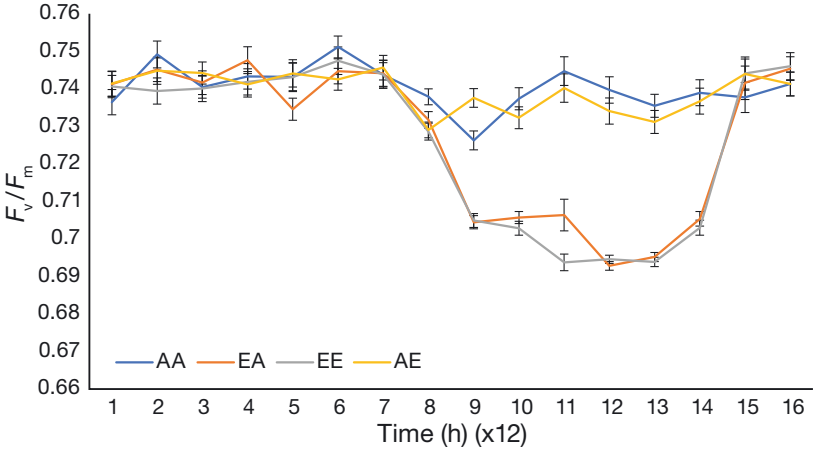

Fig. 2. Average photosynthetic efficiency ($F_v/F_m \pm$ SE) values of *Acropora cervicornis* fragments among 4 treatments over the course of an 8 d acclimation period involving approximately 3 d at either an ambient or elevated temperature, followed by a 5 d recovery period at ambient temperature. See Fig. 1 for definition of treatment groups

while tank contributed little of the variation (Table 1).

In treatment groups AA and EA, all fragments survived the entire moderate-duration exposure and their F_v/F_m remained consistent throughout the course of the experiment. All fragments in treatment groups AE and EE, however, died prior to the 30 d mark. Investigation of F_v/F_m yields across the moderate-duration high temperature exposure showed a steady decrease that was similar for the first 5 d, with AE averaging 0.54 ± 0.10 (mean ± SE) and EE averaging 0.57 ± 0.13 at the end of that period. The F_v/F_m patterns of the AE and EE treatments then diverged, with the EE treatment showing a plateau for approximately 10 d before falling sharply below 0.2 F_v/F_m, while the F_v/F_m in the AE treatment continued to show a steady decline throughout the moderate-term elevated temperature exposure (Fig. 3). A linear regression of F_v/F_m fit the data fairly well, yielding R^2 = 0.73 and 0.72 for AE and EE, respectively, both with slopes of −0.01 ($p < 0.05$). Due to the presence of the apparent plateau in F_v/F_m observed in the EE treatment, the trajectories were also plotted nonparametrically under the time-varying effect model (Tan et al. 2012) using the R package tvem (Dziak et al. 2021). This non-parametric model showed a slight improvement over the linear regression according to the lower Akaike's information criterion (AIC) values derived from both treatments, with the penalized B-spline (AIC_{EE} = 9736.92, AIC_{EA} = 17215.63) slightly smaller than the linear regression (AIC_{EE} = 9810.36, AIC_{AE} = 17800.54) (Fig. 3).

Mean lifespan differed significantly among treatments ($H(3) = 72.4$, $p < 0.001$) with lower lifespans in both AE and EE groups. All fragments in the AA and EA treatments survived for the duration of the experiment (528.00 ± 0.00 h) and all fragments in the AE and EE treatments perished within 30 d (mean lifespan 179.31 ± 2.84 and 321.26 ± 72.11 h, respectively). While acclimation temperature did not affect survival of fragments maintained at ambient temperature for the duration of the experiment (AA and EA), it had a significant effect on survival at elevated temperature (Cox PH model, $p < 0.001$) (Fig. 4). The estimated hazard ratio of the AE group vs. the EE group was 4.57 (95% CI 2.41 to 8.63), implying that fragments treated with the elevated acclimation temperature have an estimated 4.57-fold lower risk of death compared to those with the ambient temperature acclimation. Fragments that were acclimated at the elevated temperature (EE) survived a second heat exposure an average of 141 h longer than those acclimated at ambient temperatures (AE).

Some genotypes exhibited significantly greater plasticity than others ($H(19) = 33.05$, $p = 0.02$) (Fig. 5), suggesting genotype significantly influences plasticity of thermal tolerance. A Dunn's post-hoc revealed that genotypes U11, U106, and M21 had significantly

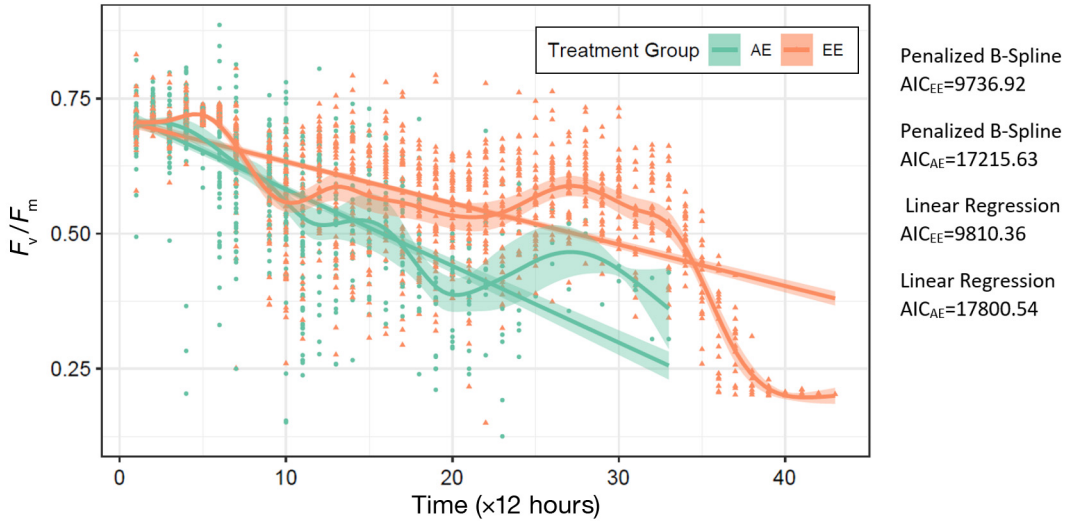

Fig. 3. Estimation of the trajectory of photosynthetic efficiency (F_v/F_m) of *Acropora cervicornis* fragments acclimated at ambient temperature (AE) and fragments acclimated at elevated temperature (EE) throughout the second heat treatment. The trajectories were plotted nonparametrically under the time-varying effect model (Tan et al. 2012) using the R package tvem (Dziak et al. 2021). Akaike's information criterion (AIC) for linear regression and the penalized B-spline are displayed for each line of fit. Lower AIC values describe a model that better fits the data. These values show that a non-parametric model is only a slightly better fit to the data. Dots: longitudinal measurements of photosynthetic efficiency (F_v/F_m) for fragments in treatment groups AE and EE; solid lines: estimates of average F_v/F_m along the timeline; ribbons: 95% pointwise confidence bands

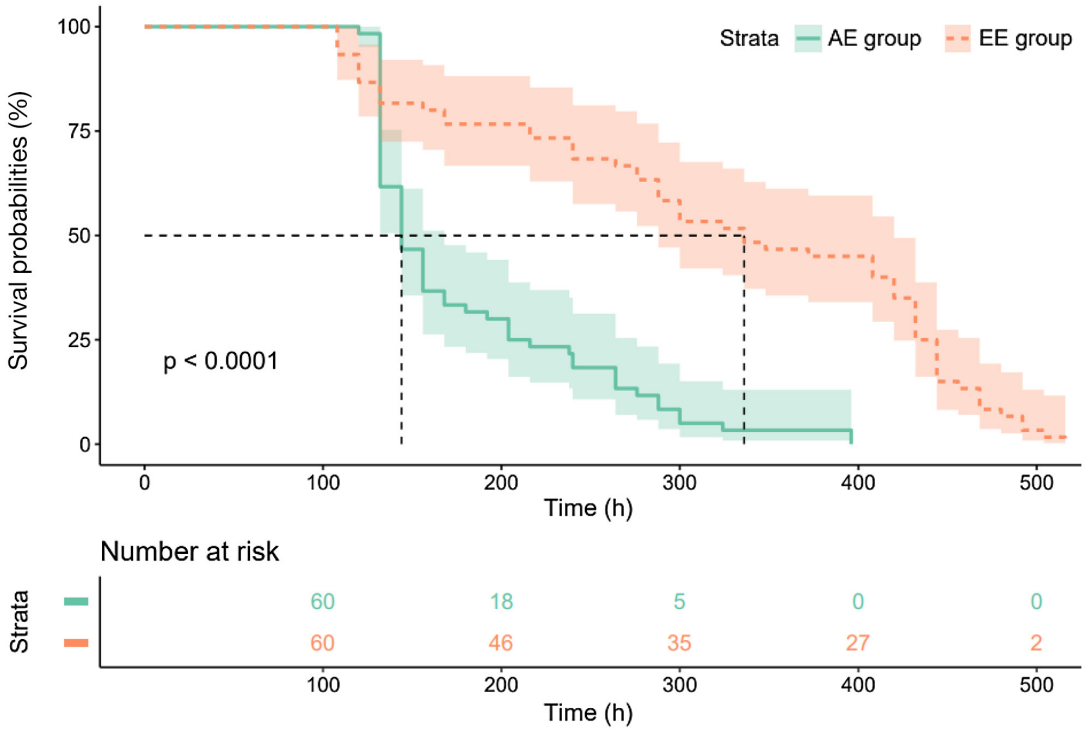

Fig. 4. Survival probabilities of fragments of *Acropora cervicornis* during a moderate-term elevated temperature treatment. Fragments in the AE group were previously acclimated at ambient temperatures while those in the EE group had received a short-term heat stress. (Ribbons) 95% pointwise confidence bands; (dashed black lines) times to 50% survival for each treatment. Actual number of fragments remaining at each 100 h time point in each treatment are provided in the bottom panel

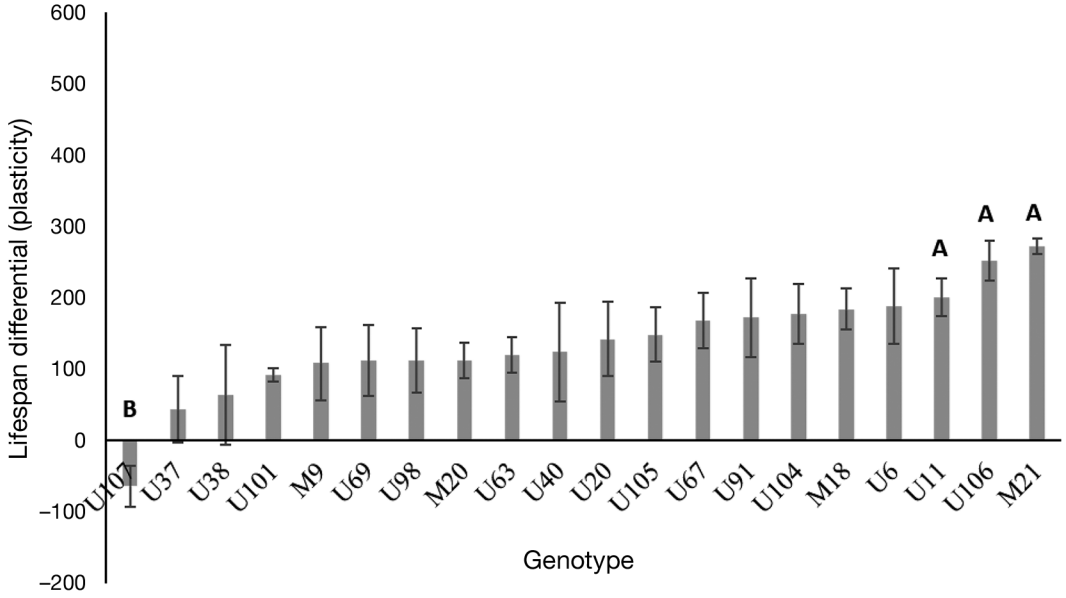

Fig. 5. Plasticity in thermal tolerance, measured as the average difference (±SE) in h survived under an elevated temperature treatment between ambient and heat acclimated fragments for each *Acropora cervicornis* genotype. Significant pairwise differences are denoted by different letters above bars

greater mean thermal plasticity than genotype U107 ($p < 0.05$). The observed differences in thermal plasticity among genotypes resulted in a broad-sense heritability (H^2) estimate of 0.19 (SE = 0.0754). Estimates of narrow-sense heritability (h^2) of plasticity in thermal tolerance were essentially the same for all 7 models of relatedness, ranging from 0.003 to 0.013, none of which were significantly different from zero (Table 2).

Table 2. Seven different estimators of pairwise relatedness among fragments were utilized to estimate the narrow sense heritability (h^2) for 3 different categories of thermal tolerance: (1) plasticity (the difference between 2 and 3), (2) when corals were acclimated at ambient temperature, and (3) when corals were acclimated to elevated temperature

Estimator	r	Plasticity		Ambient		Elevated	
		h^2	SE	h^2	SE	h^2	SE
TrioML	0.019	0.005	0.138	0.006	0.138	0.000	0.138
Wang	0.094	0.012	0.306	0.000	0.308	0.001	0.308
LynchLi	0.113	0.013	0.335	0.00	0.337	0.000	0.337
LynchRd	0.025	0.004	0.158	0.00	0.158	0.000	0.158
Ritland	0.07	0.003	0.265	0.00	0.266	0.001	0.266
QuellerGT	0.051	0.011	0.226	0.00	0.227	0.001	0.227

Linear regressions of thermal plasticity with ambient tolerance suggested that little of the variance observed in plasticity among genotypes could be explained by their ambient tolerance ($R^2 = 0.036$, $F_{1,18} = 0.676$, p = 0.42) (Fig. 6A). Elevated tolerance, on the other hand, was a strong predictor of differences in plasticity among genotypes ($R^2 = 0.727$, $F_{1,18} = 48.10$, p = 0.005) (Fig. 6B). While elevated tolerance of a genotype reliably predicted plasticity estimates, no significant differences in elevated tolerance were identified among genotypes ($F = 19.069$, df = 19, p = 0.452). Similarly, survival times of fragments acclimated to ambient temperatures were relatively similar among genotypes and did not differ significantly ($F = 17.959$, df = 19, p = 0.525). Estimates of both broad- and narrow-sense heritability for ambient and elevated tolerance were effectively zero in all cases (ambient tolerance, $H^2 = 0.00$, SE = 0.0785; elevated tolerance, $H^2 = 0.00$, SE = 0.0785; see Table 2 for h^2).

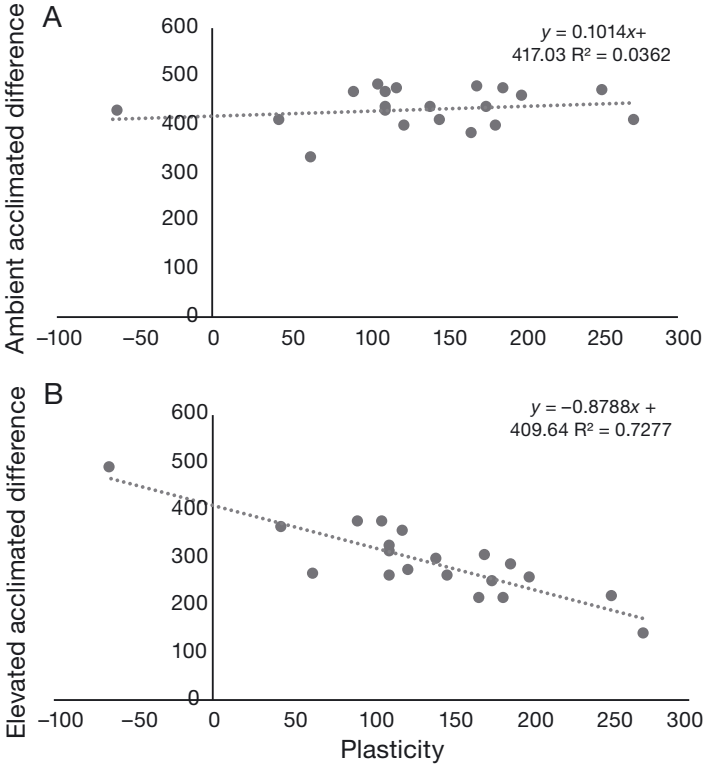

Fig. 6. (A) Regression of the average thermal tolerance of *Acropora cervicornis* genotypes when acclimated at ambient temperatures (27°C) vs. the average amount of plasticity in thermal tolerance of a genotype. (B) Regression of average thermal tolerance of genotypes when acclimated at elevated temperature (30°C) vs. the average amount of plasticity in thermal tolerance of a genotype. Thermal tolerance in each case was defined as the difference in lifespan between coral fragments under thermal stress and controls. Plasticity of thermal tolerance was defined as the difference between thermal tolerance when acclimated at ambient temperature and thermal tolerance when acclimated to elevated temperature

Since both F_v/F_m yields during acclimation and measurements of thermal plasticity differed significantly among genotypes, we also tested whether these variables correlated with each other such that measurements of F_v/F_m during the acclimation period had a significant effect on lifespan at elevated temperatures. The model tested suggested that only the acclimation treatment had a significant effect on lifespan (Table 3). Neither F_v/F_m scores during the acclimation period nor any of the other fixed factors (date, time of day) were a significant predictor of lifespan.

There were no significant differences in Symbiodiniaceae communities among treatment groups, as both the fragments that underwent ambient acclimation and the fragments that underwent the elevated acclimation had the same dinoflagellate community as the pre-acclimation fragments (ANOSIM, R = 0.01, p = 0.31). All treatment groups were dominated by DIV A3, representing about 80% of total DIVs among all 3 treatments (Fig. 7A). Dinoflagellate diversity as measured by Shannon indices varied only slightly among the treatments with no significant differences observed among the 3 groups ($F = 1.61$, df = 2, p = 0.21). Sampling depth may be inadequate according to the projected rarefaction curve of all fragments (Fig. 7C); however, the absence of an asymptote may simply be due to the proportional dominance of DIV A3 and the low relative counts and inconsistent presence of background clades (Fig. S1). NMDS displayed no significant clustering of fragments based on treatment type (Fig. 7B), revealing that heat stress did not sort dinoflagellate communities into distinct groups. Small counts of background DIVs were detected in all 3 groups, with both elevated acclimation and

Table 3. Results of a linear mixed-effects model exploring the relationship between photosynthetic efficiency (F_v/F_m) during an acclimation period and lifespan during a moderate-term temperature stress. TOD: time of day

Fixed effects	Estimate	SE	df	*t*-value	p
Intercept	1.410×10^2	6.042×10^1	5.269×10^1	2.333	0.024
Treatment EE	1.412×10^2	4.467×10^1	4.000×10^0	3.161	0.034
F_v/F_m	5.360×10^2	6.666×10^{-2}	1.898×10^3	0.804	0.422
TOD	7.984×10^2	3.801×10^0	1.892×10^3	0.021	0.983
Date	2.212×10^1	8.736×10^{-1}	1.893×10^3	0.253	0.800

Random effects	Variance	SD
Genet	2172	46.61
Tank	2971	54.51
Residual	6929	83.24

ambient acclimation corals harboring 3 unique DIVs each, while the pre-acclimation fragments were found with 4 unique DIVs (Fig. 8). These unique sequences are poorly represented among fragments within the same treatment group and are not correlated with the addition of a heat stress. It can therefore be concluded that the acclimation temperature executed in this experiment does not immediately alter the composition of dinoflagellate communities among *Acropora cervicornis* fragments. A complete list of dinoflagellate DIVs is provided in Table 4.

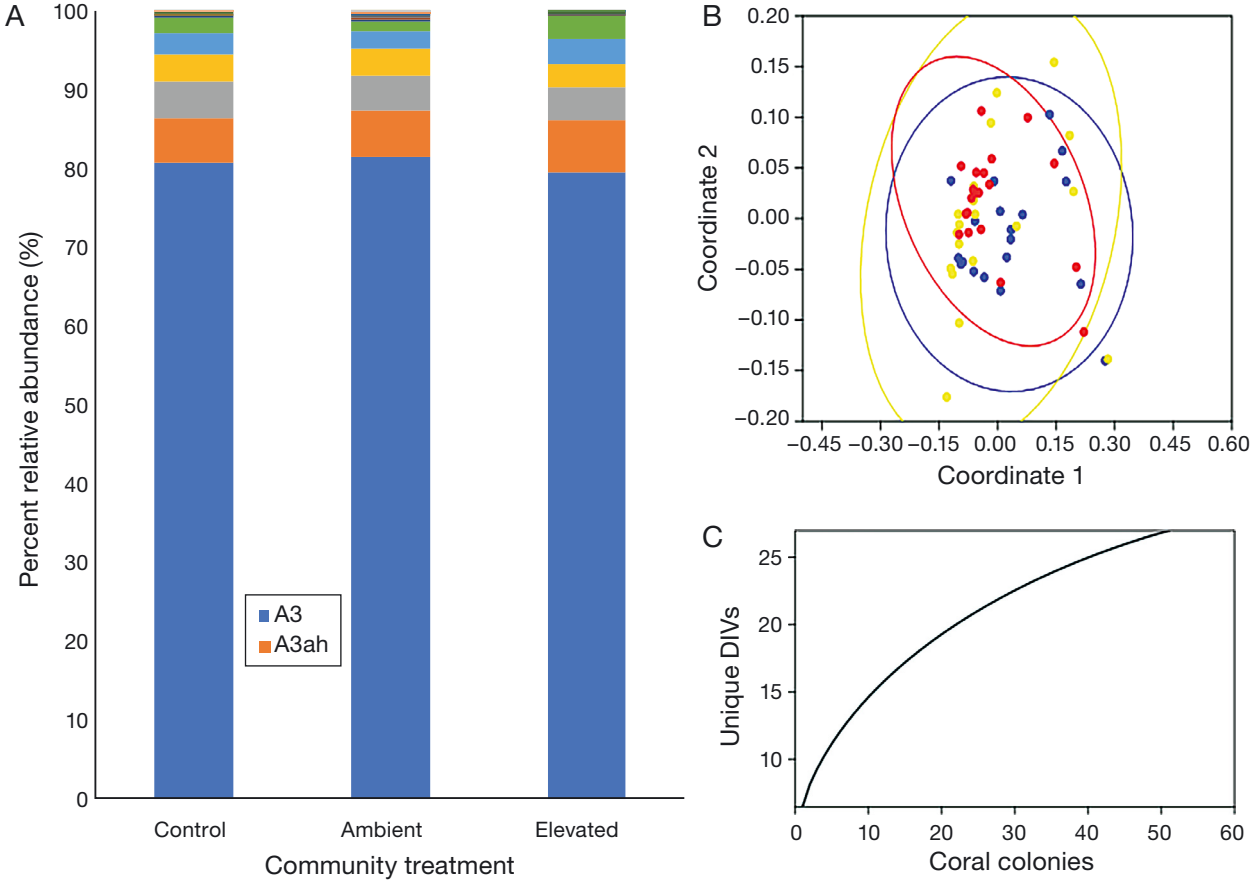

Fig. 7. (A) Relative proportion of Defining Intragenomic Variants (DIVs) of dinoflagellates detected among *Acropora cervicornis* fragments of the initial Symbiodiniaceae community, ambient acclimation, and elevated acclimation. There were no significant differences in dinoflagellate composition among the 3 groups. DIVs exhibiting >5% relative abundance are listed in the key in the key in this panel, with a complete list of DIVs provided in Table 4. (B) NMDS plot for all fragments, showing initial bacterial community (blue), ambient acclimation community (yellow), and elevated acclimation community (red). Rings delineate a 95% confidence interval for each treatment group. No clustering was observed due to treatment type. (C) Rarefaction plot of ITS2 markers showing the number of unique DIVs as a function of number of fragments sampled

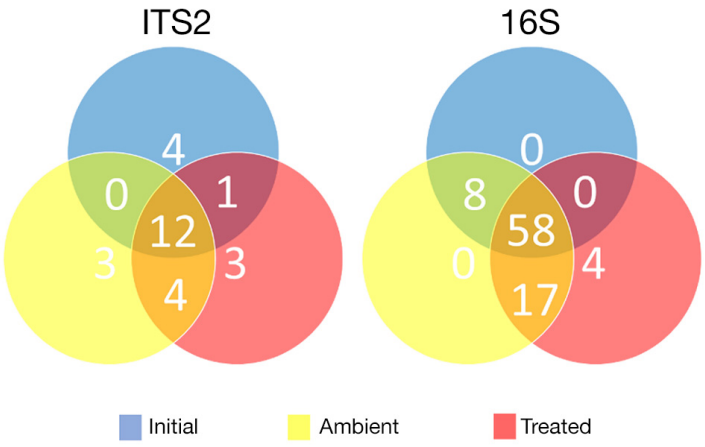

Fig. 8. Distribution of bacterial 16S rDNA Amplicon Sequence Variants (ASVs) and dinoflagellate ITS2 rDNA Defining Intragenomic Variants (DIVs) found within *Acropora cervicornis* fragments of the initial bacterial community, ambient acclimation, and elevated acclimation treatment. Blue spheres: initial community, yellow spheres: ambient community, and red spheres: elevated acclimation community. Overlapping colors represent sequences shared by 2 or more treatment groups. Unique ITS2 sequences were found in each group, while only 4 unique 16S sequences were found in the elevated group. Heat treatment had no significant effect upon the distribution of unique sequences among treatment groups

Table 4. List of dinoflagellate taxa and their relative abundance detected using ITS2 rDNA markers. Taxa are described as SymPortal's taxonomic unit, Defining Intragenomic Variants (DIVs). Relative abundance is delineated among initial bacterial community, ambient acclimation, and elevated acclimation treatment. Small proportions of rare DIVs were detected in some groups and not in others; however, these proportions were small enough to not influence the overall diversity of each treatment group. The conformity among groups suggests that heat treatment had no effect on dinoflagellate community

Taxa DIV	Relative abundance per group		
	Initial community	Ambient community	Elevated community
A3	0.807	0.814	0.796
A3ah	0.056	0.059	0.066
A3ac	0.048	0.046	0.040
A3m	0.0335	0.033	0.030
A3ae	0.028	0.023	0.031
A3ay	0.018	0.012	0.028
903838_A	0.003	0.003	0.002
33881_A	0	0.003	0.001
695980_A	0.001	0.001	0.001
22415_A	0.002	0.001	0.001
364626_A	0	0.001	0.001
364601_A	0.001	<0.001	0.001
22428_A	0	0.001	<0.001
1264834_A	0	0.002	0
703789_A	0.001	0.001	<0.001
1265367_A	0	<0.001	<0.001
1264609_A	0.001	0	0
363588_A	0	0	0.001
1760751_A	<0.001	0	<0.001
50857_A	0	<0.001	0
1147897_A	0	0	<0.001
1264614_A	<0.001	0	0
B1	0	0	<0.001
G3l	<0.001	<0.001	<0.001
36199_G	<0.001	0	0
36194_G	<0.001	0	0
1760750_G	0	<0.001	0

Similar to the results for the dinoflagellate communities, no significant changes were observed in bacterial composition among the 3 treatment groups (ANOSIM, R = 0.02, p = 0.16). The majority of ASVs were dominated by the class *Alphaproteobacteria*, comprising roughly 50% of all ASVs in both the ambient acclimation and elevated acclimation groups, and 30% of ASVs among the pre-acclimation group (Fig. 9A). The smaller proportion of *Alphaproteobacteria* in pre-acclimation fragments is likely due to a higher fraction of unidentified ASVs detected among those corals. Average bacterial diversity was not significantly different among treatment groups (F = 1.88, df = 2, p = 0.16) and NMDS revealed no clustering of fragments based on treatment (Fig. 9B). The rarefactions curve revealed adequate sampling depth to detect the majority of taxa present (Fig. 9C). Lastly, 4 unique ASVs were found in the elevated acclimation fragments, while 8 unique ASVs were found exclusively in pre-acclimation and ambient acclimation fragments (Fig. 8). Each of these unique ASVs were rare among fragments, representing <1% of the total community among the 3 groups. A complete list of bacterial ASVs is provided in Table 5 and ramet-specific bacterial communities can be viewed in Fig. S2.

4. DISCUSSION

4.1. Variation in acclimation ability

Fragments of *Acropora cervicornis* that were exposed to a short-term heat stress displayed a considerably longer average lifespan (141 h) in a moderate-duration heat stress than fragments that were acclimated to ambient conditions. In fact, of the 20

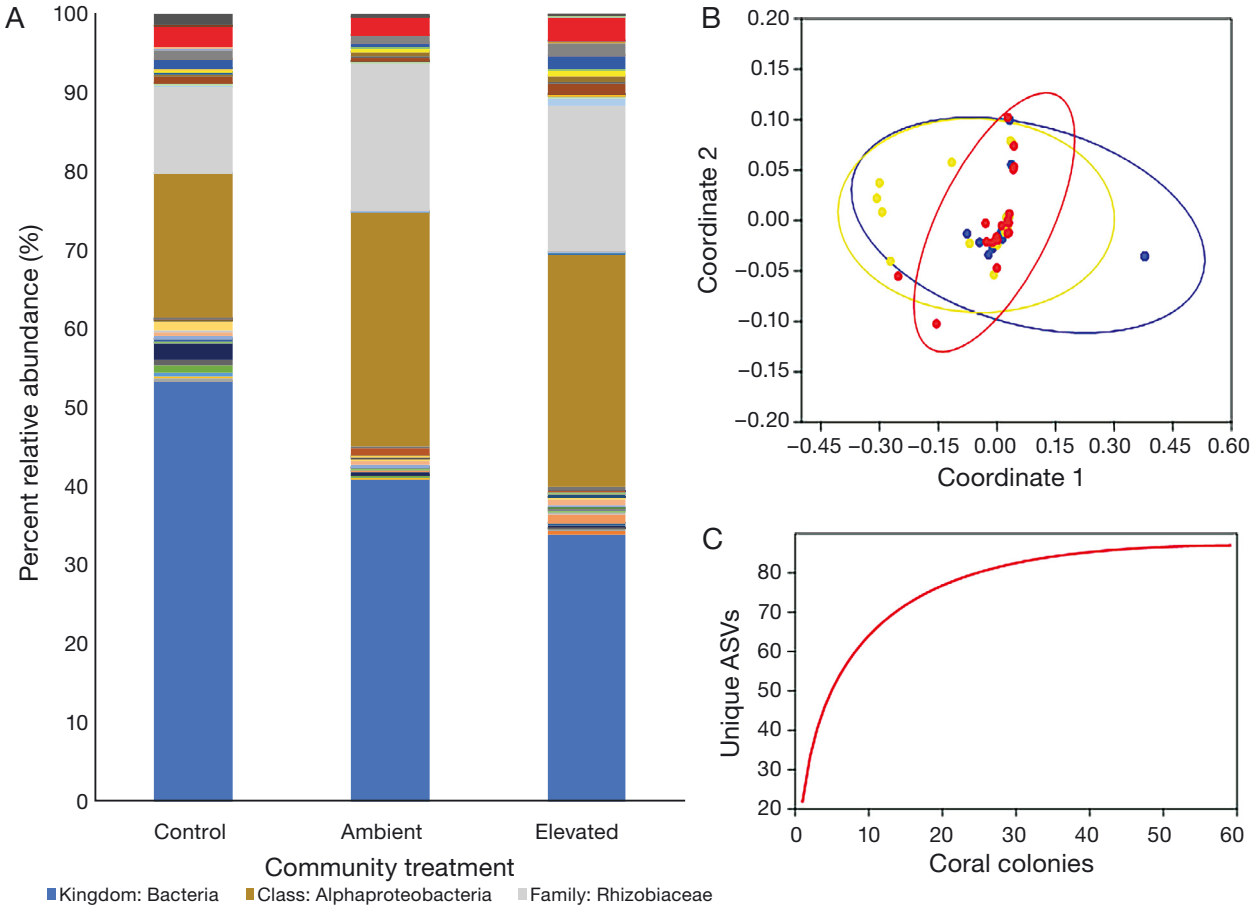

Fig. 9. (A) Relative proportion of bacterial Amplicon Sequence Variants (ASVs) as defined by 16S rDNA detected among *Acropora cervicornis* fragments of the initial community, ambient acclimation, and elevated acclimation. ASVs representing >5% relative abundance are listed in the key below the *x*-axis. A complete list of ASVs is provided in Table 5. (B) NMDS plot for all fragments, showing initial community (blue), ambient community (yellow), and elevated community (red). Rings delineate a 95% confidence interval for each treatment group. No clustering was observed due to treatment type. (C) Rarefaction plot of 16S markers showing the number of unique ASVs as a function of number of fragments sampled

genotypes tested in the present study, 19 of them showed an overall increase in average lifespan when previously acclimated to an elevated temperature, suggesting the ability to acclimate to temperature stress is a relatively common trait in *A. cervicornis*. Previous studies have typically focused on bleaching as the metric of thermal stress (as opposed to survival as done in the present study), so are not directly comparable. Still, studies in other species of *Acropora* (Maynard et al. 2008, Middlebrook et al. 2008, Bellantuono et al. 2012) have consistently shown that previous exposure to a heat stress results in better performance in a subsequent heat stress. Coles & Jokiel (1978) provided evidence of acclimation in *Montipora verrucosa* and similar responses have been documented in coral of the genera *Pocillopora* (Maynard et al. 2008) and *Porites* (Maynard et al. 2008, Edmunds 2014). The consistent finding of acclimation to elevated temperatures across multiple genera suggests that thermal acclimation is generally widespread, although several exceptions exist among particular genera (Klepac & Barshis 2020).

While the ability to acclimate appears to be widespread, the degree to which acclimatization can occur is not universal, particularly when multiple factors are introduced. Instead, the amount of acclimatization observed in studies often differs between species and between populations of the same species that experience different thermal environments. For example, Maynard et al. (2008) showed that previous exposure to heat stress resulted in a greater increase in thermal tolerance in both *Acropora* and *Pocillopora* species when compared to species of *Porites*. Studies of *A. hyacinthus* have documented greater thermal

Table 5. List of bacterial taxa and their relative abundance detected using 16S rDNA markers. Relative abundance is delineated among initial bacterial community (IBC), ambient acclimation (AA), and elevated acclimation (EA). There was no significant difference in Amplicon Sequence Variants (ASVs) among IBC, AA, or EA fragments. However, specificity of ASVs varied among the 3 groups, with the Control group harboring the highest proportion of ASVs designated as 'Unassigned', or specified only to the Kingdom *Bacteria*. NA: these ASVs could not be allocated into a particular Phylum or Class, but still represented a signific proportion of the total reads for this study at the Kingdom or Phylum level. The similar community structure among Control, Ambient, and Elevated treatment fragments suggests that heat treatment had no effect on bacterial community structure. *N. tomentosiformis*: *Nicotiana tomentosiformis*

Taxonomy							Treatment group		
Kingdom	Phylum	Class	Order	Family	Genus	Species	IBC	AA	EA
Bacteria	NA	NA	NA	NA	NA	NA	0.532	0.408	0.339
Bacteria	*Acidobacteria*	*Acidobacteriia*	*Acidobacteriales*	Uncultured	*Acidobacteria* bacterium	*Acidobacteria* bacterium	0.001	0.001	0.004
Bacteria	*Acidobacteria*	*Actinobacteria*	NA	NA	NA	NA	0.004	0.000	0.001
Bacteria	*Acidobacteria*	*Actinobacteria*	*Corynebacteriales*	NA	NA	NA	0.000	0.000	0.000
Bacteria	*Acidobacteria*	*Actinobacteria*	*Corynebacteriales*	*Mycobacteriaceae*	*Mycobacterium*	*Mycobacterium tuberculosis* UT0020	0.005	0.001	0.001
Bacteria	*Acidobacteria*	*Actinobacteria*	*Corynebacteriales*	*Nocardiaceae*	*Rhodococcus*	NA	0.010	0.001	0.000
Bacteria	*Acidobacteria*	*Actinobacteria*	*Micrococcales*	NA	NA	NA	0.000	0.000	0.001
Bacteria	*Acidobacteria*	*Actinobacteria*	*Micrococcales*	*Brevibacteriaceae*	*Brevibacterium*	*Brevibacterium* sp. AB	0.000	0.000	0.000
Bacteria	*Acidobacteria*	*Actinobacteria*	*Micrococcales*	*Micrococcaceae*	NA	NA	0.007	0.001	0.001
Bacteria	*Acidobacteria*	*Actinobacteria*	*Propionibacteriales*	*Propionibacteriaceae*	*Cutibacterium*	Uncultured bacterium	0.020	0.004	0.004
Bacteria	*Bacteroidetes*	*Bacteroidia*	NA	NA	NA	NA	0.000	0.000	0.001
Bacteria	*Bacteroidetes*	*Bacteroidia*	*Flavobacteriales*	NA	NA	NA	0.000	0.000	0.000
Bacteria	*Bacteroidetes*	*Bacteroidia*	*Flavobacteriales*	*Flavobacteriaceae*	NA	NA	0.000	0.000	0.001
Bacteria	*Bacteroidetes*	*Bacteroidia*	*Flavobacteriales*	*Flavobacteriaceae*	*Polaribacter*	*Flavobacteriaceae*	0.001	0.003	0.012
Bacteria	*Bacteroidetes*	*Bacteroidia*	*Flavobacteriales*	*Weeksellaceae*	*Cloacibacterium*	Uncultured bacterium	0.000	0.000	0.000
Bacteria	*Bacteroidetes*	*Bacteroidia*	*Sphingobacteriales*	NS11-12 marine group	Uncultured bacterium	Uncultured bacterium	0.000	0.000	0.000
Bacteria	*Chloroflexi*	*Anaerolineae*	SBR1031	A4b	*Chloroflexi* bacterium	*Chloroflexi* bacterium	0.000	0.000	0.000
Bacteria	*Cyanobacteria*	*Oxyphotobacteria*	Chloroplast	NA	NA	NA	0.003	0.001	0.003
Bacteria	*Cyanobacteria*	*Oxyphotobacteria*	Chloroplast	*N. tomentosiformis*	*N. tomentosiformis*	*N. tomentosiformis*	0.002	0.001	0.001
Bacteria	*Cyanobacteria*	*Oxyphotobacteria*	Chloroplast	*Triticum aestivum*	*Triticum aestivum*	*Triticum aestivum*	0.000	0.000	0.000
Bacteria	*Firmicutes*	NA	NA	NA	NA	NA	0.000	0.000	0.001
Bacteria	*Firmicutes*	*Bacilli*	NA	NA	NA	NA	0.000	0.000	0.001
Bacteria	*Firmicutes*	*Bacilli*	*Bacillales*	NA	NA	NA	0.000	0.000	0.000
Bacteria	*Firmicutes*	*Bacilli*	*Bacillales*	*Bacillaceae*	NA	NA	0.000	0.000	0.001
Bacteria	*Firmicutes*	*Bacilli*	*Bacillales*	*Bacillaceae*	*Bacillus*	NA	0.004	0.004	0.003
Bacteria	*Firmicutes*	*Bacilli*	*Bacillales*	*Bacillaceae*	*Bacillus*	*Bacillus amyloliquefaciens*	0.005	0.004	0.007
Bacteria	*Firmicutes*	*Bacilli*	*Bacillales*	*Bacillaceae*	*Bacillus*	*Bacillus horikoshii*	0.001	0.001	0.000
Bacteria	*Firmicutes*	*Bacilli*	*Bacillales*	*Staphylococcaceae*	*Staphylococcus*	*Staphylococcus aureus*	0.011	0.003	0.001
Bacteria	*Firmicutes*	*Bacilli*	*Lactobacillales*	NA	NA	NA	0.000	0.000	0.000
Bacteria	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Enterococcaceae*	*Enterococcus*	*Enterococcus faecalis*	0.000	0.000	0.000

Table continued on next page

Table 5 (contiued)

Taxonomy							Treatment group		
Kingdom	Phylum	Class	Order	Family	Genus	Species	IBC	AA	EA
Bacteria	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Lactobacillus*	NA	0.000	0.000	0.000
Bacteria	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Lactobacillaceae*	*Lactobacillus*	*Lactobacillus plantarum*	0.000	0.000	0.000
Bacteria	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	NA	0.001	0.000	0.000
Bacteria	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	*Streptococcus thermophilus*	0.002	0.000	0.000
Bacteria	*Firmicutes*	*Clostridia*	*Clostridiales*	NA	NA	NA	0.000	0.001	0.004
Bacteria	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae* 1	*Clostridium* sensu stricto 1	*Clostridium butyricum*	0.000	0.000	0.000
Bacteria	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	*Blautia massiliensis*	0.000	0.001	0.000
Bacteria	*Firmicutes*	*Clostridia*	D8A-2	Digester metagenome	Digester metagenome	Digester metagenome	0.000	0.000	0.000
Bacteria	*Nitrospirae*	*Thermodesulfovibrionia*	Uncultured	*Nitrospirae* bacterium	*Nitrospirae* bacterium	*Nitrospirae* bacterium	0.000	0.000	0.000
Bacteria	*Patescibacteria*	NA	NA	NA	NA	NA	0.000	0.000	0.000
Bacteria	*Patescibacteria*	ABY1	*Kuenenbacteria*	GW2011	GWA2	NA	0.000	0.000	0.000
Bacteria	*Patescibacteria*	*Microgenomatia*	*Woesebacteria*	Uncultured bacterium	Uncultured bacterium	Uncultured bacterium	0.000	0.000	0.001
Bacteria	*Patescibacteria*	*Parcubacteria*	*Portnoybacteria*	NA	NA	NA	0.000	0.000	0.000
Bacteria	*Patescibacteria*	*Parcubacteria*	*Portnoybacteria*	RIFCSPHIGHO2	RIFCSPHIGHO2	NA	0.000	0.010	0.002
Bacteria	*Proteobacteria*	NA	NA	NA	NA	NA	0.002	0.002	0.005
Bacteria	*Proteobacteria*	*Alphaproteobacteria*	NA	NA	NA	NA	0.181	0.298	0.295
Bacteria	*Proteobacteria*	*Alphaproteobacteria*	*Acetobacterales*	*Acetobacteraceae*	*Granulibacter*	*Granulibacter bethesdensis*	0.000	0.000	0.001
Bacteria	*Proteobacteria*	*Alphaproteobacteria*	*Azospirillales*	*Inquilinaceae*	*Inquilinus*	Alpha proteobacterium	0.000	0.000	0.000
Bacteria	*Proteobacteria*	*Alphaproteobacteria*	*Caulobacterales*	*Caulobacteraceae*	*Phenylobacterium*	*Phenylobacterium* sp.	0.001	0.001	0.002
Bacteria	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	NA	NA	NA	0.000	0.000	0.001
Bacteria	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Rhizobiaceae*	NA	NA	0.109	0.187	0.184
Bacteria	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Rhizobiaceae*	ANPR	[*Pseudomonas*] *geniculata*	0.000	0.000	0.000
Bacteria	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Rhizobiaceae*	*Mesorhizobium*	*Mesorhizobium* sp. LNHC252B00	0.003	0.002	0.010
Bacteria	*Proteobacteria*	*Alphaproteobacteria*	*Rhodobacterales*	*Rhodobacteraceae*	*Paracoccus*	*Paracoccus zeaxanthinifaciens*	0.001	0.000	0.001
Bacteria	*Proteobacteria*	*Alphaproteobacteria*	*Rhodobacterales*	*Rhodobacteraceae*	*Pseudophaeobacter*	*Pseudophaeobacter arcticus*	0.001	0.000	0.001
Bacteria	*Proteobacteria*	*Alphaproteobacteria*	*Rickettsiales*	Mitochondria	*Nicotiana otophora*	*Nicotiana otophora*	0.000	0.000	0.000
Bacteria	*Proteobacteria*	*Deltaproteobacteria*	NA	NA	NA	NA	0.000	0.000	0.000
Bacteria	*Proteobacteria*	*Deltaproteobacteria*	*Desulfobacterales*	*Desulfobacteraceae*	Uncultured	NA	0.000	0.000	0.000
Bacteria	*Proteobacteria*	*Deltaproteobacteria*	*Desulfobacterales*	*Desulfobacteraceae*	Uncultured	Uncultured bacterium	0.000	0.000	0.000
Bacteria	*Proteobacteria*	*Deltaproteobacteria*	PB19	Metagenome	Metagenome	Metagenome	0.000	0.000	0.000

Table continued on next page

Table 5 (contiued)

Taxonomy							Treatment group		
Kingdom	Phylum	Class	Order	Family	Genus	Species	IBC	AA	EA
Bacteria	*Proteobacteria*	*Deltaproteobacteria*	*Syntrophobacterales*	*Syntrophobacteraceae*	*Desulfovirga*	Delta proteobacterium	0.000	0.000	0.000
Bacteria	*Proteobacteria*	*Gammaproteobacteria*	NA	NA	NA	NA	0.009	0.006	0.014
Bacteria	*Proteobacteria*	*Gammaproteobacteria*	*Beggiatoales*	*Beggiatoaceae*	*Thiotrichaceae* bacterium	*Thiotrichaceae* bacterium IS1	0.000	0.000	0.002
Bacteria	*Proteobacteria*	*Gammaproteobacteria*	*Betaproteobacteriales*	*Burkholderiaceae*	NA	NA	0.003	0.005	0.008
Bacteria	*Proteobacteria*	*Gammaproteobacteria*	*Betaproteobacteriales*	*Burkholderiaceae*	*Achromobacter*	*Achromobacter* sp.	0.001	0.000	0.000
Bacteria	*Proteobacteria*	*Gammaproteobacteria*	*Betaproteobacteriales*	*Burkholderiaceae*	*Acidovorax*	s-d bacterium a3	0.000	0.000	0.000
Bacteria	*Proteobacteria*	*Gammaproteobacteria*	*Betaproteobacteriales*	*Burkholderiaceae*	*Acidovorax*	Uncultured bacterium	0.000	0.000	0.000
Bacteria	*Proteobacteria*	*Gammaproteobacteria*	*Betaproteobacteriales*	*Burkholderiaceae*	*Paraburkholderia*	NA	0.000	0.000	0.000
Bacteria	*Proteobacteria*	*Gammaproteobacteria*	*Betaproteobacteriales*	*Burkholderiaceae*	*Cupriavidus*	*Cupriavidus basilensis*	0.003	0.004	0.007
Bacteria	*Proteobacteria*	*Gammaproteobacteria*	*Betaproteobacteriales*	*Burkholderiaceae*	Uncultured	Uncultured bacterium	0.000	0.000	0.000
Bacteria	*Proteobacteria*	*Gammaproteobacteria*	*Betaproteobacteriales*	*Rhodocyclaceae*	*Dechloromonas*	Uncultured bacterium	0.000	0.000	0.000
Bacteria	*Proteobacteria*	*Gammaproteobacteria*	*Coxiellales*	*Coxiellaceae*	*Coxiella*	Metagenome	0.001	0.001	0.003
Bacteria	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	NA	NA	0.011	0.006	0.015
Bacteria	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Escherichia-Shigella*	NA	0.002	0.000	0.001
Bacteria	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Escherichia-Shigella*	*Escherichia coli*	0.011	0.008	0.016
Bacteria	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Pectobacterium*	*Pectobacterium carotovorum*	0.000	0.000	0.000
Bacteria	*Proteobacteria*	*Gammaproteobacteria*	*Oceanospirillales*	*Halomonadaceae*	*Halomonas*	NA	0.000	0.000	0.001
Bacteria	*Proteobacteria*	*Gammaproteobacteria*	*Oceanospirillales*	*Halomonadaceae*	*Halomonas*	*Halomonas glaciei*	0.000	0.000	0.000
Bacteria	*Proteobacteria*	*Gammaproteobacteria*	*Pseudomonadales*	*Moraxellaceae*	*Acinetobacter*	NA	0.003	0.000	0.000
Bacteria	*Proteobacteria*	*Gammaproteobacteria*	*Pseudomonadales*	*Moraxellaceae*	*Psychrobacter*	*Psychrobacter* sp. Y48	0.002	0.000	0.000
Bacteria	*Proteobacteria*	*Gammaproteobacteria*	*Pseudomonadales*	*Pseudomonadaceae*	*Pseudomonas*	NA	0.025	0.022	0.031
Bacteria	*Proteobacteria*	*Gammaproteobacteria*	*Vibrionales*	*Vibrionaceae*	NA	NA	0.000	0.000	0.000
Bacteria	*Proteobacteria*	*Gammaproteobacteria*	*Vibrionales*	*Vibrionaceae*	*Salinivibrio*	NA	0.000	0.000	0.000
Bacteria	*Proteobacteria*	*Gammaproteobacteria*	*Vibrionales*	*Vibrionaceae*	*Salinivibrio*	*Salinivibrio* sp. MA421	0.001	0.000	0.000
Bacteria	*Proteobacteria*	*Gammaproteobacteria*	*Xanthomonadales*	*Xanthomonadaceae*	NA	NA	0.000	0.000	0.001
Bacteria	*Proteobacteria*	*Gammaproteobacteria*	*Xanthomonadales*	*Xanthomonadaceae*	*Stenotrophomonas*	Bacterium CNK-7R	0.001	0.000	0.001
Unassigned	NA	NA	NA	NA	NA	NA	0.016	0.006	0.002

tolerance in samples collected from inshore locations that routinely experience large fluctuations in temperature when compared to nearby populations that encounter fewer high-temperature days (Barshis et al. 2010, Oliver & Palumbi 2011). On the other hand, Baumann et al. (2021) showed greater capacity for acclimatization in offshore populations of both *Pseudodiploria strigosa* and *Siderastrea siderea* when compared to those from more variable nearshore habitats. Howells et al. (2013) also found differences in acclimatization ability between populations of *A. millepora* obtained from different latitudes along the Great Barrier Reef. These differences in acclimatization ability among populations suggest that plasticity of thermal tolerance likely has a genetic component that can respond to natural selection.

The results of the present study provide some support for this hypothesis. The ability to acclimate to elevated temperatures varied significantly among genotypes, yielding a relatively low (H^2 = 0.19), but significantly greater than zero, broad-sense heritability estimate of the plasticity of upper thermal tolerance. This means 19% of the variation in plasticity of thermal tolerance could be attributed to genetic differences among coral genets. Narrow-sense heritability of plasticity in thermal tolerance, on the other hand, was typically <0.008 and was not significantly different from zero in any of the models. This suggests most of the variance due to genotype may not be additive, but rather due to dominance or epistatic variance. Since only additive genetic variance can respond predictably to natural selection, an h^2 of approximately 0.0 suggests that plasticity in thermal tolerance is unlikely to evolve in this population of *A. cervicornis*. That said, the present estimate is based on a relatively small sample size (20 genotypes) and heritability estimates are not necessarily constant and can be affected by a number of factors (e.g. adding new genotypes to the nursery population) (Visscher et al. 2008).

It is possible, however, that our estimates of h^2 are lower than the true heritability values. Marker-based narrow-sense heritability estimates rely heavily upon the number of microsatellite markers used and the degree of variation in relatedness among genotypes (Ritland 1996b). Our mean relatedness estimate using the Ritland estimator was negative (–0.097), which according to Ritland, may be due to using an insufficient number of loci, or to the population consisting predominantly of individuals of similar relatedness. While the number of loci utilized in the present study meets the parameters specified by Ritland (1996b), the pairwise relatedness estimates between fragments were mostly zero except for the clonal fragments. Therefore, it is probable that our narrow-sense heritability estimate for plasticity of thermal tolerance was skewed due to low relatedness among genotypes. The low relatedness among genotypes is likely due to the fact that the coral samples utilized came from a nursery and originated from several geographically distinct populations. It seems unlikely that samples collected from distinct reefs separated by >100 km would be close relatives. Therefore, future studies should focus either on natural populations, or nursery samples collected from a single location to maximize the likelihood of obtaining samples with differing degrees of relatedness.

Estimates of the heritability of plasticity in general, much less for traits related to thermal tolerance are not especially common in the literature to our knowledge. That plastic responses can evolve has been suggested from observations of greater plasticity in organisms that occupy variable environments when compared to those that occupy environments with little variability (Via & Lande 1985). That said, Gunderson & Stillman (2015) found little evidence that plasticity in upper thermal tolerance increases with latitude or with thermal seasonality, bringing these expected patterns into question. Even in cases where differences in plasticity appear to be adaptive, actual estimates of the strength of heritability tend to be absent. Logan et al. (2018) showed high heritability (h^2 = 0.41) in the breadth of temperatures tolerated in a pair of lizard species, but a review of heritability estimates for traits of thermal physiology by Logan & Cox (2020) uncovered no other studies that focused on thermal ranges or plasticity. The most common estimates of the heritability of thermal plasticity come from studies of genetic variation underlying differences in gene expression under different environmental conditions (Leder et al. 2015, McCairns et al. 2016). Logan & Cox (2020), however, warned that the magnitude of gene expression shifts may not be concordant with physiological plasticity, so it is unknown how to relate such studies.

While the present study suggests there is genetic variation underlying plasticity of upper thermal tolerance in *A. cervicornis*, which may provide the ability to evolve greater plasticity in response to future climate change, the role plasticity plays in evolution is still considered contentious (Koch & Guillaume 2020). Several authors have suggested plasticity can provide for population persistence in a changing environment by maintaining larger population sizes, effectively buying time and maintaining genetic variation necessary for future adaptive evolution (Chevin et al. 2010,

Snell-Rood et al. 2018). On the other hand, Price et al. (2003) argued that strong phenotypic plasticity could impede evolutionary responses by effectively hiding genetic variation on which selection could act. Trade-offs between plasticity and adaptation have been shown in copepods (Kelly et al. 2017) and butterflies (Oostra et al. 2018), providing evidence that greater plasticity may not be beneficial in the long-term. Whether coral management practices should focus on maximizing plasticity may depend on the population sizes of the target species. Those with small population sizes may require the protection that plasticity provides, while those with larger population sizes may benefit more from selection on increasing critical thermal maxima.

Heritability for upper thermal tolerance among corals acclimated to either ambient or elevated temperature were both estimated as zero with no significant differences among genotypes. These estimates are in stark contrast to results reported in Yetsko et al. (2020), a study in which similar methodology resulted in an estimate of $H^2 = 0.52$ for what we have defined as ambient tolerance in the present study. In the Yetsko et al. (2020) study, 3 out of 20 genotypes of *A. cervicornis* (distinct from, but obtained from the same nursery as those in the present study) showed significant differences in thermal tolerance in pairwise comparisons. Since there was no overlap in the genotypes used in the 2 studies, it would appear that the majority of genotypes do not differ significantly in their tolerance of elevated temperatures, and that random differences in sampling can result in substantial differences in heritability estimates. Therefore, it would seem that the heritability estimates of thermal tolerance reported here be viewed with some caution. That said, the majority of other studies that have estimated heritability of traits related to thermal tolerance in coral have been more in line with those estimated by Yetsko et al. (2020). Both Dziedzic et al. (2019) and Dixon et al. (2015) estimated the heritability of thermal tolerance of corals acclimated to ambient temperatures, with Dixon et al. (2015) estimating broad sense heritability of thermal tolerance of *A. millepora* as $H^2 = 0.87$, and Dziedzic et al. (2019) estimating the narrow sense heritability of thermal tolerance of *Orbicella faveolata* as $h^2 = 0.58$. Similarly, a meta-analysis of heritability estimates for thermal tolerance traits in coral by Bairos-Novak et al. (2021) suggests that significant heritability tends to exist in most coral analyzed thus far. These results suggest that the upper thermal tolerance of many coral species has at least some potential to adapt.

4.2. Mechanism of acclimation

The pattern of acclimation to elevated temperatures in *A. cervicornis* observed in the present study begs the question of how the previous exposure to a high-temperature stress resulted in greater survival during a second stress. One hypothesized mechanism for acclimation has been symbiont shuffling, where coral hosts alter the dominant dinoflagellate clade to best suit their current environment (Baker et al. 2004). Many coral species retain multiple background clades that comprise <1% of the relative symbiont abundance, which then become more abundant after a bleaching event (Sogin et al. 2006, Reid & Buckley 2011). For example, *Durusdinium* often remains in coral tissue after a bleaching event due to the clade's resistance to extreme temperatures and tendency to remain within its host coral despite varying temperatures (Silverstein et al. 2017).

We detected no change in dinoflagellate endosymbiont populations among pre-acclimation and post-acclimation fragments, regardless of the acclimation protocol. All fragments were dominated by DIV A3 (~95%) in all treatment groups. A3 has been reported as the dominant endosymbiont type in other *A. cervicornis* populations, including in the Dominican Republic (Lirman et al. 2014) and the Florida Keys (O'Donnell et al. 2018). There are 2 possible explanations for similarity in dinoflagellate communities between pre-acclimation and post-acclimation fragments. First, no bleaching was observed during the initial heat stress, suggesting that endosymbionts were not expelled from the fragments to any large extent. As exemplified by Silverstein et al. (2015), if no endosymbionts are expelled, relative proportions of endosymbionts before and after acclimation should remain stable. Second, it is possible *A. cervicornis* is unable to alter endosymbiont populations when encountering elevated temperatures. Dinoflagellate communities are often dependent on host family and exhibit a significant degree of heritability ($h^2 = 0.3$), even in a broadcast spawning species like *A. cervicornis* (Quigley et al. 2017). Furthermore, Goulet (2006) states that species that favor a dominant clade of endosymbionts (including *Acropora*) have never been observed collecting new clades from the environment or from cryptic subpopulations within the colony. The act of collecting new clades from the environment would seem especially unlikely in a laboratory experiment such as the present study.

Bacterial communities are another component of the coral holobiont that may influence the plasticity of thermal tolerance. Ziegler et al. (2017) detected

changes in bacterial populations among corals grown in highly variable and moderately variable thermal environments. Not only were bacteria found in highly variable environments more resilient to thermal stress, but they also enriched functions related to metabolism, which may contribute to plasticity within the coral host. Some bacterial clades such as *Vibrio* sp. have been shown to disrupt microbiome metabolism, while others including *Endozoicomonas* sp. are positively correlated with Symbiodiniaceae density and provide probiotic benefits to the coral host (Bayer et al. 2013, Ding et al. 2016).

In the present study, no change in bacterial communities was observed between the pre-acclimation and post-acclimation samples in either acclimation treatment. All 3 treatment groups were dominated by the Phylum *Proteobacteria*, with classes *Alphaproteobacteria* and *Gammaproteobacteria* representing the majority of proportional ASVs, similar to previous findings for *A. cervicornis* and other Atlantic coral (Carlos et al. 2013, Godoy-Vitorino et al. 2017). *Vibrio* was found among all treatment groups; however, their proportion relative to the abundance of other ASVs was extremely small and did not significantly change as a result of heat stress. Curiously, no *Endozoicomonas* were detected in any coral fragments in the present study, although it is possible this clade was detected but not accurately categorized, as a significant portion of raw ASV reads were superficially allocated to the Kingdom *Bacteria*.

While data from the present study revealed no change in bacterial or dinoflagellate communities in response to a short-term heat stress, it is possible that shuffling occurs at some point after we sampled the symbiont communities. In the present study, symbiont communities were sampled approximately 24 h after the acclimation period. Given the patterns observed in F_v/F_m scores throughout the study, however, it may be prudent for future studies to investigate symbiont communities at later time points. For example, in the present study, F_v/F_m scores showed significant divergence between the AE and EE treatments after the first approximately 120 h during the moderate-duration heat stress, with continued decline in the AE treatment and a plateau in the EE treatment. This pattern suggests that there was a change within the host or endosymbionts after the first 5 d of the second heat stress that altered the photosynthesis occurring in the coral fragments. Therefore, it is possible that shuffling occurred during the moderate-duration heat stress that was not apparent after the initial acclimation period. If shuffling at this time point is ultimately shown, it would suggest that the first heat stress during acclimation somehow primed the coral to shuffle symbionts during the second heat exposure.

A still untested hypothesis to explain the observed thermal acclimation is changes to host gene expression patterns. Changes to gene expression or protein concentrations in response to heat stress have been studied extensively in a variety of coral species (DeSalvo et al. 2008, Császár et al. 2009, Barshis et al. 2010, Bellantuono et al. 2012, Bay & Palumbi 2015, Seneca & Palumbi 2015, Mayfield et al. 2018, Parkinson et al. 2018, Yetsko et al. 2020). The majority of these studies, however, focus on gene expression changes in the short-term and not necessarily across a time frame that involves multiple alterations between ambient and heated conditions. One of the most analogous studies was by Bay & Palumbi (2015), where gene expression patterns in *A. nana* during an acute heat stress were compared among colonies that experienced different acclimation treatments. Gene expression patterns were considerably different among the acclimation treatments showing substantial plasticity in the response depending on previous environmental conditions. Since symbiont shuffling seems an unlikely explanation for the acclimation observed in the present study, future work should focus on how gene expression patterns differ between subsequent heat stress events. Seneca & Palumbi (2015) showed that coral genets collected from highly variable environments that repeatedly experience heat stress had greater resistance to bleaching when expression of many genes returned to baseline levels more quickly (transcriptome resilience). Investigating gene expression patterns across time will be necessary to determine if greater acclimation ability typically involves a quick return to baseline levels of gene expression following a stress event.

Differences in ability to acclimate to thermal stress among genotypes may be due to differences in gene expression patterns. Several studies have shown differences in gene expression patterns among coral genotypes, suggesting that alternative patterns may result in different physiological responses to elevated temperature (DeSalvo et al. 2008, Bellantuono et al. 2012, Barshis et al. 2013, Granados-Cifuentes et al. 2013, Yetsko et al. 2020). Unfortunately, gene expression patterns often vary among genotypes even if they have similar thermal tolerance phenotypes, making identification of gene expression biomarkers to distinguish tolerant and susceptible genotypes problematic (Parkinson et al. 2018, Yetsko et al. 2020). These results conform to the hypothesis that

thermal tolerance is an extremely polygenic trait where many distinct combinations of alleles can lead to similar phenotypes.

4.3. Photosynthetic efficiency patterns

While the present study did not focus on photosynthetic efficiency, we used measurements of F_v/F_m to estimate the health and time-of-death of coral fragments utilized in the experiments. This allowed us to observe patterns in photosynthetic efficiency under a moderate-duration stress and to determine if F_v/F_m could be a useful metric to predict thermal plasticity of a coral genet. Unfortunately, the differences in tolerance to elevated temperature were not predictable through the metric of F_v/F_m. Estimates of F_v/F_m during the acclimation portion of the study did not significantly influence lifespan. While variability among genets was high, there is no evidence to suggest that it can be used as a predictor of temperature tolerance in any way. Therefore, it appears that longer-term studies of response to temperature, or other biomarkers will be necessary for managers seeking to identify resistant genotypes.

More interestingly, however, were the patterns by which F_v/F_m scores dropped throughout the study. Instead of a linear decline, there was a distinct asymptote in the EE treatment. While the drop in F_v/F_m in both the AE and EE experimental groups dropped along the same trajectory for the first approximately 120 h, the patterns diverged in the 2 treatment groups, with F_v/F_m continuing to decline in the AE treatment while the scores plateaued in the EE treatment. This pattern suggests that something happened after the first 5 d of the second heat stress that stabilized the photosynthesis occurring in the coral fragments. We can conceive of 3 potential explanations for this plateau in F_v/F_m in the EE treatment. (1) It is possible that symbiont shuffling occurred as the coral neared the 5 d time point, resulting in greater photosynthetic efficiency of the remaining community. (2) The initial short-term heat stress experienced by the EE treatment may have acclimated the dinoflagellate community. (3) Alterations of host gene expression patterns may have protected the symbiont community during the second heat stress. Unfortunately, none of these hypotheses can be tested with the data on hand.

What is especially intriguing is the timing of divergence in F_v/F_m between the AE and the EE treatment, since it suggests that alterations that occur during the first heat stress in the EE treatment have a delayed effect on stress levels experienced in the second heat treatment. If the acclimation is ultimately due to alterations in gene expression as opposed to symbiont shuffling, it would seem that some important alterations may not occur until several days after experiencing the second heat stress. Since most studies of gene expression changes in response to elevated temperatures concentrate on alterations that occur in the first 1 to 3 d, it is possible that important changes that enable the observed increase in tolerance are being missed. This suggests that future studies may want to explore gene expression patterns of thermal tolerance over a greater span of time.

4.4. Environmental influences on plasticity

The low estimates of broad-sense heritability in this experiment were due to high variability in lifespan among fragments of the same genet. Because clones are genetically identical, we can attribute the difference in lifespan among fragments of the same genet to environmental factors. Differences in thermal tolerance of different portions of the same coral genet are not necessarily surprising. Several studies have shown that portions of the same coral colony that have been exposed to different light and temperature environments (e.g. exposed vs. shaded sides of a colony) can have different temperature tolerances or susceptibility to bleaching (Brown et al. 2000, 2002). This same factor could alter the tolerance of elevated temperatures in a nursery environment as well. Nursery trees of the CRF can be several meters in length, with tree-tops averaging approximately 5 m below the surface and the lowest branches about 8 m deep. Some fragments of the same genotype may have originated from different parts of the nursery tree or have been placed in different locations on the tree (i.e. lower or higher in the water column), resulting in a shading effect of fragments that originated or were placed lower on the tree. Even the position of a coral fragment in a tank, or the position of the tank during the experiment could induce differences in light exposure, potentially resulting in environmentally induced variation. These small-scale environmental gradients may induce differential gene expression among fragments, as observed in the wild by Bay et al. (2009) and Durante et al. (2019).

Acclimatization to local stressors in coral may be partially attributed to differential methylation, where genes are methylated differently depending on immediate environmental factors (Putnam 2021). Methylation refers to epigenetic changes, or modifica-

tions in DNA and chromatin that do not change the DNA sequence (Duncan et al. 2014). Addition of methyl groups to histones can decrease the tension with which the proteins wind DNA, allowing for easier access to transcription factors and thereby increasing the transcription of genes responsible for ameliorating thermal stress.

4.5. Summary

In conclusion, *A. cervicornis* exhibits phenotypic plasticity of thermal tolerance, the strength of which differs significantly among and within genotypes. While significant heritability of thermal plasticity is observed, the heritability is relatively low when compared to other estimates of traits related to thermal tolerance in species of coral. The strong environmental component to the variation in thermal plasticity suggests that previous environmental exposure likely plays a role in much of the observed differences both within and among genets. While the mechanism of the observed plasticity has not been confirmed, there was no evidence in the present study to support the hypothesis of symbiont shuffling during heat stress, at least among fragments of the same genotype subjected to the same conditions as in the earlier heritability experiment. Therefore, it is more likely that differences in gene expression of the host or its symbionts are responsible for the differences in acclimation ability among genotypes. Additional studies focused on patterns of gene or protein expression will be necessary to determine if this is indeed the case. Future studies should also consider the timing of sampling of symbionts or samples for gene expression since there appears to be a delayed effect of stress levels as estimated by the decline in F_v/F_m.

Acknowledgements. We thank the Florida 'Protect Our Reefs' fund (POR-2016-10) and the University of North Florida Graduate School and Coastal Biology Program for providing funding for this research. Thanks to the Coral Restoration Foundation for providing the coral fragments and the Keys Marine Laboratory for assistance with experimental setup. We also thank C. Ross and M. Aspinwall for their help in addressing questions pertaining to coral physiology and plasticity of thermal tolerance.

LITERATURE CITED

Aronson RB, Precht WF (2001) White-band disease and the changing face of Caribbean coral reefs. Hydrobiologia 460:25–38

Babcock RC (1991) Comparative demography of three species of scleractinian corals using age-and size-dependent classifications. Ecol Monogr 61:225–244

Bairos-Novak KR, Hoogenboom MO, van Oppen MJ, Connolly SR (2021) Coral adaptation to climate change: Meta-analysis reveals high heritability across multiple traits. Glob Change Biol 27:5694–5710

Baker AC (2003) Flexibility and specificity in coral-algal symbiosis: diversity, ecology, and biogeography of *Symbiodinium*. Annu Rev Ecol Evol Syst 34:661–689

Baker AC, Starger CJ, McClanahan TR, Glynn PW (2004) Corals' adaptive response to climate change. Nature 430: 741

Barshis DJ, Stillman JH, Gates RD, Toonen RJ, Smith LW, Birkeland C (2010) Protein expression and genetic structure of the coral *Porites lobata* in an environmentally extreme Samoan back reef: does host genotype limit phenotypic plasticity? Mol Ecol 19:1705–1720

Barshis DJ, Ladner JT, Oliver TA, Seneca FO, Traylor-Knowles N, Palumbi SR (2013) Genomic basis for coral resilience to climate change. Proc Natl Acad Sci USA 110:1387–1392

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

Baums IB, Hughes CR, Hellberg ME (2005) Mendelian microsatellite loci for the Caribbean coral *Acropora palmata*. Mar Ecol Prog Ser 288:115–127

Baums IB, Devlin-Durante MK, Brown L, Pinzón JH (2009) Nine novel, polymorphic microsatellite markers for the study of threatened Caribbean *Acroporid* corals. Mol Ecol Resour 9:1155–1158

Bay RA, Palumbi SR (2015) Rapid acclimation ability mediated by transcriptome changes in reef-building corals. Genome Biol Evol 7:1602–1612

Bay LK, Nielsen HB, Jarmer H, Seneca F, Van Oppen MJ (2009) Transcriptomic variation in a coral reveals pathways of clonal organization. Mar Genomics 2:119–125

Bayer T, Neave MJ, Alsheikh-Hussain A, Aranda M and others (2013) The microbiome of the Red Sea coral *Stylophora pistillata* is dominated by tissue-associated *Endozoicomonas* bacteria. Appl Environ Microbiol 79:4759–4762

Bellantuono AJ, Granados-Cifuentes C, Miller DJ, Hoegh-Guldberg O, Rodriguez-Lanetty M (2012) Coral thermal tolerance: tuning gene expression to resist thermal stress. PLOS ONE 7:e50685

Berkelmans R, Van Oppen MJ (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 R Soc B 273:2305–2312

Berkelmans R, Willis BL (1999) Seasonal and local spatial patterns in the upper thermal limits of corals on the inshore Central Great Barrier Reef. Coral Reefs 18: 219–228

Bligh J, Cloudsley-Thompson JL, Macdonald AG (eds) (1976) Environmental physiology of animals. Halsted Press, New York, NY

Boulotte NM, Dalton SJ, Carroll AG, Harrison PL, Putnam HM, Peplow LM, Van Oppen MJ (2016) Exploring the *Symbiodinium* rare biosphere provides evidence for symbiont switching in reef-building corals. ISME J 10: 2693–2701

Brown BE, Dunne RP, Goodson MS, Douglas AE (2000) Bleaching patterns in reef corals. Nature 404:142–143

Brown BE, Dunne RP, Goodson MS, Douglas AE (2002)

Experience shapes the susceptibility of a reef coral to bleaching. Coral Reefs 21:119–126
Byler KA, Carmi-Veal M, Fine M, Goulet TL (2013) Multiple symbiont acquisition strategies as an adaptive mechanism in the coral *Stylophora pistillata.* PLOS ONE 8:e59596
Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 13: 581–583
Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL (2009) BLAST+: architecture and applications. BMC Bioinformatics 10:421
Camp EF, Schoepf V, Mumby PJ, Hardtke LA, Rodolfo-Metalpa R, Smith DJ, Suggett DJ (2018) The future of coral reefs subject to rapid climate change: lessons from natural extreme environments. Front Mar Sci 5:4
Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K and others (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335–336
Carlos C, Torres TT, Ottoboni LM (2013) Bacterial communities and species-specific associations with the mucus of Brazilian coral species. Sci Rep 3:1624
Carpenter KE, Abrar M, Aeby G, Aronson RB and others (2008) One-third of reef-building corals face elevated extinction risk from climate change and local impacts. Science 321:560–563
Chevin LM, Lande R, Mace GM (2010) Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. PLOS Biol 8:e1000357
Coles SL, Jokiel PL (1978) Synergistic effects of temperature, salinity and light on the hermatypic coral *Montipora verrucosa.* Mar Biol 49:187–195
Császár NBM, Seneca FO, van Oppen MJH (2009) Variation in antioxidant gene expression in the scleractinian coral *Acropora millepora* under laboratory thermal stress. Mar Ecol Prog Ser 392:93–102
Császár NB, Ralph PJ, Frankham R, Berkelmans R, van Oppen MJH (2010) Estimating the potential for adaptation of corals to climate warming. PLOS ONE 5:e9751
Csilléry K, Johnson T, Beraldi D, Clutton-Brock T, Coltman D, Hansson B, Pemberton JM (2006) Performance of marker-based relatedness estimators in natural populations of outbred vertebrates. Genetics 173:2091–2101
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
DeMerlis A, Kirkland A, Kaufman ML, Mayfield AB and others (2022) Pre-exposure to a variable temperature treatment improves the response of *Acropora cervicornis* to acute thermal stress. Coral Reefs 41:435–445
DeSalvo MK, Voolstra CR, Sunagawa S, Schwarz JA and others (2008) Differential gene expression during thermal stress and bleaching in the Caribbean coral *Montastraea faveolata.* Mol Ecol 17:3952–3971
DeWitt TJ, Sih A, Wilson DS (1998) Costs and limits of phenotypic plasticity. Trends Ecol Evol 13:77–81
Ding JY, Shiu JH, Chen WM, Chiang YR, Tang SL (2016) Genomic insight into the host–endosymbiont relationship of *Endozoicomonas montiporae* CL-33T with its coral host. Front Microbiol 7:251
Dixon GB, Davies SW, Aglyamova GV, Meyer E, Bay LK, Matz MV (2015) Genomic determinants of coral heat tolerance across latitudes. Science 348:1460–1462
Duncan EJ, Gluckman PD, Dearden PK (2014) Epigenetics, plasticity, and evolution: How do we link epigenetic change to phenotype? J Exp Zool B Mol Dev Evol 322: 208–220
Durante MK, Baums IB, Williams DE, Vohsen S, Kemp DW (2019) What drives phenotypic divergence among coral clonemates of *Acropora palmata?* Mol Ecol 28:3208–3224
Dziak JJ, Coffman DL, Li R, Litson K, Chakraborti Y (2021) tvem: time-varying effect models.. R package version 1.3. https://CRAN.R-project.org/package=tvem
Dziedzic KE, Elder H, Tavalire H, Meyer E (2019) Heritable variation in bleaching responses and its functional genomic basis in reef-building corals (*Orbicella faveolata*). Mol Ecol 28:2238–2253
Edmunds PJ (2014) Is acclimation beneficial to scleractinian corals, *Porites* spp.? Mar Biol 161:1531–1542
Eren AM, Esen ÖC, Quince C, Vineis JH, Morrison HG, Sogin ML, Delmont TO (2015) Anvi'o: an advanced analysis and visualization platform for 'omics data. PeerJ 3:e1319
Falconer DS, MacKay TFC (1996) Introduction to quantitative genetics, 4th edn, Addison Wesley Longman, Harlow
Fisher R (1930) The genetical theory of natural selection. Clarendon Press, Oxford
Fitt WK, Gates, RD, Hoegh-Guldberg O, Bythell JC and others (2009) Response of two species of Indo-Pacific corals, *Porites cylindrica* and *Stylophora pistillata,* to short-term thermal stress: the host does matter in determining the tolerance of corals to bleaching. J Exp Mar Biol Ecol 373: 102–110
Fregley MM (1996) Adaptations: some general characteristics. In: Fregly MJ, Blatties CM (eds) Handbook of physiology, section 4: environmental physiology. Oxford University Press, New York, NY, p 3–15
Ghalambor CK, Angeloni LM, Carroll SP (2010) Behavior as phenotypic plasticity. In: Westneat DF, Fox CW (eds) Evolutionary behavioral ecology. Oxford University Press, New York, NY, p 90–107
Godoy-Vitorino F, Ruiz-Diaz CP, Rivera-Seda A, Ramírez-Lugo JS, Toledo-Hernández C (2017) The microbial biosphere of the coral *Acropora cervicornis* in Northeastern Puerto Rico. PeerJ 5:e3717
Goulet TL (2006) Most corals may not change their symbionts. Mar Ecol Prog Ser 321:1–7
Granados-Cifuentes C, Bellantuono AJ, Ridgway T, Hoegh-Guldberg O, Rodriguez-Lanetty M (2013) High natural gene expression variation in the reef-building coral *Acropora millepora*: potential for acclimative and adaptive plasticity. BMC Genomics 14:228
Greenstein BJ, Pandolfi JM (2008) Escaping the heat: range shifts of reef coral taxa in coastal Western Australia. Glob Change Biol 14:513–528
Grottoli AG, Toonen RJ, van Woesik R, Vega Thurber R and others (2021) Increasing comparability among coral bleaching experiments. Ecol Appl 31:e02262
Gunderson AR, Stillman JH (2015) Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. Proc R Soc B 282:20150401
Hackerott S, Martell HA, Eirin-Lopez JM (2021) Coral environmental memory: causes, mechanisms, and consequences for future reefs. Trends Ecol Evol 36:1011–1023
Hammer Ø, Harper DA, Ryan PD (2001) PAST: paleontological statistics software package for education and data analysis. Palaeontol Electronica 4:4
Hoegh-Guldberg O (1999) Climate change, coral bleaching and the future of the world's coral reefs. Mar Freshw Res 50:839–866

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS and others (2007) Coral reefs under rapid climate change and ocean acidification. Science 318:1737–1742

Howells EJ, Beltran VH, Larsen NW, Bay LK, Willis BL, van Oppen MJH (2012) Coral thermal tolerance shaped by local adaptation of photosymbionts. Nat Clim Chang 2: 116–120

Howells EJ, Berkelmans R, van Oppen MJ, Willis BL, Bay LK (2013) Historical thermal regimes define limits to coral acclimatization. Ecology 94:1078–1088

Hume B, D'Angelo C, Burt J, Baker A, Riegl B, Wiedenmann J (2013) Corals from the Persian/Arabian Gulf as models for thermotolerant reef-builders: prevalence of clade C3 *Symbiodinium*, host fluorescence and *ex situ* temperature tolerance. Mar Pollut Bull 72:313–322

Hume BCC, D'Angelo C, Smith EG, Stevens JR, Burt J, Wiedenmann J (2015) *Symbiodinium thermophilum* sp. nov., a thermotolerant symbiotic alga prevalent in corals of the world's hottest sea, the Persian/Arabian Gulf. Sci Rep 5:8562

Hume BCC, Ziegler M, Poulain J, Pochon X and others (2018) An improved primer set and amplification protocol with increased specificity and sensitivity targeting the *Symbiodinium* ITS2 region. PeerJ 6:e4816

IPCC (Intergovernmental Panel on Climate Change) (2014) Climate change 2014. Mitigation of climate change. Edenhofer O, Pichs-Madruga R, Sokona Y, Farahani E and others (eds) Contribution of Working Group III to the fifth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge

Jokiel PL, Coles SL (1990) Response of Hawaiian and other Indo-Pacific reef corals to elevated temperature. Coral Reefs 8:155–162

Jones AM, Berkelmans R, van Oppen MJH, Mieog JC, Sinclair W (2008) A community change in the algal endosymbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. Proc R Soc B 275:1359–1365

Kelly MW, Pankey MS, DeBiasse MB, Plachetzki DC (2017) Adaptation to heat stress reduces phenotypic and transcriptional plasticity in a marine copepod. Funct Ecol 31: 398–406

Kenkel CD, Matz MV (2017) Gene expression plasticity as a mechanism of coral adaptation to a variable environment. Nat Ecol Evol 1:0014

Kenkel CD, Meyer E, Matz MV (2013) Gene expression under chronic heat stress in populations of the mustard hill coral (*Porites astreoides*) from different thermal environments. Mol Ecol 22:4322–4334

Kenkel CD, Setta SP, Matz MV (2015) Heritable differences in fitness-related traits among populations of the mustard hill coral, *Porites astreoides.* Heredity 115:509–516

Klepac CN, Barshis DJ (2020) Reduced thermal tolerance of massive coral species in a highly variable environment. Proc R Soc B 287:20201379

Klindworth A, Pruess E, Schweer T, Peplies J, Quast C, Horn M, Glöckner FO (2013) Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res 41:e1

Koch EL, Guillaume F (2020) Additive and mostly adaptive plastic responses of gene expression to multiple stress in *Tribolium castaneum*. PLOS Genet 16:e1008768

Lande R, Shannon S (1996) The role of genetic variation in adaptation and population persistence in a changing environment. Evolution 50:434–437

Leder EH, McCairns RJS, Leinonen T, Cano JM and others (2015) The evolution and adaptive potential of transcriptional variation in sticklebacks — signatures of selection and widespread heritability. Mol Biol Evol 32: 674–689

Legendre P, Gallagher ED (2001) Ecologically meaningful transformations for ordination of species data. Oecologia 129:271–280

Leichter JJ, Helmuth B, Fischer AM (2006) Variation beneath the surface: quantifying complex thermal environments on coral reefs in the Caribbean, Bahamas and Florida. J Mar Res 64:563–588

Lirman D, Schopmeyer S, Galvan V, Drury C, Baker AC, Baums IB (2014) Growth dynamics of the threatened Caribbean staghorn coral *Acropora cervicornis*: influence of host genotype, symbiont identity, colony size, and environmental setting. PLOS ONE 9:e107253

Logan ML, Cox CL (2020) Genetic constraints, transcriptome plasticity, and the evolutionary response to climate change. Front Genet 11:538226

Logan ML, Curlis JD, Gilbert AL, Miles DB, Chung AK, McGlothlin JW, Cox RM (2018) Thermal physiology and thermoregulatory behaviour exhibit low heritability despite genetic divergence between lizard populations. Proc R Soc B 285:20180697

Lynch M, Ritland K (1999) Estimation of pairwise relatedness with molecular markers. Genetics 152:1753–1766

Mayfield AB, Chen YJ, Lu CY, Chen CS (2018) The proteomic response of the reef coral *Pocillopora acuta* to experimentally elevated temperature. PLOS ONE 13:e0192001

Maynard JA, Anthony KRN, Marshall PA, Masiri I (2008) Major bleaching events can lead to increased thermal tolerance in corals. Mar Biol 155:173–182

McCairns RJS, Smith S, Sasaki M, Bernatchez L, Beheregaray LB (2016) The adaptive potential of subtropical rainbowfish in the face of climate change: heritability and heritable plasticity for the expression of candidate genes. Evol Appl 9:531–545

Meyer E, Davies S, Wang S, Willis BL, Abrego D, Juenger TE, Matz MV (2009) Genetic variation in responses to a settlement cue and elevated temperature in the reef-building coral *Acropora millepora.* Mar Ecol Prog Ser 392:81–92

Middlebrook R, Hoegh-Guldberg O, Leggat W (2008) The effect of thermal history on the susceptibility of reef-building corals to thermal stress. J Exp Biol 211:1050–1056

Mieog JC, van Oppen MJH, Cantin NE, Stam WT, Olsen JL (2007) Real-time PCR reveals a high incidence of *Symbiodinium* clade D at low levels in four scleractinian corals across the Great Barrier Reef: implications for symbiont shuffling. Coral Reefs 26:449–457

Muscatine L (1990) The role of symbiotic algae in carbon and energy flux in reef corals. In: Dubinsky Z (ed) Coral reefs. Ecosystems of the world, Vol 25. Elsevier, New York, NY, p 75–87

Nakagawa S, Schielzeth H (2010) Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. Biol Rev Camb Philos Soc 85:935–956

O'Donnell KE, Lohr KE, Bartels E, Baums IB, Patterson JT (2018) *Acropora cervicornis* genet performance and symbiont identity throughout the restoration process. Coral Reefs 37:1109–1118

Oliver TA, Palumbi SR (2011) Do fluctuating temperature

environments elevate coral thermal tolerance? Coral Reefs 30:429–440
Oostra V, Saastamoinen M, Zwaan BJ, Wheat CW (2018) Strong phenotypic plasticity limits potential for evolutionary responses to climate change. Nat Commun 9:1005
Parkinson JE, Bartels E, Devlin-Durante MK, Lustic C and others (2018) Extensive transcriptional variation poses a challenge to thermal stress biomarker development for endangered corals. Mol Ecol 27:1103–1119
Pigliucci M (2005) Evolution of phenotypic plasticity: where are we going now? Trends Ecol Evol 20:481–486
Precht WF, Aronson RB (2004) Climate flickers and range shifts of reef corals. Front Ecol Environ 2:307–314
Price TD, Qvarnstrom A, Irwin DE (2003) The role of phenotypic plasticity in driving genetic evolution. Proc R Soc B 270:1433–1440
Putnam HM (2021) Avenues of reef-building coral acclimatization in response to rapid environmental change. J Exp Biol 224:jeb239319
Quast C, Pruesse E, Yilmaz P, Gerken J and others (2012) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41:D590–D596
Quigley KM, Willis BL, Bay LK (2017) Heritability of the *Symbiodinium* community in vertically- and horizontally-transmitting broadcast spawning corals. Sci Rep 7:8219
R Core Team (2021) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
Rasher DB, Hay ME (2010) Chemically rich seaweeds poison corals when not controlled by herbivores. Proc Natl Acad Sci USA 107:9683–9688
Reid A, Buckley M (2011) The rare biosphere. American Academy of Microbiology, Washington, DC
Ritland K (1996a) A marker-based method for inferences about quantitative inheritance in natural populations. Evolution 50:1062–1073
Ritland K (1996b) Estimators for pairwise relatedness and individual inbreeding coefficients. Genet Res 67:175–185
Ritland K (2004) MARK — genetic marker analysis program. University of British Columbia, Vancouver, BC
Russello MA, Amato G (2004) *Ex situ* population management in the absence of pedigree information. Mol Ecol 13:2829–2840
Rützler K, Macintyre IG (1982) The habitat distribution and community structure of the barrier reef complex at Carrie Bow Cay, Belize. The Atlantic barrier reef ecosystem at Carrie Bow Cay, Belize. I. Structure and communities. Smithson Contrib Mar Sci 12:9–45
Schloss PD, Westcott SL, Ryabin T, Hall JR and others (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537–7541
Seneca FO, Palumbi SR (2015) The role of transcriptome resilience in resistance of corals to bleaching. Mol Ecol 24:1467–1484
Seneca FO, Forêt S, Ball EE, Smith-Keune C, Miller DJ, van Oppen MJH (2010) Patterns of gene expression in a scleractinian coral undergoing natural bleaching. Mar Biotechnol (NY) 12:594–604
Silverstein RN, Cunning R, Baker AC (2015) Change in algal symbiont communities after bleaching, not prior heat exposure, increases heat tolerance of reef corals. Global Change Biol 21:236–249
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
Snell-Rood EC, Kobiela ME, Sikkink KL, Shephard AM (2018) Mechanisms of plastic rescue in novel environments. Annu Rev Ecol Evol Syst 49:331–354
Sogin ML, Morrison HG, Huber JA, Welch DM and others (2006) Microbial diversity in the deep sea and the underexplored 'rare biosphere'. Proc Natl Acad Sci USA 103: 12115–12120
Stoffel M, Nakagawa S, Schielzeth H (2017) rptR: repeatability estimation and variance decomposition by generalized linear mixed-effects models. Methods Ecol Evol 8: 1639–1644
Sultan SE (1995) Phenotypic plasticity and plant adaptation. Acta Bot Neerl 44:363–383
Tan X, Shiyko MP, Li R, Li Y, Dierker L (2012) A time-varying effect model for intensive longitudinal data. Psychol Methods 17:61–77
Thornhill DJ, Xiang YU, Fitt WK, Santos SR (2009) Reef endemism, host specificity and temporal stability in populations of symbiotic dinoflagellates from two ecologically dominant Caribbean corals. PLOS ONE 4:e6262
Tunnicliffe V (1981) Breakage and propagation of the stony coral *Acropora cervicornis.* Proc Natl Acad Sci USA 78: 2427–2431
Van De Casteele T, Galbusera P, Matthysen E (2001) A comparison of microsatellite-based pairwise relatedness estimators. Mol Ecol 10:1539–1549
Via S (1993) Adaptive phenotypic plasticity: target or by-product of selection in a variable environment? Am Nat 142:352–365
Via S, Lande R (1985) Genotype-environment interaction and the evolution of phenotypic plasticity. Evolution 39: 505–522
Visscher PM, Hill WG, Wray NR (2008) Heritability in the genomics era — concepts and misconceptions. Nat Rev Genet 9:255–266
Voolstra CR, Valenzuela JJ, Turkarslan S, Cárdenas A and others (2021) Contrasting heat stress response patterns of coral holobionts across the Red Sea suggest distinct mechanisms of thermal tolerance. Mol Ecol 30:4466–4480
Wang J (2011) COANCESTRY: a program for simulating, estimating, and analyzing relatedness and inbreeding coefficients. Mol Ecol Resour 11:141–145
Weis VM (2010) The susceptibility and resilience of corals to thermal stress: adaptation, acclimatization, or both? Mol Ecol 19:1515–1517
Yamano H, Sugihara K, Nomura K (2011) Rapid poleward range expansion of tropical reef corals in response to rising sea surface temperatures. Geophys Res Lett 38: L04601
Yetsko K, Ross M, Bellantuono A, Merselis D, Rodriquez-Lanetty M, Gilg MR (2020) Genetic differences in thermal tolerance among colonies of threatened coral *Acropora cervicornis*: potential for adaptation to increasing temperature. Mar Ecol Prog Ser 646:45–68
Ziegler M, Seneca FO, Yum LK, Palumbi SR, Voolstra CR (2017) Bacterial community dynamics are linked to patterns of coral heat tolerance. Nat Commun 8:14213

Editorial responsibility: Inna Sokolova, Rostock, Germany
Reviewed by: C. Kenkel and 1 anonymous referee

Submitted: March 3, 2022
Accepted: October 5, 2022
Proofs received from author(s): November 18, 2022