Coral Reefs (2022) 41:1681–1692
https://doi.org/10.1007/s00338-022-02300-4

REPORT

Cross-generational heritability analysis of physiological traits in *Porites astreoides* across an inshore-offshore gradient in the Lower Florida Keys

Yingqi Zhang[1] · **Shelby J. Barnes**[1] · **Carly D. Kenkel**[1]

Received: 9 March 2022 / Accepted: 23 August 2022 / Published online: 15 September 2022

Abstract Estimating the heritable genetic variation in fitness-related traits is key to projecting the adaptive evolution of organisms in response to a changing environment. While heritability studies on reef-building corals to date support adaptive capacity, little is known about the dynamics of trait heritability across life stages in which distinct selective pressures can have long-lasting effects both within and across generations. In this study, we obtained heritability estimates for energetic and thermal stress response traits in adult, larval, and recruit *Porites astreoides* from two populations in the Lower Florida Keys. To induce bleaching phenotypes among individual families, larvae were exposed to a 4-day thermal stress at 32 °C, whereas adults and recruits received the same treatment for 22 days. Origin-dependent tolerance was observed in two life stages where offshore recruits lost more symbiont cells under heat than inshore recruits compared to their respective controls and heat-treated offshore adults suffered a greater loss in total protein content. Surprisingly, larvae appeared to be largely insensitive to heat regardless of origin. Broad sense heritability (H^2) estimates varied greatly among traits and life stages, which may reflect changes in the relative importance of genetic and environmental variation throughout development. Over 50% of the variation in all larval traits, adult symbiont density and chlorophyll a concentration, and recruit protein content can be attributable to genetic factors. The overall moderate to high H^2 estimates measured here suggest family-level variation can persist across different life stages and these corals may be equipped with considerable potential to adapt to environmental change.

Keywords Coral · Life stage · Bleaching · Thermal tolerance · Protein content

Introduction

Understanding the adaptive potential of marine organisms is essential to predict population dynamics and persistence in light of rapidly changing ocean temperature and chemistry (Munday et al. 2013; IPCC 2021). For quantitative traits, heritability, which is defined as the proportion of total phenotypic variation attributed to genetic variation, is often used as a metric to estimate the evolvability of those traits under selection (Falconer and Mackay 1996). Specifically, broad sense heritability (H^2) includes all genetic factors, including both additive genetic effects and non-additive genetic effects, such as dominance and epistasis. In contrast, narrow sense heritability (h^2) is a fraction of H^2 and only accounts for additive genetic factors, which are guaranteed to be inherited by the next generation via sexual reproduction. Therefore, h^2 describes the true adaptive potential of a given trait but requires knowledge of relatedness or pedigree.

Scleractinian corals are foundation species in reef ecosystems but have undergone severe population declines in recent decades due to increased anthropogenic activities (Hughes et al. 2017; Hoegh-Guldberg et al. 2007). The most common stress response in corals is bleaching,

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00338-022-02300-4.

✉ Yingqi Zhang
yingqizh@usc.edu

[1] Department of Biological Sciences, University of Southern California, 3616 Trousdale Parkway, Los Angeles, CA 90089, USA

which refers to the whitening of tissues resulting from the dissociation of endosymbiotic algae from the coral host (Lesser 2011). Elevated temperature has been repeatedly shown to be a major cause of mass bleaching events worldwide (Hughes et al. 2018; Jokiel and Coles 1990). Factors that influence the thermal tolerance of corals include host genetics (Bay and Palumbi 2014), diversity and flexibility of endosymbiont (family Symbiodiniaceae) (Berkelmans and van Oppen 2006), other members of the microbial community (Voolstra et al. 2021), and prior acclimatization to thermal stress (Ainsworth et al. 2016), which may be mediated by epigenetic changes (Dixon et al. 2018). Each component of the coral holobiont is characterized by vastly different generation time (e.g., 3–74 days for symbiont *in hospite* vs. 4–20 yrs for coral host) (Muscatine et al. 1984; Babcock 1991), thus the rate of evolutionary change in each symbiont partner may not align, further complicating the projection of future reefs as sea surface temperature continues to increase.

Similar to other marine invertebrates, corals exhibit complex life cycles that oscillate between the planktonic larval stage and the benthic juvenile/adult stages (Richmond and Hunter 1990). Different life stages encounter distinct selective pressures but phenotypic changes in a given life stage resulting from interactions with its environment can have long-lasting effects both within and across generations (Marshall and Morgan 2011; Putnam 2021). The majority of work to date has focused on the effects of abiotic stressors on the physiology and critical developmental processes of one distinct life stage and only a handful of studies have compared phenotypic response to similar stress among multiple life stages (Wong et al. 2021; Kenkel et al. 2015a, b; Putnam et al. 2010; Putnam and Gates 2015). Even less is known about how variable the heritability of a given trait may be across a coral's life span (Bairos-Novak et al. 2021). Additive genetic variance (and hence h^2) of fitness-related traits has been shown to increase with age in birds (mute swan and collared flycatcher) and terrestrial mammals (red deer and soay sheep), likely explained by the accumulation of deleterious mutations, or positive selection on genes that increase fitness at earlier life stages but have the opposite effects later in life (Wilson et al. 2008). Life history traits highly related to fitness can also undergo stabilizing selection in a specific life stage and become less heritable due to reduced additive genetic variance (Gustafsson 1986). Carlon et al. (2011) documented lower h^2 estimates of coral skeletal traits in juvenile *Favia fragum* than in adult colonies and attributed the differences to environmental effects. More work is needed to shed light on the dynamics of heritability over distinct coral life stages to obtain a holistic understanding of the adaptive potential of critical phenotypes, as only focusing on a single life stage can be misleading (Albecker et al. 2021).

Heritability estimates across life stages may also be impacted by other aspects of coral life-history or environmental variation, including when and how symbionts are acquired. Lingering maternal effects may inflate heritability estimates in larvae, particularly for coral species in which fertilization and larval development are completed within the maternal colony, termed 'brooding' species (Richmond and Hunter 1990). Given the role that algal endosymbionts play in holobiont thermal tolerance traits (Fuller et al. 2020), heritability of such traits will likely also be driven by the extent of genomic fidelity between partners. For species in which offspring directly inherit algal endosymbionts from maternal colonies, known as 'vertical transmitters', there may be greater correlation in thermal tolerance between generations and thus higher heritability (Baker 2003). However, more recent work has suggested that symbiont communities in vertical transmitters may be more flexible (Quigley et al. 2018) and moreover, that symbiont acquisition in species which must acquire algal endosymbionts anew each generation likely has a genetic and thus heritable component to symbiont specificity (Quigley et al. 2017). Finally, heritability is dependent on population-specific parameters and it is not unusual for heritability estimates to vary substantially among populations (Visscher et al. 2008). Given increasing examples of environmental variation influencing coral phenotypes among populations, even across small spatial scales (Thomas et al. 2018; Kenkel et al. 2015a, b), it will be imperative to consider how heritability estimates may be affected by these different drivers.

In this study, we aimed to quantify thermal performance of *P. astreoides* at adult, larval, and recruit life stages as well as estimate broad sense heritability of various physiological traits shared across all stages. We utilized two distinct populations originating from an inshore and an offshore reef environment in the Lower Florida Keys. Compared to offshore reefs at similar latitudes, inshore reefs experienced greater thermal variation and more extreme summer temperatures (Kenkel et al. 2015a, b; Lirman and Fong 2007). Nonetheless, inshore coral populations were characterized by greater thermal tolerance, higher cover, higher growth and calcification rate (Kenkel et al. 2013; Manzello et al. 2015, 2019). Prior studies on inshore and offshore *P. astreoides* populations revealed distinct host genetic structures and transcriptomic profiles but shared symbiont type (ITS2 type A4/A4a), indicating that the animal host might play a bigger role in conferring thermal tolerance (Kenkel et al. 2013; Kenkel and Matz 2017). Although it is important to note that physiological variation among symbiont strains can exist despite a lack of variation in ITS2-type profiles (Hoadley et al. 2021). We aimed to take advantage of standing genetic variation and origin-specific thermal traits between the two populations to answer the following two questions: 1. Are inshore larvae and recruits also more heat resistant than the offshore

offspring? 2. Are traits associated with thermal tolerance more or less heritable depending on life stage?

Methods

Spawning collection and larval thermal stress experiment

A total of 30 adult *Porites astreoides* colonies were collected from an inshore reef site (Summerland Shoals Patch: 24° 36.346′ N, 81° 25.742′ W, $n = 15$) and an offshore reef site (Big Pine Ledges: 24° 33.174′ N, 81° 22.809′ W, $n = 15$) five days before the new moon on April 29, 2019 under permit #FKNMS-2018-033. Colonies were transported to Mote Marine Laboratory's Elizabeth Moore international center for coral reef research and restoration and kept in a shaded (70% PAR reducing) raceway to monitor for larval release. Plastic numerical tags were attached to the bottom of colonies using marine epoxy putty (All-Fix) to track individual identities. Colonies were then placed into flow-through larval collection chambers before sunset according to Kuffner et al. (2006) and exposed to ambient moonlight to mimic the natural planulation processes. Beaker traps were checked for larvae the following morning (April 30, 2019). Most inshore colonies produced enough larvae to be included in the subsequent experiment while only one offshore colony (Family 34) had sufficient planulation. As a result, collection chambers were deployed for a second night to capture more spawning colonies that originated from the offshore site.

A total of ten colonies (five from inshore and five from offshore) were used in the following cross-generational thermal stress experiments. Ten larvae from each colony were immediately fixed in 5% formalin post release and photographed under a stereomicroscope for size estimate. Three groups of ten larvae ($n = 3 \times 10$ per family) from each colony were then subjected to a moderate heat stress challenge beginning on May 1 following the same methods described in Zhang et al. (2019) (Fig. 1). Briefly, three replicate plastic bins were set up for both control (26 °C) and treatment (32 °C) groups under a 12 h:12 h light:dark cycle (Figure S1). Treatment was set to 32 °C because inshore reefs commonly experience this temperature during late summer months (Aug–Sept) when bleaching is likely to occur (Kenkel et al. 2015a, b). Additionally, 32 °C induces a level of stress that can produce distinct bleaching responses among individuals (Zhang et al. 2019). Each bin was filled with 30 L seawater and equipped with a SL381 submersible water pump to maintain circulation, a 100 W aquarium heater, and a HOBO temperature logger (Onset). One group of 10 larvae per spawning colony was added to a 70 uM cell strainer (Grenier Bio-One) which served as a floating netwell in each replicate bin ($n = 3 \times 10$ larvae per family per temperature). Target temperature for the heat treatment bins was achieved by increasing the temperature by 0.5 °C per hour over a 12-h window and maintained for 4 days. No water change was performed due to the short experimental duration and high water volume to biomass ratio, but bins were periodically topped off with distilled water to compensate for evaporation. At the end of the exposure period, swimming larvae in each netwell were sampled on the same day (with seawater removed) and frozen at − 20 °C for subsequent physiological assays.

Remaining larvae were settled onto aragonite plugs preconditioned with crustose coralline algae (CCA) in ambient temperature by family, with the goal of recruiting between 3 and 4 individuals on the top side of each plug to avoid fusion of recruits. For each larval family, 19 plugs were secured by two staggered egg crates placed in a 1.2 L plastic food storage container. After adding seawater to the containers, larvae were gently pipetted onto the plugs and into the water column. Containers were monitored daily and water changes were performed every two days, after which new larvae were introduced to facilitate further recruitment. To securely transport recruits to the University of Southern California (USC), plugs with ideal settlement densities were superglued to the caps of 50 mL falcon tubes, which were then filled up with seawater to displace any air bubbles. Adult colonies that produced larvae were wrapped in wet bubble wrap and transported to USC along with the recruits.

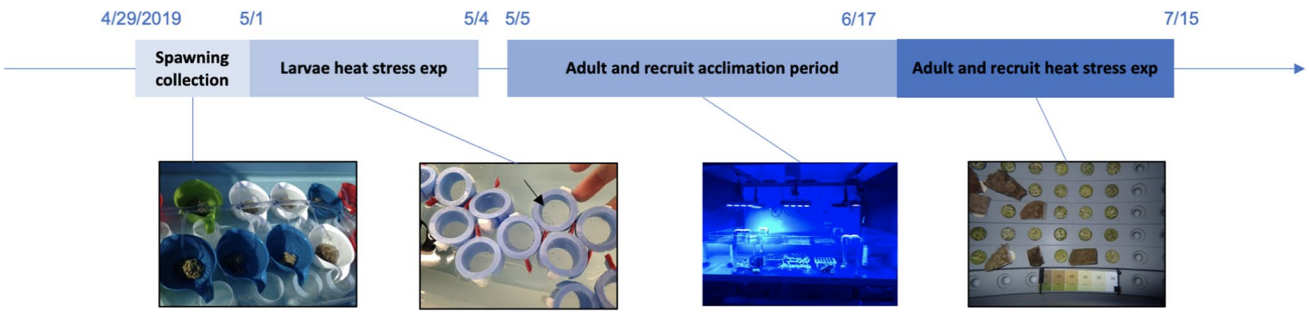

Fig. 1 Experimental design and timeline including ten *Porites astreoides* families

Adult and recruit thermal stress experiments

Adult coral fragments and their recruits were allowed a 37-day recovery and acclimation period in a 500 L holding tank at the Cnidarian Evolutionary Ecology Lab aquarium room at USC (26 °C, 80 µmol photons m^{-2} s^{-1}, 5 cm s^{-1}). After a month of recovery (June 5), six ca. 15 cm^2 fragments were obtained from each adult colony and mounted on clean aragonite plugs using superglue. Adult fragments were then returned to the acclimation tank. To quantify the thermal tolerance of adult and recruit life stages, three replicate 50 L tanks were set up for both control (26 °C) and treatment (32 °C) groups. On June 13, 2019, one adult fragment per colony and an average of 8.5 recruits per family were randomly assigned to each tank. Recruit numbers as well as settlement patterns varied across families (Table S1), thus each tank received between 1 and 6 plug(s) to achieve an even distribution of recruits across tanks. The experimental tanks were individually outfitted with submersible water pumps (Sicce Syncra Silent 0.5, 185 GPH), 150 W HMA-S thermal-regulated heaters (Finnex), and AI Prime HD lights (Aqua Illumination) programmed to imitate the light cycle in FL, with an average daytime PAR of 85–115 µmol photons m^{-2} s^{-1} reaching the tank bottom. Adult and recruit plugs were acclimated to their individual tank conditions with recirculating flow for 4 days. Adult fragments and recruit plugs were cleaned using a small brush every two days for the duration of the experiment to remove any filamentous algal growth. The thermal stress challenge started on June 17, when recirculating flow was turned off for each tank to prevent pseudo replication. Temperature in the individual heat treatment tanks was slowly ramped to 32 °C over 6 days (1 °C increase per day) and maintained for 22 more days until July 15 (Figure S2). HOBO temperature loggers were deployed to track the temperature profile throughout the exposure. A 10% water change was performed every week and the concentrations of potential nitrogen metabolic wastes (including NH_4^+, NO_3^-, and NO_2^-) were measured daily using commercial aquarium test kits. Levels of NH_4^+, NO_3^-, and NO_2^- were consistently under 0.25, 1, 0.05 ppm, respectively. Salinity was maintained at 35 ppt by topping off with distilled water (300–500 ml for control tanks and 500–1300 ml for heat tanks per day).

To track bleaching status and recruit growth over the course of the thermal stress exposure, photographs were taken from a top-down view under identical illumination using an Olympus Tough camera TG-4/TG-5 (Olympus America Inc.) at the initial timepoint (T_0), final timepoint (T_{28}), and 6 additional time points in between (T_7, T_{11}, T_{14}, T_{18}, T_{21}, T_{25}). Starting with the control group, PVC racks were removed from each tank and immediately placed into a Nally bin filled with seawater. To minimize the warping effect along the edges of the photos, racks were divided into 4 quadrants and each quadrant was photographed individually to make sure the corals were centered (Fig. 1). A subset of the Coral Health Chart (Siebeck et al. 2006), E1-E6 and B1-B6, was zip tied to an empty rack that was placed against the coral racks to serve as a color and size reference. Each quadrant was photographed three times with the camera lens just submerged. After all four quadrants were covered, the coral racks were immediately transferred back to their respective treatment tank. The Nally bin was then heated to 32 °C before corals from the heat treatment group were transferred and photographed.

After 22 days of exposure, recruits were scraped off the plugs using a razor blade and pooled into 1.5 ml Eppendorf tubes by family per replicate tank. Adult fragments were removed from their plugs and wrapped in pre-labeled tin foil. All samples were frozen immediately at − 80 °C for subsequent physiological assays.

Physiological assays

Similar to Zhang et al. (2019), larvae (3 replicates of 10 larvae per family) and recruit (3 replicates of between 2 and 15 recruits per family, see Table S1) samples were thawed on ice and mixed with 100 µl extraction buffer (50 mM phosphate buffer, pH 7.8, with 0.05 mM dithiothreitol). The mixture was further homogenized by back pipetting to free symbiont cells from host tissues and the final volume of the homogenate was recorded to account for residual seawater. For recruit samples, skeletal debris was allowed to settle for a minute and the aqueous tissue layer was further transferred to a new set of tubes. To quantify symbiont cell density, 20 µl of the homogenate was fixed in 20 µl of a 20% formalin solution (10% final concentration) and triplicate 10 µl aliquots were assessed under a compound microscope at 100 × magnification using a hemocytometer. The remaining slurry was centrifuged for 3 min at 1500 × g at 4 °C to pellet symbiont cells for chlorophyll quantification and isolate host tissue supernatant for protein analysis. Symbiont cell pellets were resuspended in 90% acetone and further broken down by shaking with metal beads in a TissueLyser II (Qiagen) for 90 s. After an overnight incubation at − 20 °C, the solution was centrifuged for 5 min at 10,000 × g at 4 °C. Triplicate 50 µl aliquots of the resultant supernatant were measured for absorbance at 630, 647, and 664 nm using Synergy H1 microplate reader (Biotek). Chlorophyll a concentration was determined using the equation specified in Ritchie (2008). Soluble host protein was measured colorimetrically using triplicate 10 ul aliquots of the host supernatant with the RED 660™ protein assay kit (G-Biosciences) following the manufacturer's instructions. Measured concentrations were multiplied by initial homogenate volume to account for differential dilution. Larval traits were normalized by total volume of larvae as the average larval size across different colonies

varied (i.e., family #34 produced substantially bigger larvae than the rest, Figure S3). Recruit traits were normalized by total recruit surface area (see details below) as individual recruit size was also quite variable.

The same workflow also applied to adult sample processing with a few modifications: each thawed adult fragment was mixed with 10 ml extraction buffer in a plastic zipper bag (Plymor) over ice and airbrushed to remove the tissue, which was then homogenized by a tissue homogenizer (VWR® Model 200) for 1 min following (Palmer et al. 2010) and the final volume was recorded. Symbiont cells were fixed by mixing 250 µl of homogenate with 125 µl 20% formalin solution (6.7% final concentration). One ml of homogenate was aliquoted to conduct chlorophyll analysis. Host tissue was isolated by centrifuging the remaining homogenate (ca. 8.75 ml) at 1500 xg. Skeletons were cleaned using 10% bleach and air dried. Surface area was assessed using the single wax dipping method (Veal et al. 2010) and was used to standardize adult traits.

Adult and recruit photos were analyzed in ImageJ (Schneider et al. 2012). To assess visual bleaching, color scores were assigned to individuals at each time point following Siebeck et al. (2006). Briefly, the Coral Health Chart color reference was used to generate a standard curve of mean grayness values (or an average of R, G, B values) from the standardized color scores (D1-D6) within each photo (Grottoli et al. 2021). The entire surface area of recruits was traced and three unshaded subsets of adult fragments were selected to measure mean grayness value, which was then converted to a color score using the standard curve. A recruit was considered dead (recorded as NA) if no visible tissue remained. Recruit size was determined by tracing the outline of each individual and recording the pixel area. A line of known distance on the Coral Health Chart was used as a reference to convert pixel area to metric area. Growth of individual recruits was calculated as the difference in size between initial and final timepoints.

Statistical analyses

All statistical analyses were conducted in R 4.0.3 (R Core Team 2020). Linear mixed effects models (*lme4* package, Bates et al. 2015) were used to analyze all physiological traits, including symbiont density, chlorophyll a concentration, total protein content, change in color score, and recruit growth. Treatment (levels: control and heat) and origin (levels: inshore and offshore) were included as fixed effects. The interaction of these two effects was also included in the model. Family and tank (for adults and recruits only) were included as random effects. Larval volume was modeled as a function of origin, with family included as random effect. Models were evaluated for normality and homoscedasticity using diagnostic plots. Natural logarithmic transformation was performed on trait data that did not satisfy the model assumptions of normality and absence of heteroscedasticity of residuals. The null hypothesis was rejected at an alpha of 0.05. Survival analysis was used to model time of death in the recruit heat stress experiments as a function of treatment and origin, including a random effect of family and tank using the coxme package (Therneau 2018). Specifically, mortality was coded as a binary trait (dead = 1, alive = 0) and time of death as an integer (timepoints at which photos were taken in days). Correlation coefficients were calculated for traits that were shared between life stages to investigate the strength of familial effect across stages (*Hmisc* package, Harrell and Harrell 2019). Trait data were grouped by family and treatment and thus each comparison consisted of 20 data points.

Broad sense heritability (H^2) of measured traits was estimated using MCMC models (*MCMCglmm* package, Hadfield 2010) for each life stage, including treatment and origin as fixed effects and family as a random effect. Traits were log-transformed as necessary to meet the normality requirement. All models were run for 100,000 iterations, with the first 10,000 discarded to ensure convergence and every 20 subsequent parameter values sampled to minimize autocorrelation. Model convergence and autocorrelation were evaluated by plotting trace of mean and variances. The resulting effective sample size was 4500. H^2 values were calculated as the ratio of variance attributable to the random familial effect over total variance.

Results

There was a significant interactive effect of treatment x origin on recruit symbiont density, where recruits originating from inshore families lost fewer symbionts than offshore recruits, by 5% on average ($t = -2.86$, df = 48, $p < 0.01$, Fig. 2c). Adults, larvae, and recruits all experienced a significant decline in symbiont density in response to heat stress ($p < 0.05$, Fig. 2a, b, c). Specifically, heat-exposed adult corals lost 62% of their symbionts compared to the control group, followed by 9% in both larvae and recruits. Mean adult symbiont density correlated positively with mean recruit symbiont density across families ($r = 0.66$, $p < 0.01$) but no correlations with larvae were apparent.

Chlorophyll a concentration declined by 85% ($t = -3.86$, $df = 48$, $p < 0.001$, Fig. 2d) in heat-treated adults and tended to decrease ($t = -1.97$, $df = 45$, $p = 0.055$, Fig. 2f) in heat-treated recruits compared to their respective controls. Larval chlorophyll a was not affected by treatment, origin, or the interaction between the two factors. No significant correlations were found between any two life stages in terms of chlorophyll a concentration.

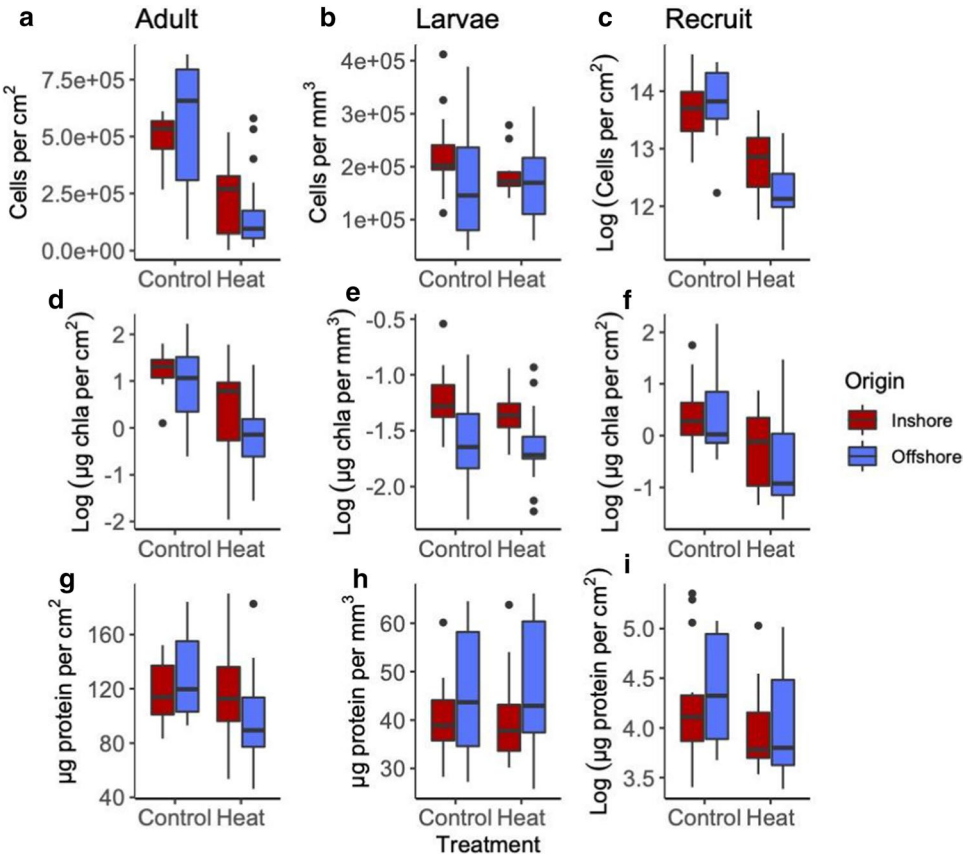

Fig. 2 Standardized physiological parameters in response to experimental conditions shared across all life stages separated by reef origin. Boxplots represent the 50th (median), 25th, and 75th percentiles (± 1.5 × IQR). Each column represents a distinct life stage (left to right: adult, larvae, and recruit) and each row represents a distinct trait (top to bottom: symbiont density, chlorophyll a concentration, total soluble protein content). Natural logarithmic transformation was performed on values that were not normally distributed

Significant interaction was detected in adults where offshore coral lost 23% more protein after the heat exposure period compared to inshore coral ($t = -2.18$, df = 48, $p < 0.05$, Fig. 2g). However, no fixed effect of origin or treatment was observed for total soluble host protein content in any life stage, save for a marginal treatment effect in recruits where protein content tended to be reduced in response to heat stress ($p = 0.059$, Fig. 2i). No significant correlations were found between any two life stages in terms of protein content.

The overall survival rate of recruits after the 28-day heat stress was 89%. No significant difference in mortality risk was found between treatment or origin. The standardized color score of adult and recruit corals decreased over time (Figure S4). Heat-treated individuals experienced a greater degree of paling, by more than twofold in both life stages at the end of the experiment compared to the beginning ($p < 0.05$, Figure S5). The correlation between change in color score over time in adults and recruits was not significant. Individual recruit size also decreased after the exposure period, but recruits from the heat group suffered less of a decrease than those from the control group ($t = 3.05$, $df = 48$, $p < 0.01$, Figure S6).

Significant heritability estimates were detected for multiple physiological traits but varied greatly across life stages. The average broad sense heritability estimate for symbiont density was highest in the larval stage ($H^2 = 0.75$, 95% confidence interval (CI): 0.54, 0.93), followed by adult ($H^2 = 0.65$, 95% CI: 0.27, 0.96) and recruit stages ($H^2 = 0.32$, 95% CI: 0.0009, 0.61) (Fig. 3). High H^2 value was also detected for chlorophyll a concentration in larvae ($H^2 = 0.67$, 95% CI: 0.43, 0.90), indicating 67% of the variance in chlorophyll a was explained by genetic factors, which include additive genetic effects, epistasis, and maternal effects. In comparison, the H^2 estimate for chlorophyll a yielded a lower value of 0.53 (95% CI: 0.27, 0.84) in adults and a much lower value of 0.07 (95% CI: 3×10^{-5}, 0.22) in recruits. Heritability estimates for protein content exhibited the opposite trend where recruits had the highest value of 0.67 (95% CI: 0.42, 0.92), followed by larvae ($H^2 = 0.61$, 95% CI: 0.35, 0.88) and adults ($H^2 = 0.26$, 95% CI: 3×10^{-7}, 0.58). The H^2 of change in color score between final and initial time points was 0.35 (95% CI: 0.03, 0.66) for adults and 0.11 (95% CI: 0.01, 0.25) for recruits.

Discussion

Heat induced a significant bleaching response in all three life stages, as indicated by the reduction in symbiont

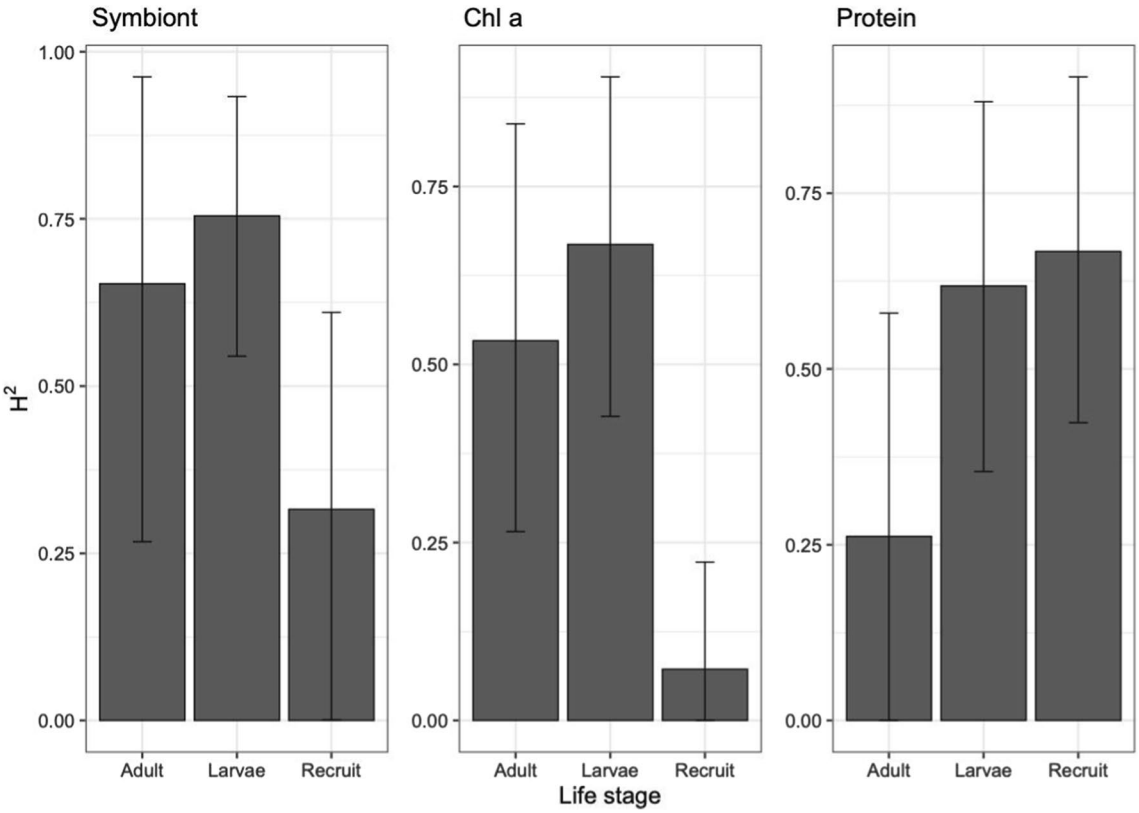

Fig. 3 Broad-sense heritability estimate (mean ± 95% C.I.) for three shared physiological traits at different life stages

density and/or chlorophyll a concentration after treatment (Fig. 2a–f). Adult corals and their recruits in the 32 °C group also lost more pigmentation as indicated by larger decreases in color score (Figures S4 and S5). Interestingly, larvae appeared to be more heat resistant compared to adults and recruits. In particular, symbiont density was unaffected by treatment in several larval families (5, 17, 38, 39) and even slightly elevated in two offshore families 32 and 34 (Figure S7b). No treatment effect was detected at all for larval chlorophyll concentration (Fig. 2e). One possible explanation for the muted response to heat in larvae is a shorter exposure period (i.e., 4 days as opposed to 22 days for adults and recruits). From a developmental standpoint, it would be difficult to extend the exposure window as *P. astreoides* larvae become competent quickly after release (Ritson-Williams et al. 2016) and high temperature tends to expedite settlement (Nozawa and Harrison 2005). Another possibility may be that larvae are indeed more tolerant of thermal stress than later life stages, although prior evidence has been mixed and studies emphasizing both the vulnerability and robustness of coral larvae can be found. Earlier studies showed that larval survivorship and settlement in broadcast spawning coral species *Diploria strigosa* and *Acropora palmata* were significantly reduced when subjected to sublethal thermal stress (30–32 °C) (Bassim and Sammarco 2003; Randall and Szmant 2009). Symbiotic larvae of *Pocillopora damicornis*, a brooding coral like *P. astreoides,* suffered from impaired metabolism under a combination of high temperature and CO_2 stresses (Rivest & Hofmann 2014). Another study on the same *Pocillopora* species reported an over 50% reduction in dark-adapted maximum quantum yield of photosystem II of heat-exposed larvae compared to adults under the same treatment (Putnam et al. 2010). Conversely, our previous study highlighted incredible thermal tolerance of *P. astreoides* larvae where significant mortality under 36 °C was only observed after > 24 h of exposure (Zhang et al. 2019), mirroring the survival data for aposymbiotic *Acropora millepora* larvae subjected to a similar level of stress (Dixon et al. 2015). *A. millepora* larvae also survived well and underwent normal development after a 5-day exposure to 32 °C (Meyer et al. 2009). Larval thermal tolerance is therefore likely species- and phenotype-specific. More cross-generational or even multi-generational studies are needed to clarify these conflicting results. Ultimately, the performance of larvae also needs to be contextualized in a wider developmental framework, as short-term sublethal thermal stress experienced by larvae can negatively affect recruitment and post-recruitment survival (Edmunds et al. 2001; Ross et al.

2013). In turn, the environment that the parents experience during the brooding period can also have carry-over effects on newly-released larvae (Wong et al. 2021).

Population-specific responses to heat stress were observed in both adults and recruits. Notably, inshore adult corals lost less protein content and inshore recruits lost fewer symbiont cells, respectively, in comparison to their offshore counterparts (Fig. 2g and c). Adult *P. astreoides* colonies from the inshore environment in the Lower Florida Keys have been repeatedly shown to exhibit higher bleaching tolerance based on multiple phenotypes, including bleaching color score, brightness in the red channel, and photochemical yield of the algal endosymbiont (Kenkel et al. 2013; Kenkel et al. 2015a, b). In this study, surprisingly, inshore adults failed to outperform the offshore adults in response to heat based on the two measured bleaching phenotypes. Altered environmental pressures may have induced different physiological responses among populations, for example, repeated thermal stress events could reduce the resilience of inshore populations or increase the tolerance of offshore populations through acclimation (Ainsworth et al. 2016). The current study did occur after the back-to-back mass bleaching events of 2014 and 2015 (Manzello et al. 2019). Paling and partial bleaching was repeatedly observed in late August to early September between 2016 and 2019 although both inshore and offshore reefs appeared to be equally impacted (The Florida Keys BleachWatch Program). This result could also be explained by the comparatively smaller sample size and noticeable variation among individual colonies; families 11 and 17 were more similar to offshore families whereas family 39 was more inshore-like (Figure S7a). Although among-family variation in thermal tolerance of *P. astreoides* adults originating from the same location has not been investigated before, our prior study documented significant family effects on larval survival and physiology, where a greater percentage of phenotypic variation was due to parental colony identity than other factors such as day of release and reef origin (Zhang et al. 2019). In addition, the significant positive correlation in symbiont density between individual adult and recruit families suggest strong family effects can persist into the following life stage. Most importantly, the maintenance of symbiont densities under elevated temperature presents the first evidence of enhanced bleaching tolerance in inshore offspring. This may indicate that difference in tolerance between inshore and offshore recruits has a heritable basis given that these recruits lacked prior exposure to different thermal regimes, which is further supported by the analysis of heritability.

Reduction in recruit size was likely due to lack of feeding throughout the experimental period (Figure S6). Feeding *Pocillopora acuta* with brine shrimp (*Artemia* spp.) three times a week doubled colony growth and increased symbiont density as well as their maximum quantum yield (Huang et al. 2020). We chose not to feed the corals mainly due to the concern of increased fouling in a small, closed system. More importantly, neither treatment group was provisioned with external food sources, so it is still valid to make conclusions on population level differences in heat tolerance by comparing control and heat-treated corals, although results may differ under replete conditions. Corals derive the majority of their daily energetic requirements from symbiotic Symbiodiniaceae (Muscatine and Porter 1977). However, certain species rely more heavily on heterotrophic feeding during bleaching events when their autotrophic source is limited (Grottoli et al. 2006). It is unclear whether our focal species *P. astreoides* has flexible heterotrophic capacities and therefore hard to predict the actual effects of feeding. Based on the limited trophic plasticity in its Pacific congeners *P. compressa* and *P. lobata* (Grottoli et al. 2006), feeding might have a minor role in mitigating the effect of bleaching in *P. astreoides.*

In general, we found moderate ($H^2 = 0.25 \sim 0.50$) to high ($H^2 > 0.50$) broad sense heritability estimates across all traits examined (Fig. 3). This finding reinforces the main conclusion of a recent meta-analysis by Bairos-Novak et al. (2021) which found that the majority of coral physiological traits (with few exceptions such as gene expression and photochemistry) exhibited relatively high heritability. For instance, our H^2 estimate of adult symbiont density (0.65) is on par with an estimate of 0.71 for the same trait in adult *Orbicella faveolata* during a natural bleaching event (Manzello et al. 2019). These findings may indicate adaptive capacity, which will project a positive outlook for the persistence of coral communities under changing environmental conditions. One important caveat, however, is that the heritability values derived from this study likely overestimate the true adaptive potential of our focal traits because h^2 is only a subset of H^2 (Falconer and Mackay 1996). Nonetheless, h^2 for highly heritable traits in certain life stages, for instance, symbiont density and chlorophyll a in larvae and protein in larvae and recruits, is expected to be significant as well, as h^2 is typically only 1.4–2.5 fold lower than H^2 (Bairos-Novak et al. 2021). High fidelity inheritance of symbiont communities and host-symbiont co-evolution will be important for rapid adaptation, although additional high-resolution genotyping and phenotyping of symbionts is needed to verify both heritability and the extent to which symbionts contribute to holobiont thermal tolerance in this system (Quigley et al. 2018; Hoadley et al. 2021). Another factor that may bias estimates is the assumption that each spawning colony possesses a unique genetic identity. Given the rapid propensity for settlement in *P. astreoides* larvae, they are expected to have short dispersal potential and therefore high local retention (Jones et al. 2009). It is likely that clones or sibling clusters group over a small spatial scale as larvae of other brooding species have been documented to

settle within meters from their parents (Carlon and Olson 1993). If phenotypically similar colonies were clones and their larval/recruit families were siblings, true heritability values could possibly be higher than our estimates. Conversely, true heritability for a given trait may be lower than estimated if phenotypically dissimilar individuals shared the same genetic identity. In the absence of genotyping data we are unable to rule out the possibility of clones, however, it is unlikely that we collected clonal or sibling adult colonies because we sampled individuals that were at least 10 m apart in the field, a greater distance compared to 1 m used in other studies (Riquet et al. 2021; Serrano et al. 2016).

Despite overall moderate to high heritability levels, estimates differed among life stages and across different physiological traits within a given stage. Although the confidence interval associated with most heritability values is not trivial, it is comparable to similar error estimates in previous analyses (Jury et al. 2019; Kenkel et al. 2015a, b) and in future work can be potentially reduced by larger sample sizes and increased replication. Symbiont density tended to be more heritable in the adult and larval stages in comparison to the recruit stage (Fig. 3). Similarly, recruits had the lowest heritability estimate for chlorophyll a concentration ($H^2 = 0.07$), whereas over half of the variation in adults and larvae was attributed to genetics. In general, older age in various vertebrate species is associated with high genetic variance in fitness-related traits due to the accumulation of somatic mutation over time (Wilson et al. 2008). A recent study estimated that the table coral *Acropora hyacinthus* accumulates mutations at a similar rate (2.6 mutations per gigabase per year in its coding region) as human somatic cell lines (López and Palumbi 2020). Given this parallel, it is reasonable to expect increased mutations and genetic variation in older corals with bigger colony sizes. Moreover, those potentially deleterious mutations are suspected to be purged before gamete production (Orive 2001), which likely contributes to the relatively lower genetic variation in the offspring. A potential explanation for high heritability estimate of larval symbiont and chlorophyll traits is that maternal effects may be dominant during early life stages as energetic reserves are maternally provisioned (Richmond and Hunter 1990), thus inflating the overall estimate of genetic effects. Moreover, brooding species like *P. astreoides* likely experience larger maternal effects since brooded larvae are subject to maternal environment during early development and inherit symbionts directly from the mother (Richmond and Hunter 1990). However, the rank order of H^2 estimates is completely reversed for soluble protein content, where higher values were observed in larvae and recruits rather than adults (Fig. 3). Based on an interspecific comparison, the genetic component of protein content was about four times higher in *P. astreoides* larvae ($H^2 = 0.60$) than in *A. millepora* adults ($H^2 = 0.15$) (Bairos-Novak et al. 2021), which could be determined by the lack of maternal effects in *A. millepora* as it reproduces by broadcasting gametes, leading to external fertilization and embryonic development. Interestingly, despite the general expectation that maternal effects attenuate over time (Dufty et al. 2002), the estimate of heritability of protein content was similar between recruits and larvae. Studies on small mammals and birds have found that phenotypic variance components constantly fluctuate across developmental stages, resulting in divergent heritability estimates depending on sampling time (Atchley 1984; Bourret et al. 2017). Additive genetic variance (V_A), in particular, can be modified by relative expression at specific loci and/or the phenotypic effects of those loci especially during early ontology (Atchley 1984). Indeed, loci involved in metabolism and proteolysis were differentially expressed in *A. millepora* planula larvae and newly-settled polyps (Hayward et al. 2011). It is also possible that different life stages in *P. astreoides* use different sets of genes to respond to environmental stimuli (Ruggeri et al. 2022), which may further contribute to the difference in V_A observed between larvae and recruits.

In summary, we observed population-level differences in the response to heat stress in both adult and offspring generations of *P. astreoides* derived from two distinct reef zones in the Lower Florida Keys, highlighting the potential contribution of genetic adaptation and physiological acclimatization to thermal tolerance. Larvae tended to be more bleaching tolerant than adults and recruits from the same lineages, although more studies involving multiple life stages need to be conducted to validate this hypothesis. It is also important to identify an appropriate level and duration of heat stress for each life stage so that responses are comparable. Moreover, we also found that broad sense heritability of a given trait is widely divergent among different life stages. Two bleaching phenotypes, symbiont density and chlorophyll a concentration, were highly heritable in adults and larvae, whereas protein content was highly heritable in larvae and recruits, which likely reflects the fluctuating dynamics between genetic variation and environmental variation as organisms undergo different developmental phases. The overall significant heritability levels of bleaching- and nutrient- associated traits suggest strong familial influence on those traits that is evident across generations. If these traits are truly heritable (determined by additive genetic effects), it bodes well for the local *P. astreoides* populations, as they may be capable of keeping up with the rapidly changing ocean temperatures through adaptation.

Acknowledgements We would like to thank E. Bartels and other staff members at Mote Marine Laboratory's International Center for Coral Reef Research and Restoration for their amazing field and logistical support, W. Million and E. Aguirre for their assistance with field sampling and conducting the experiments, C. Timmons for her help with aquarium maintenance, S. O'Donnell for her dedication to color score

analysis. Lastly, we greatly appreciate the Manahan lab (especially M. DellaTorre) at USC for lipid protocol training and troubleshooting despite unpublishable results. This research was supported by start-up funds from the University of Southern California to C. Kenkel.

Author contributions YZ and CDK: designed the study and performed field collection and larval experiments. YZ and SJB: conducted adult and recruit experiments and end sampling. SJB: processed adult samples and YZ: finished the remaining samples and analysis. YZ: wrote the first draft of the manuscript. All authors reviewed, approved of and are accountable for this submission.

Funding University of Southern California, PR1003881, Carly D. Kenkel.

Data availability Raw data and R scripts for statistical analysis are available at https://github.com/yingqizhang/Porites2022.

Declarations

Conflict of interest The authors declare no conflict of interest.

References

Ainsworth TD, Heron SF, Ortiz JC, Mumby PJ, Grech A, Ogawa D, Eakin CM, Leggat W (2016) Climate change disables coral bleaching protection on the Great Barrier Reef. Science 352(6283):338–342

Albecker MA, Wilkins LG, Krueger-Hadfield SA, Bashevkin SM, Hahn MW, Hare MP, Reitzel AM (2021) Does a complex life cycle affect adaptation to environmental change? Genome-informed insights for characterizing selection across complex life cycle. Proc R Soc B 288(1964):20212122

Atchley WR (1984) Ontogeny, Timing of Development, and Genetic Variance-Covariances Structure. *The American Naturalist*, 22

Babcock RC (1991) Comparative Demography of Three Species of Scleractinian Corals Using Age- and Size-Dependent Classifications. Ecol Monogr 61(3):225–244. https://doi.org/10.2307/2937107

Bairos-Novak KR, Hoogenboom MO, Oppen MJH, Connolly SR (2021) Coral adaptation to climate change: Meta-analysis reveals high heritability across multiple traits. *Global Change Biology*, gcb.15829. https://doi.org/10.1111/gcb.15829

Baker AC (2003) Flexibility and specificity in coral-algal symbiosis: diversity, ecology, and biogeography of Symbiodinium. Annual Review of Ecology, Evolution, and Systematics, 661–689.

Bassim K, Sammarco P (2003) Effects of temperature and ammonium on larval development and survivorship in a scleractinian coral (Diploria strigosa). Mar Biol 142(2):241–252. https://doi.org/10.1007/s00227-002-0953-z

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, *67*(1). https://doi.org/10.18637/jss.v067.i01

Bay RA, Palumbi SR (2014) Multilocus Adaptation Associated with Heat Resistance in Reef-Building Corals. Curr Biol 24(24):2952–2956. https://doi.org/10.1016/j.cub.2014.10.044

Berkelmans R, van Oppen MJH (2006) The role of zooxanthellae in the thermal tolerance of corals: A 'nugget of hope' for coral reefs in an era of climate change. Proceedings of the Royal Society b: Biological Sciences 273(1599):2305–2312. https://doi.org/10.1098/rspb.2006.3567

Bourret A, Bélisle M, Pelletier F, Garant D (2017) Evolutionary potential of morphological traits across different life-history stages. J Evol Biol 30(3):616–626. https://doi.org/10.1111/jeb.13031

Carlon DB, Olson RR (1993) Larval dispersal distance as an explanation for adult spatial pattern in two Caribbean reef corals. J Exp Mar Biol Ecol 173(2):247–263. https://doi.org/10.1016/0022-0981(93)90056-T

Carlon DB, Budd AF, Lippé C, Andrew RL (2011) The quantitative genetics of incipient speciation: heritability and genetic correlations of skeletal traits in populations of diverging Favia fragum ecomorphs. Evolution 65(12):3428–3447. https://doi.org/10.1111/j.1558-5646.2011.01389.x

Dixon GB, Davies SW, Aglyamova GV, Meyer E, Bay LK, Matz MV (2015) Genomic determinants of coral heat tolerance across latitudes. Science 348(6242):1460–1462. https://doi.org/10.1126/science.1261224

Dixon G, Liao Y, Bay LK, Matz MV (2018) Role of gene body methylation in acclimatization and adaptation in a basal metazoan. Proc Natl Acad Sci 115(52):13342–13346

Dufty A (2002) Hormones, developmental plasticity and adaptation. Trends Ecol Evol 17(4):190–196. https://doi.org/10.1016/S0169-5347(02)02498-9

Edmunds P, Gates R, Gleason D (2001) The biology of larvae from the reef coral Porites astreoides, and their response to temperature disturbances. Mar Biol 139(5):981–989. https://doi.org/10.1007/s002270100634

Falconer D, Mackay T (1996) Introduction to Quantitative Genetics. Longman, New York

Fuller ZL, Mocellin VJ, Morris LA, Cantin N, Shepherd J, Sarre L, Przeworski M (2020) Population genetics of the coral Acropora millepora: Toward genomic prediction of bleaching. Science, 369(6501), eaba4674

Grottoli AG, Rodrigues LJ, Palardy JE (2006) Heterotrophic plasticity and resilience in bleached corals. Nature 440(7088):1186–1189. https://doi.org/10.1038/nature04565

Grottoli AG, Toonen RJ, van Woesik R, Vega Thurber R, Warner ME, McLachlan RH, Wu HC (2021) Increasing comparability among coral bleaching experiments. Ecol Appl 31(4):e02262

Gustafsson L (1986) Lifetime Reproductive Success and Heritability: Empirical Support for Fisher's Fundamental Theorem. The American Naturalist, 128(5), 761–764. http://www.jstor.org/stable/2461955

Hadfield JD (2010) MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmm *R* Package. *Journal of Statistical Software*, *33*(2). https://doi.org/10.18637/jss.v033.i02

Harrell Jr FE, Harrell Jr MFE (2019) Package 'hmisc'. *CRAN2018*, *2019*, 235–236.

Hayward DC, Hetherington S, Behm CA, Grasso LC, Forêt S, Miller DJ, Ball EE (2011) Differential Gene Expression at Coral Settlement and Metamorphosis—A Subtractive Hybridization Study. PLoS ONE 6(10):e26411. https://doi.org/10.1371/journal.pone.0026411

Hoadley KD, Pettay DT, Lewis A, Wham D, Grasso C, Smith R, Warner ME (2021) Different functional traits among closely related algal symbionts dictate stress endurance for vital Indo-Pacific reef-building corals. Glob Change Biol 27(20):5295–5309

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K, Knowlton N, Eakin CM, Iglesias-Prieto R, Muthiga N, Bradbury RH, Dubi A, Hatziolos ME (2007) Coral Reefs Under Rapid Climate Change and Ocean Acidification. Science 318(5857):1737–1742. https://doi.org/10.1126/science.1152509

Huang Y-L, Mayfield AB, Fan T-Y (2020) Effects of feeding on the physiological performance of the stony coral Pocillopora acuta. Sci Rep 10(1):19988. https://doi.org/10.1038/s41598-020-76451-1

Hughes TP, Barnes ML, Bellwood DR, Cinner JE, Cumming GS, Jackson JB, Scheffer M (2017) Coral reefs in the Anthropocene. Nature 546(7656):82–90

Hughes TP, Anderson KD, Connolly SR, Heron SF, Kerry JT, Lough JM, Baird AH, Baum JK, Berumen ML, Bridge TC, Claar DC, Eakin CM, Gilmour JP, Graham NAJ, Harrison H, Hobbs J-PA, Hoey AS, Hoogenboom M, Lowe RJ, Wilson SK (2018) Spatial and temporal patterns of mass bleaching of corals in the Anthropocene. Science 359(6371):80–83. https://doi.org/10.1126/science.aan8048

IPCC (2021) Climate change 2021: the physical science basis. In V. Masson-Delmotte, P. Zhai, A. Pirani, SL. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, MI. Gomis, M. Huang, K. Leitzell, E. Lonnoy, JBR. Matthews, TK Maycock, T. Waterfield, O. Yelekçi, R. Yu, & B. Zhou (eds) Contribution of working group I to the sixth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 2391 pp. https://doi.org/10.1017/9781009157896

Jokiel PL, Coles SL (1990) Response of Hawaiian and other Indo-Pacific reef corals to elevated temperature. Coral Reefs 8(4):155–162. https://doi.org/10.1007/BF00265006

Jones GP, Almany GR, Russ GR, Sale PF, Steneck RS, van Oppen MJH, Willis BL (2009) Larval retention and connectivity among populations of corals and reef fishes: History, advances and challenges. Coral Reefs 28(2):307–325. https://doi.org/10.1007/s00338-009-0469-9

Jury CP, Delano MN, Toonen RJ (2019) High heritability of coral calcification rates and evolutionary potential under ocean acidification. Sci Rep 9(1):20419. https://doi.org/10.1038/s41598-019-56313-1

Kenkel CD, Matz MV (2017) Gene expression plasticity as a mechanism of coral adaptation to a variable environment. Nature Ecology & Evolution 1(1):0014. https://doi.org/10.1038/s41559-016-0014

Kenkel CD, Goodbody-Gringley G, Caillaud D, Davies SW, Bartels E, Matz MV (2013) Evidence for a host role in thermotolerance divergence between populations of the mustard hill coral (*Porites astreoides*) from different reef environments. Mol Ecol 22(16):4335–4348. https://doi.org/10.1111/mec.12391

Kenkel CD, Almanza AT, Matz MV (2015a) Fine-scale environmental specialization of reef-building corals might be limiting reef recovery in the Florida Keys. Ecology 96(12):3197–3212. https://doi.org/10.1890/14-2297.1

Kenkel CD, Setta SP, Matz MV (2015b) Heritable differences in fitness-related traits among populations of the mustard hill coral. Porites Astreoides Heredity 115(6):509–516. https://doi.org/10.1038/hdy.2015.52

Kuffner I, Walters L, Becerro M, Paul V, Ritson-Williams R, Beach K (2006) Inhibition of coral recruitment by macroalgae and cyanobacteria. Mar Ecol Prog Ser 323:107–117. https://doi.org/10.3354/meps323107

Lesser MP (2011) Coral Bleaching: Causes and Mechanisms. In Z. Dubinsky & N. Stambler (Eds.), *Coral Reefs: An Ecosystem in Transition* (pp. 405–419). Springer Netherlands. https://doi.org/10.1007/978-94-007-0114-4_23

Lirman D, Fong P (2007) Is proximity to land-based sources of coral stressors an appropriate measure of risk to coral reefs? An example from the Florida Reef Tract. Mar Pollut Bull 54(6):779–791. https://doi.org/10.1016/j.marpolbul.2006.12.014

López EH, Palumbi SR (2020) Somatic Mutations and Genome Stability Maintenance in Clonal Coral Colonies. Mol Biol Evol 37(3):828–838. https://doi.org/10.1093/molbev/msz270

Manzello D, Enochs I, Kolodziej G, Carlton R (2015) Recent decade of growth and calcification of Orbicella faveolata in the Florida Keys: An inshore-offshore comparison. Mar Ecol Prog Ser 521:81–89. https://doi.org/10.3354/meps11085

Manzello DP, Matz MV, Enochs IC, Valentino L, Carlton RD, Kolodziej G, Serrano X, Towle EK, Jankulak M (2019) Role of host genetics and heat-tolerant algal symbionts in sustaining populations of the endangered coral Orbicella faveolata in the Florida Keys with ocean warming. Glob Change Biol 25(3):1016–1031. https://doi.org/10.1111/gcb.14545

Marshall DJ, Morgan SG (2011) Ecological and Evolutionary Consequences of Linked Life-History Stages in the Sea. Curr Biol 21(18):R718–R725. https://doi.org/10.1016/j.cub.2011.08.022

Meyer E, Davies S, Wang S, Willis B, Abrego D, Juenger T, Matz M (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. https://doi.org/10.3354/meps08208

Munday PL, Warner RR, Monro K, Pandolfi JM, Marshall DJ (2013) Predicting evolutionary responses to climate change in the sea. Ecol Lett 16(12):1488–1500. https://doi.org/10.1111/ele.12185

Muscatine L, Falkowski PG, Porter JW, Dubinsky Z (1984) Fate of photosynthetic fixed carbon in light-and shade-adapted colonies of the symbiotic coral Stylophora pistillata. Proceedings of the Royal Society of London. *Series B. Biological Sciences, 222*(1227), 181–202

Muscatine L, Porter JW (1977) Reef corals: mutualistic symbioses adapted to nutrient-poor environments. Bioscience 27(7):454–460

Nozawa Y, Harrison PL (2005) Temporal settlement patterns of larvae of the broadcast spawning reef coral Favites chinensis and the broadcast spawning and brooding reef coral Goniastrea aspera from Okinawa. Japan Coral Reefs 24(2):274–282. https://doi.org/10.1007/s00338-005-0476-4

Orive ME (2001) Somatic Mutations in Organisms with Complex Life Histories. Theor Popul Biol 59(3):235–249. https://doi.org/10.1006/tpbi.2001.1515

Palmer CV, Bythell JC, Willis BL (2010) Levels of immunity parameters underpin bleaching and disease susceptibility of reef corals. FASEB J 24(6):1935–1946. https://doi.org/10.1096/fj.09-152447

Putnam HM, Gates RD (2015) Preconditioning in the reef-building coral Pocillopora damicornis and the potential for trans-generational acclimatization in coral larvae under future climate change conditions. J Exp Biol 218(15):2365–2372. https://doi.org/10.1242/jeb.123018

Putnam HM, Edmunds PJ, Fan T-Y (2010) Effect of a fluctuating thermal regime on adult and larval reef corals. Invertebr Biol 129(3):199–209. https://doi.org/10.1111/j.1744-7410.2010.00199.x

Putnam HM (2021) Avenues of reef-building coral acclimatization in response to rapid environmental change. *Journal of Experimental Biology*, *224*(Suppl_1), jeb239319. https://doi.org/10.1242/jeb.239319

Quigley KM, Willis BL, Bay LK (2017) Heritability of the Symbiodinium community in vertically-and horizontally-transmitting broadcast spawning corals. Sci Rep 7(1):1–14

Quigley KM, Warner PA, Bay LK, Willis BL (2018) Unexpected mixed-mode transmission and moderate genetic regulation of Symbiodinium communities in a brooding coral. Heredity 121(6):524–536

R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Randall CJ, Szmant AM (2009) Elevated Temperature Affects Development, Survivorship, and Settlement of the Elkhorn Coral, *Acropora palmata* (Lamarck 1816). Biol Bull 217(3):269–282. https://doi.org/10.1086/BBLv217n3p269

Richmond R, Hunter C (1990) Reproduction and recruitment of corals: Comparisons among the Caribbean, the Tropical Pacific, and the Red Sea. Mar Ecol Prog Ser 60:185–203. https://doi.org/10.3354/meps060185

Riquet F, Japaud A, Nunes FLD, Serrano XM, Baker AC, Bezault E, Bouchon C, Fauvelot C (2021) Complex spatial patterns of genetic differentiation in the Caribbean mustard hill coral Porites astreoides. Coral Reefs. https://doi.org/10.1007/s00338-021-02157-z

Ritchie RJ (2008) Universal chlorophyll equations for estimating chlorophylls a, b, c, and d and total chlorophylls in natural assemblages of photosynthetic organisms using acetone, methanol, or ethanol solvents. Photosynthetica 46(1):115–126. https://doi.org/10.1007/s11099-008-0019-7

Ritson-Williams R, Arnold S, Paul V (2016) Patterns of larval settlement preferences and post-settlement survival for seven Caribbean corals. Mar Ecol Prog Ser 548:127–138. https://doi.org/10.3354/meps11688

Rivest EB, Hofmann GE (2014) Responses of the Metabolism of the Larvae of Pocillopora damicornis to Ocean Acidification and Warming. PLoS ONE 9(4):e96172. https://doi.org/10.1371/journal.pone.0096172

Ross C, Ritson-Williams R, Olsen K, Paul VJ (2013) Short-term and latent post-settlement effects associated with elevated temperature and oxidative stress on larvae from the coral Porites astreoides. Coral Reefs 32(1):71–79. https://doi.org/10.1007/s00338-012-0956-2

Ruggeri M, Zhang Y, Aglyamova GV, Kenkel CD (2022) Divergent transcriptional response to thermal stress among life stages could constrain coral adaptation to climate change. bioRxiv

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9(7):671–675. https://doi.org/10.1038/nmeth.2089

Serrano XM, Baums IB, Smith TB, Jones RJ, Shearer TL, Baker AC (2016) Long distance dispersal and vertical gene flow in the Caribbean brooding coral Porites astreoides. Sci Rep 6(1):21619. https://doi.org/10.1038/srep21619

Siebeck UE, Marshall NJ, Klüter A, Hoegh-Guldberg O (2006) Monitoring coral bleaching using a colour reference card. Coral Reefs 25(3):453–460. https://doi.org/10.1007/s00338-006-0123-8

Therneau TM, (2018) coxme: Mixed Effects Cox Models

Thomas L, Rose NH, Bay RA, López EH, Morikawa MK, Ruiz-Jones L, Palumbi SR (2018) Mechanisms of thermal tolerance in reef-building corals across a fine-grained environmental mosaic: Lessons from Ofu. American Samoa Frontiers in Marine Science 4:434

Veal CJ, Carmi M, Fine M, Hoegh-Guldberg O (2010) Increasing the accuracy of surface area estimation using single wax dipping of coral fragments. Coral Reefs 29(4):893–897. https://doi.org/10.1007/s00338-010-0647-9

Visscher PM, Hill WG, Wray NR (2008) Heritability in the genomics era—concepts and misconceptions. Nat Rev Genet 9(4):255–266

Voolstra CR, Valenzuela JJ, Turkarslan S, Cárdenas A, Hume BCC, Perna G, Buitrago-López C, Rowe K, Orellana MV, Baliga NS, Paranjape S, Banc-Prandi G, Bellworthy J, Fine M, Frias-Torres S, Barshis DJ (2021) Contrasting heat stress response patterns of coral holobionts across the Red Sea suggest distinct mechanisms of thermal tolerance. Mol Ecol 30(18):4466–4480. https://doi.org/10.1111/mec.16064

Wilson AJ, Charmantier A, Hadfield JD (2008) Evolutionary genetics of ageing in the wild: Empirical patterns and future perspectives. Funct Ecol 22(3):431–442. https://doi.org/10.1111/j.1365-2435.2008.01412.x

Wong KH, Goodbody-Gringley G, de Putron SJ, Becker DM, Chequer A, Putnam HM (2021) Brooded coral offspring physiology depends on the combined effects of parental press and pulse thermal history. Glob Change Biol 27(13):3179–3195

Zhang Y, Million WC, Ruggeri M, Kenkel CD (2019) Family matters: Variation in the physiology of brooded Porites astreoides larvae is driven by parent colony effects. Comp Biochem Physiol a: Mol Integr Physiol 238:110562. https://doi.org/10.1016/j.cbpa.2019.110562