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REPORT

Larval thermal conditioning does not improve post-settlement thermal tolerance in the dominant reef-building coral, *Montipora capitata*

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Abstract Climate change-induced ocean warming has reshaped reef ecosystems as coral bleaching events continue to lead to mass coral die-offs globally. These thermal anomalies also negatively affect reef persistence and recovery when heat stress events continue to occur during critical reproduction and recruitment periods. As a result, recruitment (settlement and survival of early life stages) continues to decline on reefs globally, compromising the recovery of reef systems following mass bleaching and mortality events, which has led to the loss of ecological function on these reefs. One mitigation strategy within the active restoration framework includes propagated corals (i.e., adult fragments, larvae, or juveniles) produced in nurseries that are then directly out planted to the reef benthos. Restoration programs can greatly benefit from use of sexually derived coral propagules, which when optimized, increase the scalability and genetic diversity of outplanted stock. Thermal conditioning during larval rearing has the potential to be a cost-effective and scalable strategy to enhance the survival and stress tolerance of subsequent life stages. Here, we tested the potential for thermal conditioning during the larval stage to induce positive latent effects on the stress tolerance of *Montipora capitata* spat. We exposed larvae to cool (24.5 °C), ambient (27.2 °C), and warm (28.9 °C) conditions for four days and during this period we tracked larval survival. Following thermal conditioning during the larval stage, we measured settlement success and then exposed spat to a 47-day high temperature stress (+ 2 °C) and measured survival. We found that larval thermal conditioning did not provide survival benefits to spat under elevated temperature. Furthermore, larval exposure to temperatures outside of an ambient regime compromised survival, suggesting that although positive latent effects were not observed here in spat, optimizing thermal conditions is important for maximizing survival and settlement of coral larvae.

Keywords Early life history · Bleaching · Latent effects · Larvae · Spat · Coral reef

Introduction

Rising ocean temperatures is causing more frequent and severe coral bleaching events (Pandolfi et al. 2011; Hughes et al. 2017, 2018), during which the nutritional symbiosis between corals and endosymbiotic algae (family: Symbiodiniaceae) is destabilized, resulting in the loss of the coral's primary nutrition source (Grottoli et al. 2006; Weis 2008) and can lead to mass mortality events (Loya et al. 2001; Riegl and Purkis 2015; Burt et al. 2019). Reef recovery, replenishment, and mortality following bleaching depend on the survival and recruitment of coral early life history stages (Doropoulos et al. 2017). For successful recruitment to occur, offspring must pass through critical bottlenecks—larvae must survive pelagic dispersal,

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undergo settlement and metamorphosis, and progress through vulnerable, early post-settlement growth stages (Ritson-Williams et al. 2009). Local disturbances (e.g., sedimentation, eutrophication) and extreme marine heat waves caused by anthropogenically-driven climate change can severely constrict these naturally occurring bottlenecks (Olsen et al. 2014; Ritson-Williams et al. 2016; Evans et al. 2020), resulting in limited recruitment, and therefore, reduced resilience in modern reef habitats threatening the loss of ecosystem function (Hughes et al. 2019). A particularly critical bottleneck is larval survival, where elevated temperatures can further reduce recruitment pools (Richmond et al. 2018). Here, we examine the effect of elevated temperatures on larval survivorship and investigate whether thermal conditioning can increase settlement and survivorship of spat (newly settled larvae which have metamorphosed into coral polyps and adhered to substrate) under thermal stress.

Coral reef managers and practitioners are turning to active restoration strategies to combat coral loss and restore ecosystem function through supporting coral recruitment practices (Boström-Einarsson et al. 2020; Randall et al. 2020). To increase the introduction of coral propagules onto degraded and threatened reef environments, sexual reproduction strategies (i.e., ex situ fertilization, larval rearing, juvenile coral aquaculture) are being increasingly relied upon as tools to increase stock material via improved survival and growth (Vanderklift et al. 2020; Hein et al. 2021). These strategies, which involve rearing coral larvae and spat from laboratory or field collected gametes ex situ, offers opportunities to (1) increase genetic diversity, (2) scale-up the propagation of coral spat, and (3) increase survival through early life stage bottlenecks by facilitating favorable conditions (i.e., controlled tank conditions, substrate cleaning, supplemental feeding) (Doropoulos et al. 2019; Ligson et al. 2020; Suzuki et al. 2020). However, survival of these propagules has often been low and is variable based on complex biological and environmental conditions (Tamura 2008; Chamberland et al. 2017; Ligson et al. 2020; Hancock et al. 2021; Ligson and Cabaitan 2021) and further optimization of rearing strategies is required to improve the scale and longevity of species-specific assisted approaches. Additionally, due to the increasing environmental challenges resulting from global climate change, restoration approaches must also consider climate-readiness of propagated stocks (Caruso et al. 2021; Hein et al. 2021).

Ex situ rearing practices present an opportunity to scale up thermal conditioning to boost reef transplantation survival. Coral thermal conditioning is the practice of exposing corals to a specific temperature regime to elicit an enhanced response to subsequent elevated thermal conditions (van Oppen et al. 2015; Dilworth et al. 2021). However, this approach has been primarily evaluated in adult colonies (Maynard et al. 2008; Bay and Palumbi 2015; Ainsworth et al. 2016; Coles et al. 2018), leaving the influence of thermal conditioning on survival and tolerance in early life stages not well understood. In adult corals, thermal conditioning profile (temperature and time) can positively impact coral performance (Bellantuono et al. 2012; Dilworth et al. 2021; Majerova et al. 2021). Exposure to warmer-than-ambient conditions may prepare corals for facing near future warming events by activating cellular stress response mechanisms for survival under warming (Bellantuono et al. 2012; Barshis et al. 2013; Bay and Palumbi 2015; Majerova et al. 2021). Alternatively, exposure to periods of cooler temperatures prior to thermal stress may provide a period of energetic savings (i.e., a period of lower metabolic rates) and biomass accumulation (Edmunds 2009). For example, in a brooding reproductive system, juveniles of the coral species *Pocillopora acuta* conditioned to cool thermal conditions exhibited enhanced thermal tolerance (Huffmyer et al. 2021).

Latent effects during development present opportunities for life stage-specific thermal exposure to impact the performance at subsequent stages (Byrne et al. 2020; Leung and McAfee 2020; Putnam et al. 2020). However, studies tracking latent effects due to thermal exposure across early life history stages in corals are limited. In one study, *Porites astreoides* larvae exposed to elevated temperature had lower survival during the spat stage (Ross et al. 2013). In a restoration context, exposing coral propagules to thermal conditioning regimes ex situ provides a potential option for elevating resilience in propagated stocks, especially if conditioning during early life stages (i.e., embryos, larvae) has positive latent effects on subsequent life stages (i.e., spat). Exposing coral propagules to thermal conditioning regimes is one potential strategy for enhancing thermal tolerance (van Oppen et al. 2015; Randall et al. 2020). It is critical to optimize thermal profiles and test the outcomes of thermal conditioning to understand the physiological and phenotypic implications in early life stages and to further develop species-specific restoration strategies. Therefore, in this study, we investigated the effects of warm and cool temperatures on larval survivorship, and the potential for thermal conditioning during the larval stage to improve thermal tolerance in the subsequent spat stage. Here, we investigated this in the coral species *Montipora capitata,* which is a reef-building broadcast spawner and vertical transmitter (passes Symbiodiniaceae from parent to offspring) in Hawai‘i, USA.

Materials and methods

Gamete collection

In this study, we tested the effects of larval thermal exposure on larval survival, settlement, and spat survival under warming conditions (Fig. 1). *Montipora capitata* (Fig. 2a) egg and sperm bundles were collected in June 2019 (on the night of the new moon), in Kāne'ohe Bay, O'ahu, Hawai'i (patch reef 11; 21° 26′ 56'' N, 157° 47′ 45'' W), also described in Hancock et al. (2021). Coral gametes were collected under Hawai'i DLNR permit SAP 2018-03 to the Hawai'i Institute of Marine Biology (HIMB). Egg sperm bundles were gathered from the ocean surface during a mass spawning event at approximately 21:00 h. Using 153 µm nets, bundles were gently scooped from the surface before being rinsed into collection containers (19 L) using 1 µm filtered seawater (FSW). After gamete collection on the reef, bundles were carefully transported back to an indoor facility at HIMB, where all subsequent steps, described below, were conducted. Bundles were placed in 0.2 µm FSW and allowed to separate and fertilize for 1 h. After fertilization, embryos were rinsed of excess sperm with FSW and transferred into either 20 L (N = 8) or 1 L (N = 21) indoor conical rearing vessels (Fig S1a) with flow-through 1 µm FSW at ambient (28 °C) temperature to develop in low light indoor conditions (< 20 µmol photons m^2 s^{-1}) (Hancock et al. 2021). Conical vessels were equipped with banjo filters, which allow for water filtration without damaging fragile coral larvae using 153 µm mesh across the surface area of the filter submerged in the conical tank (Fig S1b). Water flow was low for the first 24 h post-fertilization (approx. < 1 L per hour) to prevent embryo damage and was increased to approx. 2 L per hour thereafter (Hancock et al. 2021). Embryos reached the swimming planula stage at five days post-fertilization, as visualized on a dissecting microscope (Fig. 2b).

Larval exposure and survivorship

At five days post-fertilization, larvae from all rearing conicals were pooled and evenly divided between temperature treatments: cool (24.5 ± 1.0 °C; N = 4 20 L conicals), ambient (27.2 ± 0.3 °C; N = 4 20 L conicals), and warm (28.9 ± 0.7 °C; N = 21 1 L conicals) (Figs. S1a, S2a). Temperature profiles were controlled in sumps (200 L) using a combination of titanium submersible heaters, a submersible chiller, and Apex Neptune AquaControllers (Neptune Systems, CA, USA). Sumps supplied temperature treated 25 µm FSW to larval conicals and temperature was continuously monitored in conicals using HOBO U-22 Temperature Loggers (Onset Computers, MA, USA). Larvae were exposed to temperature treatments for four days in conical systems (Fig. 1). During temperature exposure, larval survivorship was measured in each conical using concentration estimations. Larval concentration was measured by counting larvae under a dissecting microscope

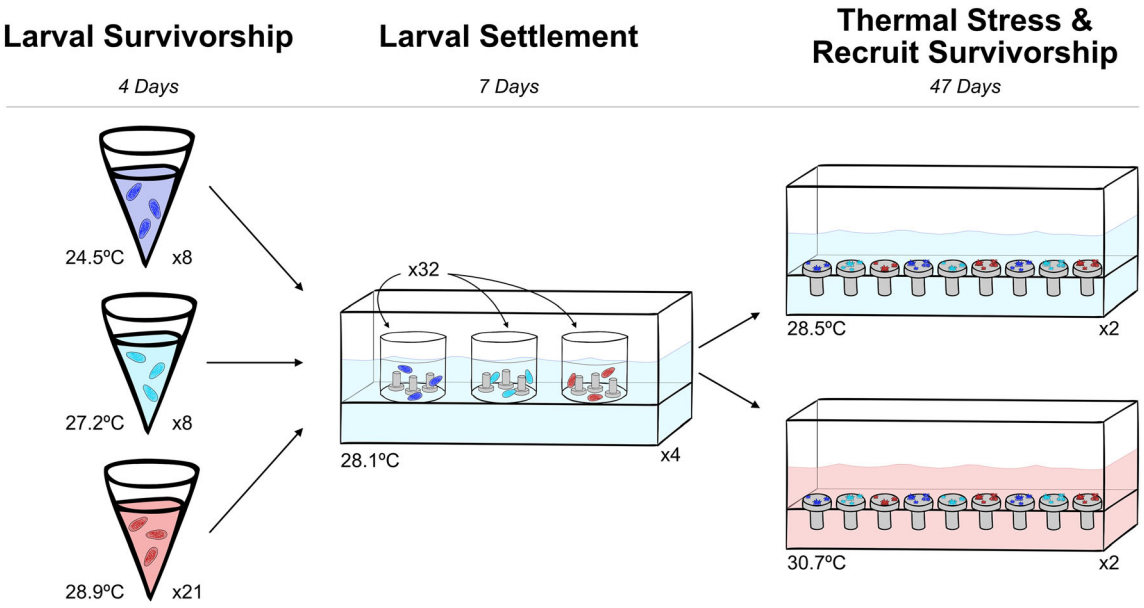

Fig. 1 Experimental design. *Montipora capitata* larvae were exposed to cool (dark blue; 24.5 °C), ambient (light blue; 27.2 °C), and warm (red; 28.9 °C) temperature treatments in conical rearing systems. After four days of exposure, larvae from each temperature treatment were held at ambient (28.1 °C) temperature and allowed to settle on aragonite plugs for seven days. Following settlement, spat from each larval exposure treatment were exposed to either ambient (light blue; 28.5 °C) or high (red; 30.7°) temperature treatments for 47 days. During this experiment, larval survival, larval settlement, and spat survival were monitored

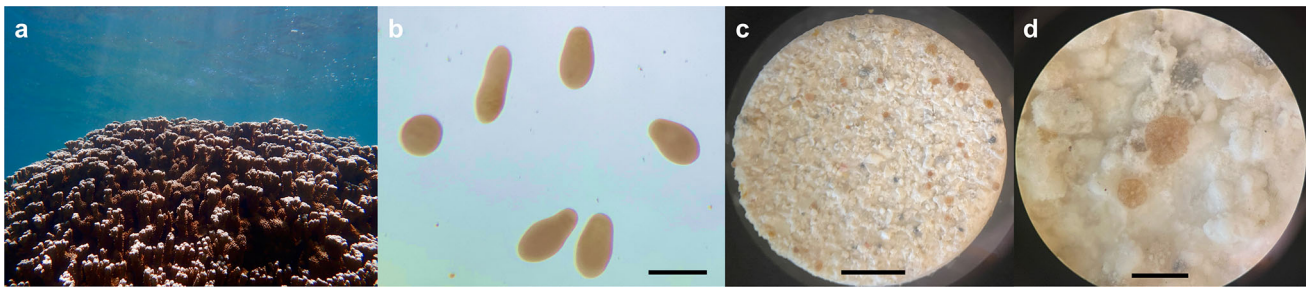

Fig. 2 Photographs of *Montipora capitata*. (**a**) Adult *Montipora capitata* colony in Kāne'ohe Bay, O'ahu, Hawaii. (**b**) Planula larvae, scale bar indicates 1 mm. (**c**) Spat on aragonite plug, scale bar indicates 0.5 cm. (**d**) Two spat on aragonite plug, scale bar indicates 1 mm

in 5 mL water samples taken from gently mixed conicals (n = 5 samples per conical) on days 0, 1, 3, and 4 of larval thermal exposure and calculated as larvae per mL.

Larval settlement

Following larval exposure to temperature treatments, larvae were moved into flow-through FSW (25 µm, 300 mL per min) 40 L tanks (N = 4) for settlement at ambient conditions (28.1 ± 0.4 °C, Fig. S2) equipped with recirculating pumps (Rio 1100 +) and temperature control using a combination of titanium submersible heaters and Apex Neptune AquaControllers (Neptune Systems, CA, USA). After larval thermal exposure, we pooled larvae within each larval temperature treatment and evenly distributed them into clear cylindrical acrylic settlement chambers (n = 100 larvae per chamber, n = 32 chambers per larval treatment, N = 96 total chambers, N = 9600 larvae) and chambers were divided between the four tanks (n = 24 chambers per tank) (Fig S1c). The bottom of each chamber was covered with 123 µm mesh to allow for water exchange. The chambers were placed on elevated racks within each tank such that the chambers were submerged at approx. 5 cm depth, while the top of the chambers was above the water to contain larvae (Fig S1c). Each chamber contained aragonite plugs (2.85 cm^2; n = 3 plugs per chamber) conditioned in 25 µm filtered seawater for 48 h prior to settlement, oriented upside-down such that the settlement surface of the plug was in contact with the mesh. Plugs were labeled with unique identification numbers to identify larval treatment and track plugs throughout the experiment.

Larvae were allowed to settle on aragonite plugs in chambers for seven days at ambient temperature (Figs. 1, S2b) with daily assessments of larval settlement rate. Settlement rate was measured by visually assessing all surfaces of each plug under a dissecting microscope and counting the number of spat, characterized by metamorphosis and formation of oral disks, septa, and/or polyp structures. The number of spat was divided by the number of larvae added to each chamber to calculate settlement rate as a percent. At the end of the settlement period (seven days), plugs with spat (Fig. 2c, d) were haphazardly placed on racks at the bottom of each tank (depth of 10 cm) in preparation for temperature exposure. Plugs that did not have spat were removed from tanks. Spat were allowed to acclimate to tank conditions for two weeks prior to starting the thermal stress experiment.

Spat exposure and survivorship

To test the effects of thermal conditioning during the larval stage on post-settlement thermal tolerance, spat originating from each larval treatment (cool, ambient, warm) were exposed to ambient (28.5 ± 0.4 °C) and high (30.7 ± 0.8 °C) temperature for 47 days (Figs. 1, S2c). Aragonite plugs with spat from each larval temperature treatment were evenly distributed between ambient and high temperature treatments and haphazardly placed in respective treatment tanks (N = 2 tanks per treatment, n = 61–67 plugs per tank per treatment; N = 255 plugs). Tanks were randomly assigned to temperature treatment. Temperature was controlled in each tank as described above during the settlement phase. High temperature tanks were ramped to the desired temperature target (approx. 31 °C) at a rate of 1 °C per day over the course of 4 days (Fig. S2c). Temperature treatments during all phases of this study underwent diel cycles to mimic a daily flux (~ 1°C per day; Fig. S2). Temperature in each tank was recorded every 15 min with HOBO U-22 temperature loggers. Light levels remained low in the indoor environment (< 20 µmol photons m^2 s^{-1}).

Photographs of the upper surface of all aragonite plugs were taken on days 1, 11, 14, 21, 28, and 47 (Fig S3). Using these photographs, spat were mapped on each plug and counted to measure survival as the percentage of alive spat per plug at each time point relative to the initial timepoint (day 1). Spat were counted as 'alive' if the following features were present: oral disk, tentacles, and well-defined shape. Spat were identified as either "individual"

spat, originating from a single settled larva, or "aggregate", originating from multiple larvae settling in an aggregation. We further describe how these classifications were utilized in the data analysis section below. After each sampling time point, aragonite plugs were haphazardly redistributed within each tank to minimize within tank effects. Plugs were not moved between tanks during the experiment.

Data analysis

Data analysis was conducted in R v3.6.1 (R Core Team 2019). Larval survivorship was calculated as proportion survival (measured as larval concentration, larvae per mL) over the course of larval temperature exposure and analyzed using a linear mixed effect model in the *lme4* package (Bates et al. 2014) with larval temperature and day as main effects. Conical nested within temperature treatment was included as a random intercept. Larval settlement was calculated as the number of larvae settled over the course of the settlement trial and was analyzed using a Poisson mixed effect model in the *lme4* package (Bates et al. 2014) with larval temperature and day as main effects. Settlement chamber nested within tank was included as a random intercept. For the above analyses, the assumption of normality was assessed using quantile–quantile plots and homogeneity of variance was examined using Levene's Test in the *car* package (Fox and Weisberg 2018). Spat survivorship was calculated as proportion survival over the course of the thermal stress test and was analyzed using a binomial mixed effect model in the *lme4* package (Bates et al. 2014) with spat temperature, larval temperature, and day as main effects. To account for the effect of aggregation (individual or aggregate spat) on survivorship, we calculated the proportion of aggregate spat on each plug. 97% of plugs had $< 50\%$ aggregate spat and 67% of plugs had $< 10\%$ aggregates. Therefore, we removed outlier plugs with $> 50\%$ aggregate spat from the analysis. To account for variation due to settlement behavior, we included the proportion aggregation for each replicate plug as a random intercept in mixed effect model analysis of spat survival. We also included plug nested within tank as a random intercept. The assumption of overdispersion was assessed in the *blmeco* package (Korner-Nievergelt et al. 2015). Significance of main effects from mixed effect models was determined in the *lmerTest* package (Kuznetsova et al. 2015) by Type II sum of squares analysis of variance (ANOVA) tests.

Results

Larval survivorship

Larval survivorship declined during the four-day temperature exposure ($p < 0.001$; Fig. 3a; Table S1) with the rate of decline significantly influenced by treatment ($p = 0.009$; Fig. 3a; Table 1). Mean survival in the cool treatment was 13% and significantly lower than mean survival of larvae in ambient conditions (60%; post hoc $p = 0.035$). There was also a trend for lower survival in the warm treatment (mean 24%) as compared to ambient larvae, but this was not significant (post hoc $p = 0.122$) (Fig. 3a; Table S1).

Larval settlement

Larval settlement significantly increased over the course of the seven-day settlement period and was modulated by larval temperature treatment ($p < 0.001$; Fig. 3b; Table 2). Overall, settlement was low in all treatment groups with $< 25\%$ settlement after seven days (Table S2). Mean larval settlement throughout the settlement period was highest in larvae exposed to warm temperature (post hoc $p < 0.001$ on days 1, 2, and 6) and lowest in those from the cool treatment (post hoc $p < 0.05$ on days 1, 2 and 3) (Fig. 3b).

Spat survivorship

Spat survival was significantly influenced by the immediate environmental conditions (spat temperature, $p < 0.001$), and was not affected by previous larval thermal conditioning ($p = 0.983$; Table 3). Further, spat survival was significantly reduced over the course of the stress test in high temperature ($p < 0.001$; Fig. 3c) but survival under high temperature was not influenced by larval conditioning ($p = 0.930$; Fig. 3c; Table 3). There was no difference in mean survivorship between spat temperature treatments for the first three weeks (post hoc $p > 0.05$); however, after 28 days there was significantly greater mortality in the high temperature treatment (post hoc $p = 0.011$), which continued to decline through day 47 (post hoc $p < 0.001$; Fig. 3c). At the end of the experiment, survivorship was 34% lower in the high treatment than ambient (Table S3). Although we did not quantify bleaching, we noticed high variation in spat pigmentation throughout the study and did not observe consistent bleaching responses in spat under high temperature (see example photographs in Fig. S3).

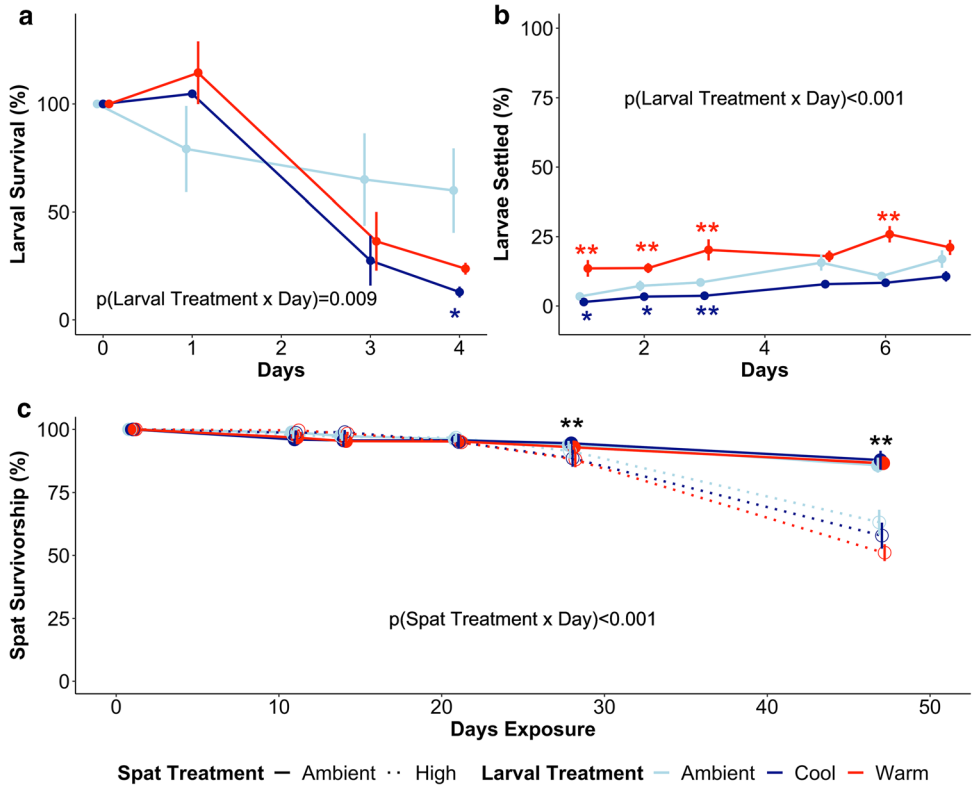

Fig. 3 Larval survival, settlement, and spat survival. (**a**) Larval survival (%) was measured in cool (dark blue), ambient (light blue) and warm (red) larval temperature treatments for four days after embryos developed to the planula stage. Means for larval survivorship were calculated as the mean larval density in replicate seawater samples, and therefore means may be higher than 100% due to artifacts in this measurement method. In all plots, *p*-values indicate significance of effects determined by mixed effect models. In (**a**) and (**b**) asterisks indicate the presence of significant differences (single asterisk, $p < 0.05$; double asterisk, $p < 0.01$) between ambient and cool (blue asterisks) or ambient and warm treatments (red asterisks) as determined by post hoc comparisons. (**b**) Larval settlement (%) was measured at ambient temperature for seven days following larval exposure. Color indicates larval temperature treatment (cool, dark blue; ambient, light blue; red, warm). (**c**) Survival of spat originating from each larval temperature treatment (cool, dark blue; ambient, light blue; red, warm) was measured in ambient (solid line) and high temperature (dashed line) treatments over 47 days. Asterisks indicate the presence of significant differences (single asterisk, $p < 0.05$; double asterisk, $p < 0.01$) in survival between ambient and high spat treatments. There were no significant differences in survival due to larval temperature treatments

Table 1 Linear mixed effect model analysis of larval survivorship. Significance determined by Type II Satterthwaite ANOVA analysis

Larval survivorship			
Main effect	SumSq	df	*p*-value
Larval temperature	0.07	2, 9.18	0.485
Day	4.33	3, 25.40	**< 0.001**
Larval temperature: Day	0.85	6, 25.51	**0.009**
Random effect	Variance	Std. Dev	
Treatment: Conical	0.02	0.15	

SumSq sum of squares; df degrees of freedom

Bold indicates statistical significance ($p < 0.05$)

Table 2 Poisson mixed effect model analysis of larval settlement. Significance determined by Type II Wald Chi-Square ANOVA

Main effect	ChiSq	df	*p*-value
Larval temperature	70.03	2	**< 0.001**
Day	280.52	5	**< 0.001**
Larval temperature: day	63.55	10	**< 0.001**
Random effect	Variance	Std. Dev	
Chamber	0.26	0.51	
Tank	0.03	0.19	

ChiSq chi-squared; df degrees of freedom; std. dev. standard deviation

Bold indicates statistical significance ($p < 0.05$)

Table 3 Binomial mixed effect model analysis of spat survivorship

Main effect	ChiSq	df	p-value
Spat temperature	143.02	15	**< 0.001**
Larval temperature	9.03	20	0.983
Day	596.67	25	**< 0.001**
Spat temperature: Larval temperature	4.68	10	0.912
Spat Temperature: day	101.01	13	**< 0.001**
Larval Temperature: day	7.21	18	0.988
Spat Temperature: Larval temperature: day	4.35	10	0.930
Random effect	Variance	Std. Dev	
Plug	1.33	1.15	
Tank	< 0.01	< 0.01	

Significance determined by Type II Wald Chi-Square ANOVA

ChiSq = chi-squared; df = degrees of freedom; std. dev. = standard deviation

Bold indicates statistical significance ($p < 0.05$)

Discussion

Ocean warming is compromising the recruitment of corals onto reefs by reducing the number of reproductive adults and the available larval pool (Hughes et al. 2019). It is critical to understand the effect of thermal stress on coral recruitment and, simultaneously, to evaluate the potential for management and restoration interventions to increase reef recruitment. Current approaches to reef restoration include the propagation of sexually produced spawned coral offspring with subsequent out planting of spat (Guest et al. 2010; Vanderklift et al. 2020). These ex situ nursery approaches are aimed at increasing survival of vulnerable life stages during critical mortality bottlenecks such as larval survival, metamorphosis, and post-settlement growth. In addition to propagating large numbers of coral spat, there is an opportunity to increase fitness in early life history stages by inducing positive latent effects through exposure to manipulated thermal regimes. Here, we evaluated the potential for thermal conditioning during the larval stage to increase the survival of spat under subsequent elevated temperature in the spawning coral *Montipora capitata*. We examined the effects of larval thermal exposure across multiple life stages (larval survival, settlement, and spat survival), providing new insights into early life history response to temperature conditioning. Although there was no larval conditioning regime that increased the thermal tolerance of spat in this study, we suggest that manipulating thermal regimes may help optimize the survival and settlement of larvae.

Montipora capitata survival was reduced under elevated temperature in both the larval and spat stages (Fig. 3a, c), highlighting the detrimental effects of elevated thermal exposure on early life stage performance. Mean survival of *M. capitata* larvae was highest at ambient temperature and reduced under both cool and warm temperature conditions (Fig. 3a). During the larval stage, corals primarily rely on energy from lipid stores that can also be supplemented by photosynthates from algal symbionts (Harii et al. 2007, 2010) but other evidence suggests this contribution may be negligible (Kopp et al. 2016). Exposure to stress strains energetic resources in early life history and therefore, exposing larvae to temperature conditions that minimize energetic demands of stress (e.g., cellular stress response) could decrease mortality risk (Marshall et al. 2020; Sokolova 2021) and it is well documented that thermal stress increases mortality at the larval stage (e.g., Bassim and Sammarco 2003; Randall and Szmant 2009; Schnitzler et al. 2012). In nursery settings, maintaining larvae at historically ambient conditions for the respective seasonal period may provide the most favorable conditions for larval survival, especially as marine heat waves continue to increase in frequency and severity.

In this study, we found that there was higher mean settlement in larvae exposed to warm temperature and lower mean settlement in larvae exposed to cool temperature (Fig. 3b). Previous work has shown that exposure to elevated temperature can result in increased or accelerated metamorphosis, due to stimulatory effects on metabolism and development (Edmunds et al. 2001; Nozawa and Harrison 2007; Putnam et al. 2008; Marshall et al. 2020). However, marginal gains in settlement due to exposure to warm temperature exposure may not outweigh the reduction in the recruitment pool during the larval stage and subsequent spat stage (i.e., reduced recruitment overall). Therefore, in ex situ settings it may be more advantageous to rear *M. capitata* larvae at ambient temperatures to maximize the production of early life stages through the spat stage. Further studies that document physiological effects of temperature during larval metamorphosis are

crucial for our understanding of settlement under ocean warming.

Spat survival decreased by 34% when exposed to high temperatures (Fig. 3c). High mortality in spat under elevated temperature has also been observed in other species, for example in *Pocillopora acuta* (Bahr et al. 2020; Huffmyer et al. 2021), *Acropora solitaryensis* and *Favia chinensis* (Nozawa and Harrison 2007), and *Porites astreoides* (Ross et al. 2013). In this study, we also documented mortality under ambient temperature; survival rates observed here ($\sim$ 75%) closely reflect survival in *M. capitata* spat recorded in another study (Hancock et al. 2021).

We examined the potential for thermal conditioning during the larval stage to decrease the severity of mortality in the spat stage when exposed to thermal stress and found that prior exposure to thermal conditioning regimes in the larval stage did not significantly improve spat survival under elevated temperature conditions (Fig. 3c). This reflects previous work on thermal conditioning in adult *M. capitata* that found no protective effect of multiple temperature-time profiles (Dilworth et al. 2021). In a study tracking spat survival in *M. capitata* conducted during the same reproductive event as the present study, Hancock et al. (2021) found that larval thermal experience had a small, but significant role in the bleaching response of 2.5-month-old spat. Specifically, spat that were exposed to warm temperatures as larvae were more susceptible to bleaching than those exposed to ambient or cool temperatures. It is possible that in *M. capitata*, latent effects from larval exposure to elevated temperature conditions may manifest later in development than recorded in the present study. Additionally, we reared spat under low light conditions while spat in Hancock et al. (2021) were under outdoor higher light conditions, likely exacerbating negative temperature effects (Abrego et al. 2008). Future work should continue to investigate the potential for latent effects in coral early life history and the strength and length of exposure required to induce conditioning effects.

Response to thermal conditioning is dependent on life stage, duration, and intensity of exposure—for example, positive effects of conditioning to high temperature were documented during embryonic development in *Acropora pulchra* (Puisay et al. 2018). Due to the variation in exposure timing, duration, and intensity across studies of coral thermal response (McLachlan et al. 2020) it is challenging to make broad conclusions and recommendations on the potential for thermal conditioning to elevate coral thermal tolerance during early development. Rather, these effects are likely species, reproductive mode, and life stage-specific and should be considered in the context of local thermal history. In our study, increased stress duration and intensity may have been required to elicit measurable latent effects on spat thermal tolerance. Evaluating the influence of thermal conditioning across reproductive systems is a critical area of future research.

Complementary approaches that utilize both conditioning and selection of desirable traits, such as thermal tolerance, are worthwhile approaches to consider in propagation of coral offspring. In addition to conditioning and physiological hardening to stress, subjecting larvae to acute thermal experiences in nursery settings can result in only the highest performing corals surviving through to subsequent recruitment, providing a pathway to selecting the most tolerant individuals for restoration (Caruso et al. 2021). Thermal effects also apply to the symbiont partner; *M. capitata* is known to associate with both thermally sensitive *Cladocopium* sp. and thermally tolerant *Durusdinium* sp. and the composition of symbiont communities can impact the performance of the host (Cunning et al. 2016; Innis et al. 2018; Wall et al. 2020). Future work should continue to investigate the impact of symbiont community on early life history survival and response to thermal stress and consider potential trade-offs. Specifically, a valuable area of future research is to compare and evaluate the potential for thermal conditioning between vertical and horizontal transmission coral species.

In this study, exposure to elevated temperature reduced *M. capitata* larval and spat survival with no significant positive benefit of thermal conditioning during the larval stage on spat thermal tolerance. These results suggest that investing in maintaining ex situ conditions below stress thresholds can maximize the survival of coral offspring. Thoroughly evaluating and developing tools for maximizing early life history survival under climate change stress is an important component of reef restoration and management that should occur simultaneously with climate change mitigation.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00338-022-02234-x.

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Data availability Data and scripts are available at DOI: https://doi.org/10.5281/zenodo.6079286

Declarations

Conflict of interest The authors declare no conflicts of interest.

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