Effects of elevated temperature on reproduction and larval settlement in *Leptastrea purpurea*

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Abstract As global ocean temperatures continue to rise, severe declines in coral reef health and diversity are reported on a global scale. Recovery of coral reefs relies on reproduction and increased rates of successful recruitment, which can vary tremendously across coral species. We investigated the effects of increased temperatures in the environment of parental colonies on larval production, size, settlement and survival, in the heat-resistant coral *Leptastrea purpurea* in Guam. Thanks to two tank experiments (eleven and four weeks, respectively) conducted over two consecutive years we found that larvae released by heat-treated parents (30°C) were significantly smaller in size but greater in number, had normal settlement behavior and increased post-settlement survival rates compared to those released by control parent colonies (28°C). We conclude that changes in the environment of parental *L. purpurea* colonies trigger an anticipatory maternal effect which leads to the release of preconditioned larvae with an increased chance of survival.

Keywords Scleractinia • Recruitment • Guam • Coral bleaching • Preconditioning

Introduction

Increases in sea surface temperature have led to severe declines in coral reef health and diversity on a global scale (Spalding and Brown 2015; Hughes et al. 2018; Smale et al. 2019). Between 2013 and 2017, consecutive worldwide mass coral bleaching events struck reefs around the island of Guam and resulted in losses of up to 60% in coral cover (Raymundo et al. 2019). Coral bleaching can have various consequences for corals and their surrounding ecosystems in both the short and long term. During and shortly after bleaching, corals often experience shifts in Symbiodiniaceae composition, compromised photosynthetic efficiency, reduced skeletal growth, and are more susceptible to disease and predation (Lesser 2006; Baird et al. 2009). In the long term, corals can exhibit increased risk of partial and full mortality as well as decreased fecundity and growth (Ward et al. 2002). Additionally, a coral depleted reef may be overtaken by algae and experience shifts in fish community composition potentially reducing fishery productivity (Graham et al. 2007).

Recovery of coral reefs, including the rebuilding of depleted adult populations and maintaining resilience in the face of increasing environmental pressures, relies heavily on reproduction and increased rates of successful recruitment (Holbrook et al. 2018). However, coral reproduction, settlement and recruitment can all be affected by thermal stress to various extents depending on the species. For example, increased temperatures may lead to a decrease in larval survivorship and settlement in spawning corals, particularly in Acroporids (Hughes et al. 2019), as well as in the brooding coral *Favia fragum* (Randall and Szmant 2009). Interestingly, *Cyphastrea japonica*, *Favites* and *Acropora millepora* species show increased larval development coupled with increased larval mortality rates at
higher temperatures (Figueiredo et al. 2014). In Porites astreoides, elevated seawater temperature can affect larval motility or reduce photosynthesis and energy supplies, ultimately causing an increase in larval mortality (Edmunds et al. 2001). Elevated temperatures can also shorten the pelagic larval phase and skew larval settlement from their preferred to a less suitable substrate in Stylophora pistillata (Putnam et al. 2008). Few studies have identified examples of trans-generational acclimation to stressful environmental conditions through anticipatory parental or maternal effects in corals (Putnam and Gates 2015). This rapid-response mechanism assumes that parents or mothers shift the allocation of resources to the offspring when brooded in a stressful environment to increase offspring fitness (Crean and Marshall 2009). Corals that can pass on greater heat tolerance to their offspring may adapt better to the predicted environmental conditions of the future and dominate future reefs. Given the variation in corals’ response to heat stress, broadening the number of studied scleractinian species is essential to refine our understanding of coral reproduction under climate change, and better predict recovery patterns after disturbance events (McLachlan et al. 2020).

Here, we focused on Leptastrea purpurea, an encrusting coral that forms small colonies and is commonly found throughout the Indo-Pacific region (Veron 2000; Arrigoni et al. 2020). In Guam, L. purpurea is found in abundance on back-reefs (Nietzer et al. 2018). Leptastrea purpurea is a brooding coral, producing and releasing larvae daily, which makes it ideal for reproduction experiments (Nietzer et al. 2018). Like most other corals, L. purpurea larvae settle preferably on crustose coralline algae (CCA) and larval metamorphosis is induced in the presence of the CCA Hydrolithon reinboldii (Nietzer et al. 2018). Dispersal abilities of L. purpurea larvae are unknown and could vary from a couple hundred to thousands of meters. In fact, larvae of the brooding coral P. astreoides, can travel distances up to 1,900 km in favorable current conditions (Serrano et al. 2016), while the larvae of brooding Helio- pora coerulea can only disperse throughout their natal reef, within 350 m of parent populations (Harii et al. 2002). Leptastrea purpurea is relatively resistant to thermal stress and there is anecdotal evidence of a surge in larval production in response to sea surface temperature increase (van Woesik et al. 2011; Nietzer et al. 2018). Over the course of four years of survey during recurrent bleaching events (2013–2017) on Guam, L. purpurea was recorded to experience bleaching, but showed no mortality despite significant losses in other coral species. In addition, 2017 surveys recorded new recruitment and growth of L. purpurea colonies (Raymundo et al. 2019).

In the present study, we investigate the effects of parental heat treatment on larval production and fitness (quantity, size, settlement and recruit survival rates) in order to better understand the effects of increased seawater temperature on L. purpurea reproduction. On small islands such as Guam, understanding coral reproduction is vital to predicting future coral assemblages after disturbances and restoring healthy ecosystems.

Methods

Two experiments were conducted one year apart. The first experiment, referred to as “Experiment 1,” tested whether an increase in seawater temperature led to an increase in larval production. The second experiment, referred to as “Experiment 2,” characterized the fitness of larvae produced by heat-treated parental colonies.

Sample collection, experimental design, and larvae collection

Experiment 1: In March 2019, 45 adult colonies of L. purpurea (all between 8 and 10 cm² in size) were collected on Luminao Reef, Guam (13.465092, 144.647424) and transported back to the University of Guam Marine Laboratory where they were given three days to recover in flow-through seawater tables. Colonies were then placed in six opaque flow-through tanks covered with shade cloth. Each tank was randomly assigned four L. purpurea colonies elevated on a PVC platform following the protocol detailed in Nietzer et al. (2018) and a digital thermometer (Risepro®). Half of the tanks (randomly chosen) were gradually heated from 28 °C to Guam’s warmest recorded temperature in 2018 (30.5 °C; personal observation, C. Sartor) and bleaching threshold (NOAA Coral Reef Watch) over an hour using Finnex® 800 W titanium water heaters with temperature controllers. The Maximum Monthly Mean (MMM, i.e., average daily temperature of the hottest month of the year) for 2018 in Luminao was 29.3 °C (pers. comm. L. Raymundo). According to Grottoli et al. (2021)’s recommendations to increase comparability among coral heat stress experiments, heat-stress temperatures should be 1 °C to 4 °C above the MMM of the site where corals were collected, which is in accordance with our chosen temperature of 30.5 °C. Over the course of 11 weeks, the average temperature in the heated tanks was 30.5 °C (SE = 0.17 °C). The other three tanks served as controls and were maintained at ambient seawater temperature (average 27.9 °C, SE = 0.07 °C; Fig. 1). In all tanks the incoming flow was set to 0.5 L/min, the average daylight lux was 342.9 (SE = 3.6; min = 10.8 and max = 6200; Onset HOBO Pendant Temperature/Light Logger), and average salinity was 35,105.8 uS/cm (SE = 2.56; min = 34,463.8 and max = 36,356.2; Onset HOBO
Conductivity/Salinity Logger U24-002-C). For 11 weeks, coral tissue color was measured and recorded regularly for each colony by visual comparison to values on a Coral-Watch Coral Health card (Siebeck et al. 2006). Color values were collected by the same individual to maintain consistency, and if the tissue color appeared to be between two values, an intermediate value was given (i.e., E3.5). Differences in colony tissue color were assessed with a Kruskal–Wallis test (Supplementary Table 1). Corals were fed 60 ml of live \textit{Artemia nauplii} in solution between 1800 and 2000 h., every three days. During that time, the inflow of seawater was turned off in the evening until the next morning, ensuring that both Artemia nauplii and coral larvae remained in the tank, thus enhancing coral feeding opportunities and facilitating larvae collection. The following morning (15 h after feeding), larvae were collected by filtering the water from each tank through a 30 \textmu m mesh. Larvae caught in the mesh were resuspended in small containers with 0.2 \textmu l M filtered seawater.

\textbf{Experiment 2:} In February 2020, 40 adult colonies of \textit{L. purpurea} (all between 8 and 10 cm\textsuperscript{2} in size) were collected as described above. After the recovery period, colonies were randomly placed in two large flow-through seawater tables to maximize similarity of experimental conditions (common garden approach, Fig. 1). The control seawater table was maintained at ambient temperature (average $= 27.9$ °C, SE $= 0.06$ °C). In 2019, the MMM for Luminao was 29.5 °C and the warmest recorded seawater temperature for Guam was 30.0 °C (pers. comm. C. Sartor). The heated water table was gradually heated over three hours and maintained at an average temperature of 30.0 °C (SE $= 0.07$ °C) using three Finnex® 800 W titanium heaters with temperature controllers and two digital thermometers (Risepro®). A shade cloth was draped over the seawater tables and each table contained three PVC platforms each holding six to seven colonies (Fig. 1). Incoming water flow, lux and salinity in the water table were similar to Experiment 1. Colonies were fed every three days as described above for 24 days. To obtain large numbers of larvae at once for the settlement and survival experiments, larvae were collected three times at 8-day intervals (i.e., three collection batches total). To do so, the trays of \textit{L. purpurea} colonies were transferred overnight into three smaller tanks maintained at the same temperature as the seawater tables. The next morning, larvae were collected as described above and colonies were returned to their respective flow-through seawater tables. Adult colony tissue color was measured and recorded regularly as described for Experiment 1.

\textbf{Larval counts, size, and settlement rates}

\textbf{Experiment 1:} The total number of larvae released in each tank was counted under a dissecting microscope by shining a fluorescent blue light on the filtered resuspended material. The influence of temperature on larval count data was assessed using a mixed effect model including “day of release” as random and “size” and “treatment” as fixed effects in the R package “lmer” (Bates et al. 2014; R Core Team 2020). Significance level was set at $p < 0.05$ (Supplementary Table 2).

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**Fig. 1** Experimental designs for Experiment 1 (top) and Experiment 2 (bottom). The picture illustrates two adult colonies of \textit{Leptastrea purpurea} on a PVC platform in a water table.
Experiment 2: Larvae obtained from each collection batch were counted as described above. Larval size (length) was measured under an Olympus SZX16 microscope with an optical scale following the protocol described in Nietzer et al. (2018). Larvae were then transferred to six-well plastic plates filled with 10 mL of 0.2 μM filtered seawater at ambient temperature. Each well contained chips of H. reinboldii (1 cm² total) and up to four L. purpurea larvae. Every day two thirds of the water content in each well was replaced with fresh filtered seawater. After collection day, every settlement well was checked daily under a dissecting microscope for three days and the number of settled larvae (on CCA or plastic well) present in each well was recorded. After the third day, settlement wells were observed every other day, again under ambient temperature until the end of the experiment to account for late settlements and report overall settlement rates and recruit survival rates. Observations were interrupted after four weeks when the University closed in response to the spread of the SARS-CoV-2 virus. As a result, the first batch of larvae collected was observed for four weeks, the second batch for three weeks and the last batch for only two weeks. Recorded data were tested for normality with a Shapiro–Wilks test. Significant effects of heat treatment were assessed as follows:

Larval counts and sizes overall the three collection batches were tested with a mixed effect model including “day of release” as random and “size” and “treatment” as fixed effects (Supplementary Tables 2 and 3). Differences in larval sizes per batch were assessed with a Kruskal–Wallis test (Supplementary Table 4). Differences in settlement rate between treatment groups were assessed with a mixed effect model including “day of release” as random and “size” and “treatment” as fixed effects (Table 1, Supplementary Figure 1; Supplementary Table 2). Noticeably, throughout both experiments the number of larvae released in both treatments decreased with time (Table 1, Supplementary Figure 2) most likely caused by a combination of handling-induced stress and life in captivity, as reported previously (Nietzer et al. 2018). During Experiment 1, heat-treated colonies released a total of 234 larvae (3.7 larvae/day on average), whereas the control colonies only released 115 larvae (1.8 larvae/day) over 64 days of collection. During Experiment 2, the heat-treated colonies released a total of 55 larvae (3.7 larvae/day), while the control colonies only released 25 larvae (1.7 larvae/day), over 15 days of collection. We noticed that the increased time between larval collections in Experiment 2 (8 days vs. 3 days in Experiment 1), which could allow some larvae to either escape or settle in the tank prior to collection day, did not affect the total number of larvae collected across the two experiments. Only a few other species of brooding corals such as S. pistillata, Pocillopora damicornis or Echinopora lamellosa are known to release larvae when temperatures are within their upper survival threshold (Fan and Dai 1999; Edmunds et al. 2011; Crowder et al. 2014, 2017). These studies showed that elevated temperatures accelerate gametogenesis and the timing of planulae release in coral species that follow a reproductive cycle (e.g., lunar, monthly or seasonal, etc.; Crowder et al. 2014). Here, we show that elevated temperatures can also influence planulation in species that release larvae in a non-seasonal and non-cyclic manner, like L. purpurea (Nietzer et al. 2018). In the context of climate change, understanding how elevated temperatures affect reproduction in various coral species is essential to better predict patterns of reproductive success and colonization.

The average size of larvae released by heat-treated colonies over all collection batches was significantly smaller than that of the larvae obtained from the control colonies (p < 0.05; Table 1, Fig. 3c, Supplementary Table 3). Average sizes per batch were also smaller for larvae released by heat-treated colonies compared to controls but this difference was only significant in the first batch (which is also the batch that produced the largest number of larvae; Table 1, Supplementary Figure 2 and
Table 4). Under stressful environmental conditions organisms may produce smaller eggs or offspring; this phenomenon has been documented in several marine invertebrates including polychaetes, bryozoans, the brooding coral *P. damicornis* and other broadcast spawning corals (summarized in Putnam and Gates 2015). There is

![Fig. 2](image-url) Average coral tissue color of heated and control parental colonies for Experiment 1 (a) and Experiment 2 (b). Color was measured by visual comparison to values on a CoralWatch Coral Health card (Siebeck et al., 2006), where 6 is the darkest hue and 0 corresponds to white or bleached tissue. None of the comparisons between control and heat-treated colonies are significant.

**Table 1** Summary of data collected in Experiments 1 and 2

| Experiment | Control | Heat treated |
|------------|---------|--------------|
| Nb Larvae Collected | Average Larvae Size | Total Nb Larvae Settled |
| Experiment 1 | | |
| Control | 115 | / | / | / | / |
| Heat treated | 234 | / | / | / | / |
| Experiment 2 | | |
| Control Batch 1 | 15 | 0.7 (SE = 0.05) | 15 | 8 | 7 |
| Control Batch 2 | 6 | 0.49 (SE = 0.02) | 6 | 4 | 2 |
| Control Batch 3 | 4 | 0.59 (SE = 0.08) | 4 | 4 | 0 |
| Control Overall | 25 | 0.64 (SE = 0.04) | 25 | 16 | 9 |
| Heat-treated Batch 1 | 21 | 0.55 (SE = 0.03) | 20 | 10 | 10 |
| Heat-treated Batch 2 | 18 | 0.52 (SE = 0.04) | 18 | 15 | 3 |
| Heat-treated Batch 3 | 16 | 0.54 (SE = 0.04) | 14 | 13 | 1 |
| Heat-treated Overall | 55 | 0.54 (SE = 0.02) | 52 | 38 | 14 |
also evidence that larvae released early within the spawning cycle of the brooders *P. damicornis*, *P. asteroides* and *Seriatopora caliendrum* are smaller in size than larvae released later (Putnam et al. 2010; Cumbo et al. 2012; de Putron et al. 2017; but see Zhang et al. 2019). This observation combined with the small larvae released by heat-treated *L. purpurea* colonies, could be another indication that elevated temperatures accelerate either gametogenesis or the timing of larval release in this species.

Smaller larvae have lower lipid content, higher respiration rates and larger energy demands which leads to an accelerated development (Harii et al. 2002; Edmunds et al. 2011; Putnam and Gates 2015). Our results suggest that in response to a potentially stressful environment, *L. purpurea* colonies modulate their larval number and size most likely to increase settlement success. The phenotypic plasticity observed in larval number and size in *L. purpurea* could be attributed to an anticipatory maternal effect (Marshall and Uller 2007). In response to a change in the environment, anticipatory maternal effect allows mothers to increase offspring fitness with regards to the predicted new environment; thus, increasing offspring’s chance of survival (Marshall and Uller 2007). In our context this means that the smaller and more numerous larvae produced by *L. purpurea* colonies in elevated temperatures should fare better in warmer water as well, which we did not test for in the present study. However, this could explain why the number of recruits of *L. purpurea* increased after the Guam 2017 bleaching event caused by increased seawater temperatures (Raymundo et al. 2019). In the context of global climate change, corals that are capable of anticipatory maternal effects have a clear advantage over other species (Crean and Marshall 2009). Further studies aiming at exploring this effect in stress-resistant corals species will help us better predict the future composition of coral reefs.

Parental heat-treatment does not affect larval settlement but increases survival of recruits

Despite being smaller in size, the larvae released by heat-treated parental colonies settled at similar rates as larvae released by control colonies (63.6% and 68% after 72 h, respectively, p = 0.05; Table 1, Fig. 4a). When the settlement assays were interrupted due to the covid19 pandemic, 94.5% of the larvae released by heated colonies had settled versus 100% for the control larvae (p = 0.9; Fig. 4a). Similarly, substrate preference was not affected by the parental treatment. Within both treatment groups, larvae significantly preferred to settle on CCA over the plastic wells (p < 0.05; Fig. 4b, 4c; Supplementary Table 6). Overall, 64% and 73% of control and treated larvae settled on CCA, respectively. Moreover, larvae produced by heat-treated parents settled on CCA over plastic at the same rate than larvae produced by control parents (p > 0.05; Fig. 4b; Supplementary Table 5). Elevated temperatures can cause larval developmental aberrations or larvae that do not properly settle or mature into adulthood after settling (Diploria strigosa Bassim and Sammarco 2003; Fungia scutaria Schnitzler et al. 2012). The similarity in settlement rates and substrate choice between the two larval types under ambient temperatures highlights the fact that increased temperatures during reproduction of *L. purpurea* do not have detrimental effects on larval settlement in this species. This remarkable observation most likely contributes to explaining why this species was reported to be mildly affected by recent and recurrent thermal anomalies (Raymundo et al. 2019).

Interestingly, batch 1 larvae released by heat-treated colonies tended to have a significantly higher survival rate post-settlement than controls when observed under ambient seawater temperatures (p < 0.0001, Fig. 5, Supplementary Table 7). The differences in survival rate between larvae released by heat-treated and control parents were, however,
Fig. 4 Larval settlement (Experiment 2). a Percent settlement of larvae released by heat-stressed and control parental colonies, after 72 h. and overall, b Preferred settlement substrate of larvae released by heat-treated and control parental colonies, c Photograph of an 11-day old recruit on CCA

Fig. 5 Larval survival (Experiment 2). Kaplan–Meier survival curves of *Leptastrea purpurea* recruits produced by control (black) and heated (gray) parental colonies
not significant for batches 2 and 3 (p = 0.25 and p = 0.99, respectively). We suspect that the absence of significance between treatments in batches 2 and 3 is due to the combined reduced amount of time we could run the survival experiment (3 weeks and 2 weeks, respectively) and the reduced number of larvae produced by both batches that made it to the survival experiment (24 and 18, respectively) compared to batch 1 (which ran for 4 weeks with 35 larvae total). Brooded embryos and larvae may be able to acclimate to the environmental conditions their parents experienced during their development within the parental colony, a phenomenon otherwise called preconditioning (Putnam and Gates 2015). Our experiment showed that despite being preconditioned to high temperatures, larvae produced by heat-treated parents are not at any significant disadvantage compared to control larvae when exposed to a different and less stressful environment than the one in which they were initially released in. In the longest survival experiment (i.e., 4 weeks), recruits from heated-treated parents actually displayed a significant advantage compared to controls, which raises the question of whether these preconditioned recruits might in fact have a higher fitness regardless of the thermal environment they found themselves in. There is evidence that some corals modify their endosymbiotic Symbiodiniaceae community in response to thermal stress and transfer these modifications to their offspring, increasing the offspring's chance to respond to thermal stress and transfer these modifications to their endosymbiotic Symbiodiniaceae community in the experimental tanks. As a result, some of the observed difference between treatments could be attributed to coral size or other factors such as genotype. Nonetheless, our findings reveal that adult L. purpurea colonies can unequivocally display plasticity in their reproduction strategy and that these changes can happen very rapidly. Whether this plasticity is beneficial over the long term (for adults, larvae, and recruits) still needs to be confirmed with long-term experiments. Furthermore, understanding and characterizing levels of reproductive plasticity across various coral species is a pressing question because plasticity can determine species chances to survive in an ever and fast changing environment (Via et al. 1995). Despite having a large geographical distribution spanning the whole Indo-Pacific, L. purpurea are not major reef-builders. Instead, they are encrusting corals capable of growing on fragments of dead coral skeletons or other hard and bare substrates. As seawater temperatures continue to rise, the need to increase the scope of studied coral species beyond the more commonly studied reef-builders (e.g., Acroporids, Poritids or Pocilloporids) becomes more pressing, as less common, and smaller species might dominate future reefs.

### Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s00338-022-02241-y.

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### Declarations

**Conflict of interest** On behalf of all authors, the corresponding author states that there is no conflict of interest.

**Data accessibility** Supplementary files and raw data are available on the publicly accessible data repository https://dataverse.harvard.edu/dataverse/Leptastrea_purpurea.

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