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Family matters: Variation in the physiology of brooded *Porites astreoides* larvae is driven by parent colony effects

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ABSTRACT

The planktonic larval phase of scleractinian coral life-history represents a crucial stage when dispersal takes place and genetic diversity among populations is maintained. Understanding the dynamics influencing larval survival is especially relevant in the context of climate change, as larvae may be more vulnerable to environmental disturbances than adults. Several physiological parameters of coral larvae have been shown to vary by release time and past environmental history. However, the contribution of parental or genetic effects is largely unknown. To investigate these potential familial effects, we collected adult *Porites astreoides* colonies in April 2018 from two reef zones in the lower Florida Keys and quantified physiological traits and thermal tolerance of the newly released larvae. Family accounted for more variation than day of release and reef origin, with > 60% of the variation in chlorophyll *a* and protein content explained by family. The survivorship of larvae under 36 °C acute temperature stress was also tightly linked to what parent colony they were released from. During a 32 °C moderate temperature stress experiment, inshore larvae tended to bleach less than offshore larvae, mirroring the enhanced bleaching resistance previously observed in inshore adult coral populations. The significant familial effects identified in the present study suggest that researchers should be cautious when interpreting results of studies which pool larvae among families, and that future studies should take care to account for this variation.

1. Introduction

Modern oceans are currently undergoing a period of rapid environmental change (Burrows et al., 2011; Zalasiewicz et al., 2011). Marine organisms have the potential to cope with these challenges through some combination of genetic adaptation, physiological acclimatization, and/or migration to a more suitable environment or microclimate (Foo and Byrne, 2016; Gienapp et al., 2008; Palumbi et al., 2014). The general consensus is that the accumulation of beneficial mutations occurs slowly in nature unless selection is strong and recombination rate is high (Barrick and Lenski, 2013; Kimura, 1964). An increasing body of literature has identified phenotypic plasticity as an important acclimatization mechanism that enables populations to persist in new regimes, while buying time for adaptation to occur over multiple generations (Hendry, 2016; Munday et al., 2013). Migration or dispersal to more suitable habitats will facilitate the persistence of a species, but may result in localized extinction of populations (Gienapp et al., 2008; Pellerin et al., 2019). The extent to which one or a combination of these strategies is employed depends in large part on life history (Meyers and Bull, 2002; Moreno and Møller, 2011).

Marine organisms exhibit an incredible diversity of life histories, many of which include a planktonic larval stage or stages (Marshall and Morgan, 2011; Strathmann, 1985). For species in which adults are sessile, planktonic larvae represent the only life stage in which large-scale dispersal is possible (Levin, 2006; Strathmann, 1974). When considering the impacts of environmental stress on marine organisms, larval stages are generally considered to be most sensitive (Putnam et al., 2010; Pineda et al., 2012). However, variation in larval phenotypes can facilitate survival and reproductive success in spatially and temporally variable environments (Beaumont et al., 2009; Clobert et al., 2009). When recruitment occurs, higher quality larvae produce higher quality juveniles (Emlet and Sadro, 2006; Giménez, 2010; Jarrett, 2003; Marshall et al., 2003). Consequently, variation in larval phenotypes resulting from either adaptation or acclimatization can significantly impact subsequent adult population dynamics (Burgess and Marshall, 2011; Davis and Marshall, 2014). As these mechanisms may operate at fundamentally different rates, disentangling the adaptive or acclimatory mechanisms driving the sensitivity or resilience of marine larvae to environmental change will be critical for understanding and predicting population and species-level responses to climate change.

Reef-building corals are a valuable study system in which to explore

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these mechanisms because they exemplify the classic marine life-history dichotomy of sessile adult populations linked by a dispersive planktonic larval stage (Richmond and Hunter, 1990). While coral are unquestionably susceptible to climate change stressors on a broad scale (Hughes et al., 2018), an increasing number of studies has demonstrated their capacity for adaptation and acclimatization to local environmental variation (Barshis et al., 2013; Dixon et al., 2015; Kenkel and Matz, 2016; Palumbi et al., 2014). The capacity for dispersal is largely dependent on the reproductive type of the focal coral species (Ayre and Hughes, 2004). The majority of corals reproduce by broadcast spawning eggs and sperm during an annual reproductive event, which fertilize externally and must develop in the water column for some days before reaching recruitment competency (Kerr et al., 2011; Richmond and Hunter, 1990). Brooding corals, on the other hand, release sperm into the water column, but fertilization and early larval development are internal, with maternal colonies releasing competent larvae over many days per reproductive cycle, and typically exhibiting multiple reproductive cycles per year (McGuire, 1998; Richmond and Hunter, 1990).

In brooding species, the timing of planulation has significant implications for post-release performance. Several qualities of planula larvae, including size, energetic status, symbiont density, and photosynthetic potential, are known to vary by day of release (Edmunds et al., 2001; Putnam et al., 2010; Cumbo et al., 2012; Rivest and Hofmann, 2015; de Putron et al., 2017). Larvae released early within a spawning cycle were shown to be smaller and less likely to settle compared to peak-release larvae and such within-brood divergence may persist into later development (Cumbo et al., 2012). On a finer time scale, larvae of *Favia fragum* released near dawn exhibited higher rates of substrate testing behaviors and settlement success than those released near sunset (Goodbody-Gringley, 2010). Temporal variation in larval performance has also been reported for four broadcasting species when comparing batches of larvae generated through bulk fertilizations over multiple nights of spawning (Nozawa and Okubo, 2011).

Multiple abiotic factors affecting the physiology and behavior of brooded larvae have been identified (reviewed in Gleason and Hofmann, 2011), but climate change related stressors have received the most attention to date. Larvae of *Porites astreoides* were found to exhibit non-linear variation in larval size and symbiont density when subjected to a 24 h exposure to a linear temperature gradient (Edmunds et al., 2005). Warmer temperatures have been consistently shown to reduce the proportion of swimming larvae but enhance their settlement (Putnam et al., 2008; Randall and Szmant, 2009; Serrano et al., 2018). Simulated ocean acidification significantly reduced metabolic rate in both *P. astreoides* and *P. damicornis* and settlement in *P. astreoides* larvae (Albright and Langdon, 2011; Rivest and Hofmann, 2014). In addition, susceptibility of brooded larvae to simulated climate change conditions (temperature and pH) has also been shown to vary by day of release (Cumbo et al., 2013).

For both brooding and broadcast spawning species, larval performance is also influenced by parental reef origins that are characterized by distinct physical environments, suggesting an important role for adaptation and/or acclimatization. Larvae of the broadcast spawning *Acropora millepora* exhibited a 10-fold increase in survival odds under heat stress when their parents came from a warmer reef (Dixon et al., 2015). Similarly, brooded *P. astreoides* from upper-mesophotic reef sites in Bermuda were found to produce more competent larvae with higher settlement and survival rates compared to shallower sites (Goodbody-Gringley et al., 2018). Brooded larvae of *Agaricia agaricites* responded to ultraviolet radiation differently depending on what depths their parents originated from (Gleason and Wellington, 1995). *Pocillopora damicornis* larvae from Taiwan and Moorea exhibited differences in lipid metabolism early in their dispersal period, which may contribute to differences in temperature tolerance (Rivest et al., 2017). Larval performance can also be modified by short-term adult pre-conditioning. Pre-conditioning adult *P. damicornis* to high temperature and pCO_2 increased larval performance in those same conditions compared to larvae released from naive parent colonies (Putnam and Gates, 2015).

Finally, variation among families within reef environments has also been shown to affect larval performance in broadcast spawning corals. Genetically unique families of *A. millepora* larvae exhibited substantial variation in metabolism and gene expression which were attributable to both fixed genetic differences among parent colonies and maternal effects (Meyer et al., 2009). Similarly, significant family and maternal effects were reported for larval survival under an acute thermal stress (Dixon et al., 2015). However, the contribution of family and/or maternal effects have rarely been quantified in any brooding coral species. Specific brooding behaviors necessary to facilitate the survival of embryos internally, for example, those involved in oxygen transport, can increase offspring fitness at the expense of maternal fecundity (Fernandez et al., 2000; Marshall et al., 2008). Consequently, maternal investment may be greater in brooding coral species as larval development occurs within the maternal colony. This suggests that on top of potential variation due to genetic differences, variation in maternal condition may substantially alter larval performance. Maternal effects may also contribute to the differential survival and growth of offspring during the first few weeks following release, although earlier work on growth rate in *P. astreoides* recruits shows that effects diminish over time (Kenkel et al., 2015b), potentially reflecting the depletion of maternal nutritional provisioning. A handful of studies have reported normalizing the contribution of different families to their experimental larval pool, but none examined differences among family groups (Cumbo et al., 2012; Hartmann et al., 2017; Rivest et al., 2017). Whereas Chamberland et al. (2017) found substantial variation in larval size, symbiont number, and symbiont density among spawning colonies, suggesting that this family-level variation may be an important driver of variation in larval performance.

This study aimed to quantify the importance of family-level variation (which we define for the purposes of this study as both maternal and additive genetic effects) on larval performance in a brooding species, *Porites astreoides*, using a system in the Florida Keys in which strong effects of reef origin have previously been established. Inshore environments in the Florida Keys are marked by higher temperature variation, turbidity, and nutrient input compared to offshore environments (Lirman and Fong, 2007; Boyer and Briceno, 2011; Kenkel et al., 2015a). *Orbicella faveolata* populations from inshore sites in the upper Keys exhibit higher calcification rates, greater resistance to and faster recovery from thermal stress events than offshore corals (Manzello et al., 2015; Manzello et al., 2019). Similarly, adult colonies of *P. astreoides* from inshore reefs in the lower Florida Keys have repeatedly been shown to exhibit higher thermal tolerance than their offshore counterparts (Kenkel et al., 2013; Kenkel et al., 2015b). We obtained parental colonies from both an inshore and an offshore reef in the lower Florida Keys and collected planula larvae released during the April 2018 spawning event. We characterized changes in physiological traits by day of larval release and examined larval thermal tolerance. For all performance metrics examined, variation attributable to larval family exceeded variation attributable to day of release or parental environment of origin, highlighting the dominant role of family in shaping physiology during the larval life history stage.

2. Methods

2.1. Coral collections and spawning conditions

Porites astreoides are hermaphroditic brooding corals which fertilize eggs internally and release competent planula larvae in monthly reproductive cycles (McGuire, 1998; Richmond and Hunter, 1990). In the Florida Keys, peak larval release months are April and May, with planulation occuring around the new moon of each month.

On 12 April 2018, three days before the new moon, 31 adult *P. astreoides* coral colonies were collected from a depth of 2–3 m in the

lower Florida Keys. Sixteen colonies were collected from an offshore site (Big Pine Ledges: 24° 33.174′ N, 81° 22.809′ W) and fifteen from an inshore site (Summerland Shoals Patch: 24° 36.346′ N, 81° 25.742′ W), under permit #FKNMS-2018-033. Colonies were transported to Mote Marine Laboratory's Elizabeth Moore International Center for Coral Reef Research and Restoration and placed in a shaded (70% photosynthetically active radiation reducing) flow-through seawater system (i.e. raceway) where water temperatures averaged 26.3 °C, consistent with seasonal temperatures naturally experienced at inshore and offshore reefs (Kenkel et al., 2015a). To track colony identity, plastic numerical labels (1–15 for inshore, 26–41 for offshore) were affixed to the bottom of each coral using marine epoxy putty (All-Fix). Each night before sunset for 5 nights following collection, corals were placed into flow through larval collection chambers following Kuffner et al. (2006). The following morning, the identity of spawning colonies was recorded, and all larvae were collected in 3-L bowls identified by parent colony number.

2.2. Daily release data

For five mornings following colony collection, ten larvae from each colony with sufficient larval release were aliquoted into individual wells in 6-well plates, fixed in 5% formalin and immediately photographed using a stereomicroscope. Digital photographs were retained for subsequent quantification of larval volume. An additional three replicates of 10 larvae per family were aliquoted into 1.5 mL tubes, excess seawater was removed and tubes were frozen at −80 °C for subsequent quantification of soluble protein content and Symbiodininaceae cell density. Remaining larvae were retained for subsequent thermal tolerance experiments in individual 3-L bowls, pooled by family, with gentle aeration and water changes occurred on alternate days.

2.3. Thermal tolerance experiments

Thermal tolerance among larval families was quantified in two ways, through an acute challenge which quantified differential mortality rates in response to 36 °C over time and a 4-day exposure to moderate thermal stress (32 °C) to quantify changes in larval physiology among families. The acute thermal challenge was conducted twice, with the first experiment beginning on 14 April and the second on 17 April. For each experiment, two Nally bins were filled with 30 L seawater and outfitted with SL381 submersible pumps. In each bin, 3 × 10 larvae per family were aliquoted into replicate 70 uM cell strainers (Grenier Bio-One, 542,070) which were used as floating netwells (sensu Dixon et al., 2015, n = 30 larvae total per family per bin). One bin was left at room temperature (mean = 24.3 °C, range = 23.4–24.8 °C, hereafter referred to as the control). This temperature is lower than the average temperature larvae would experience on reefs, but not outside of the range of temperature variation previously observed on reefs at this time of year (http://serc.fiu.edu/wqmnetwork/FKNMS-CD/). For the heat treatment, a 100-W aquarium heater was used to ramp the temperature to 36 °C at a rate of ~1 °C per hour (Fig. S1). Mortality rate was quantified by counting the number of larvae remaining in each netwell at multiple time-points over a 48 h cumulative exposure. In the first acute challenge experiment, three inshore (Families 2, 3, and 4) and three offshore (26, 27, 28) families were represented, while in the second four inshore (3, 4, 14, and 15) and eight offshore (26, 27, 28, 29, 31, 35, 39, 41) had released sufficient larvae to also be included.

In the moderate thermal challenge, three replicate 30 L Nally bins were set up for each temperature treatment as in the acute challenge, and 5 × 10 larvae per family were aliquoted into replicate netwells by bin (n = 50 larvae total per family per bin). Temperatures in the 32 °C treatment bins were ramped over the course of 24 h and maintained for four days (Fig. S2). At the end of the exposure period, swimming larvae were removed from netwells and placed into a 1.5 mL tube, seawater was removed, and larvae were frozen for subsequent physiological analyses.

2.4. Larval volume

The length and width of each larvae was measured in ImageJ (Schneider et al., 2012). Assuming an elliptical shape, volume was determined by $V = \frac{4}{3}\pi ab^2$, where a is ½ length and b is ½ width (Isomura and Nishihira, 2001).

2.5. Symbiont density, chlorophyll, and protein content

For both the daily release and thermal stress experiment samples, three batches of 10 larvae per family were frozen in −20 °C until analysis. After the samples were thawed, 100 µL extraction buffer (50 mM phosphate buffer, pH 7.8, with 0.05 mM dithiothreitol) was added to each replicate group, which was then homogenized by back pipetting to release symbionts from host animal cells. The total volume of the slurry was measured to account for any residual seawater, and 20 µL was removed and immediately mixed with 20 µL 20% formalin solution to perform Symbiodiniaceae cell counts. Symbiont density was determined using triplicate hemocytometer counts per batch. The remaining slurry was centrifuged for 3 min at 1500 xg to pellet symbiont cells for chlorophyll quantification. The supernatant was transferred to a new 1.5 mL tube for protein quantification. The three biological replicates per family were separately tested for chlorophyll and protein across different spectrophotometer runs. Symbiont cell pellets were resuspended in 90% acetone, shaken with metal beads in a TissueLyser II (Qiagen) for 90 s to further break down zooxanthellae cells, and incubated at −20 °C overnight. The sample was centrifuged for 5 min at 10,000 x g at 4 °C and 50 µL of the resultant supernatant was measured in triplicate for absorbance at 630, 647, and 664 nm using Synergy H1 microplate reader (Biotek). Chlorophyll a concentration was determined by applying the following equation, Chl$a$ (µg/ml) = $-0.3002*A_{630} + -1.7538*A_{647} + 11.9092*A_{664}$ (Ritchie, 2008). Soluble host protein was quantified with BCA Protein Assay Kit II following the manufacturer's instructions (BioVision). Protein concentrations were calculated by comparing sample absorbance to a standard curve of serial dilutions of bovine serum albumin. Symbiont density, chlorophyll a, and protein content were multiplied by the initial slurry volume to yield total content, which was then normalized per larvae.

2.6. Statistical analyses

Daily variation in physiological traits were analyzed using ANOVAs with day of release (levels: 1, 2, 3, 4, 5; specified as sequential days in which planulation was observed, with day 1 indicating the first day of larval collection and continuing for a total of 5 days of observation), reef origin (levels: inshore, offshore), and larval family as fixed effects in R 3.5.2 (R Core Team, 2017). Models were evaluated for normality and homoscedasticity using diagnostic plots, and symbiont cell density was log-transformed to meet the assumption of normality. Families 15 and 29 only produced enough larvae for one biological replicate for the symbiont density, chlorophyll a and protein content measures on Day 3 and these data were excluded prior to statistical analysis. Tukey HSD post hoc tests were used to identify significant differences among factor levels when $p < .05$. Survival analysis was used to model time of death in the acute thermal stress experiments as a function of reef origin, including a random effect of larval family and individual netwell using the *coxme* package (Therneau, 2018). Mortality was coded as a binary trait (dead = 1, alive = 0) and time of death as an integer (the re-survey time point in hours). Variation in physiological traits in the moderate stress experiment were modeled as a function of origin (levels: inshore, offshore) and treatment (levels: control, heat), including a random effect of larval family using the *lme4* package (Nakagawa and

Schielzeth, 2013). Models were again evaluated for normality and homoscedasticity, and protein concentration was log-transformed to meet the assumption of normality. A series of linear regressions was used to evaluate relationships between symbiont density and chlorophyll content, as well as between physiological trait values and hazard ratios from the survival analysis. In addition, we explored relationships between fecundity (the number of larvae released per parent daily and cumulative release over the entire observation period), physiological trait measures and familial hazard ratios.

3. Results

3.1. Daily variation in larval physiology by family

Of the 31 colonies collected, 8 inshore and 13 offshore released larvae during our window of observation (Fig. S3). Fecundity varied among planulating colonies, both in terms of the number of larvae released and days over which planulation was observed. Although both inshore and offshore colonies planulated for a similar number of days on average (inshore: 2.75 days, offshore: 2.54 days), there was significant variation among individual colonies, with some producing larvae daily for the entire observation window and others planulating only once (Fig. S3). The mean number of larvae released per colony was also similar between reef zones (inshore: 2.75 days, offshore: 2.54 days; *t*-test: $p > .05$), however, summing across all families and days of release, offshore origin corals released 40% more larvae than inshore origin corals, attributable to the greater number of planulating colonies overall. No relationships were evident between the number of larvae released per day and the resulting daily larval trait measures (volume, symbiont density, chlorophyll *a*, or protein content).

Larval volume differed significantly by day of release, reef origin, and family, with each fixed effect explaining 6%, 4%, and 17% of the variance in larval volume, respectively (day: $F = 11.263$, $df = 4$, $p < .001$; origin: $F = 31.135$, $df = 1$, $p < .001$; family: $F = 7.174$, $df = 18$, $p < .001$). Larvae became progressively smaller in later release days, except for one family (Fam 30) that displayed the opposite trend (Fig. 1). Inshore larvae were on average larger than offshore larvae by 22%. Families 1 and 12 produced larger larvae than families 28 and 40 ($p < .01$, Tukey HSD).

Day of release, reef origin, and family also had significant effects on symbiont density, explaining 19%, 8%, and 48% of the variance, respectively (day: $F = 12.337$, $df = 4$, $p < .001$; origin: $F = 23.385$, $df = 1$, $p < .001$; family: $F = 28.339$, $df = 14$, $p < .001$). Symbiont densities were increased by 39% in larvae released from the inshore families in comparison to those from offshore families. Symbiont density was generally higher later in the planulation window (Fig. S4). However, larvae from several families experienced a slight decrease in symbiont density during Day 3. Larvae from families 3 and 29 contained more symbionts on average than those from families 27 and 41 ($p < .001$, Tukey HSD).

A similar trend was observed in chlorophyll *a* content where again, all three effects were significant (day: $F = 7.728$, $df = 4$, $p < .001$; origin: $F = 34.25$, $df = 1$, $p < .001$; family: $F = 19.221$, $df = 14$, $p < .001$). Origin accounted for 8% of the variance in chlorophyll *a* content. Inshore larvae contained on average 36% more chlorophyll *a* than offshore larvae. Day of release accounted for 7% of the variance in chlorophyll *a* content, with higher concentrations associated with later releases (Fig. S5). Family accounted for 63% of the variance in chlorophyll *a* content. Larvae from families 3 and 29 contained more chlorophyll *a* than those from families 27 and 39 ($p < .001$, Tukey HSD). Symbiont density explained 61% of the variation in chlorophyll *a* content across days (Fig. S6).

Only reef origin and family had significant effects on host protein content (family: $F = 12.814$, $df = 14$, $p < .001$). Origin explained 5% of the total variance in protein content. Inshore larvae had higher protein content on average than their offshore counterparts, by 0.51 μg/larvae ($F = 13.496$, $df = 1$, $p < .001$; Fig. S7). Family explained 61% of the variance in host protein content, with larvae from families 1, 3, and 29 containing more protein on average than those from families 27 and 28 ($p < .01$, Tukey HSD).

3.2. Physiological response to moderate thermal stress

Mean survival among replicates in the moderate thermal stress experiment was 97% or greater, save for Family 29 in the control treatment, where average larval survival was 87%, and Family 27 in the heat

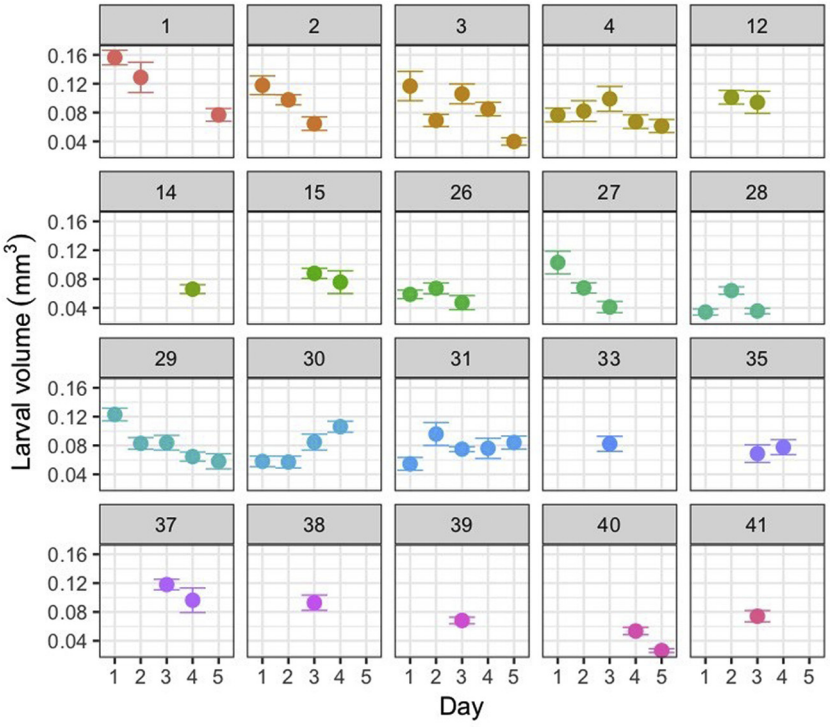

Fig. 1. Larvae volume for each family during 5 days of release (mean ± SEM). Families 1–15 are inshore-origin parents and families 26–41 are offshore-origin parents. The y-axis indicates sequential days in which planulation was observed, with day 1 indicating the first day of larval collection and continuing for a total of five days of observation.

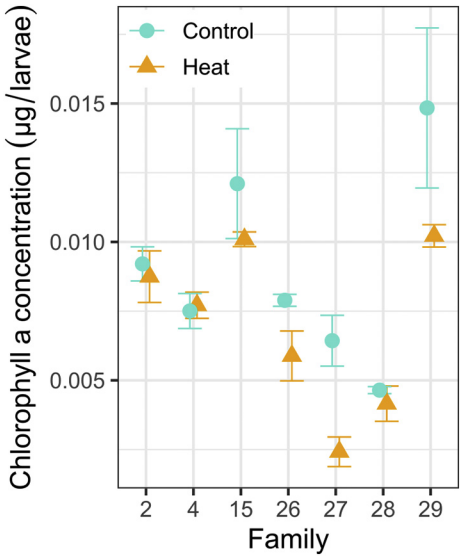

Fig. 2. Concentration of chlorophyll a during the moderate temperature stress experiment by family and treatment (mean ± SEM). Families 2, 4 and 15 are inshore-origin parents and families 26–29 are offshore-origin parents.

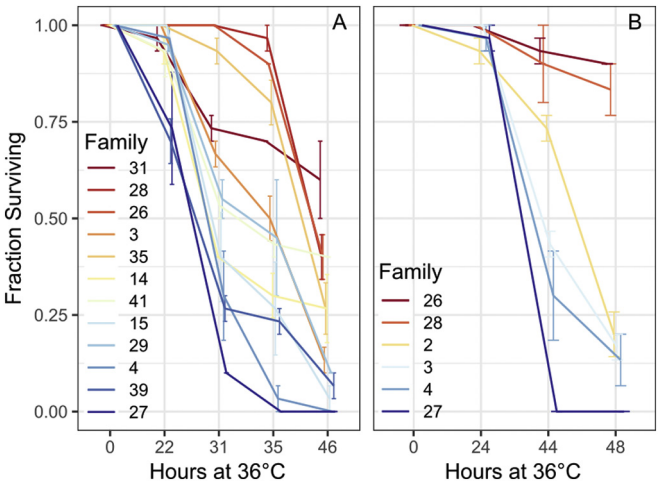

Fig. 3. Mortality curves of larvae shown as mean survival (± SEM) over time for the acute thermal stress experiments consisting of (A) twelve families and (B) six families. Families 1–15 are inshore-origin parents and families 26–41 are offshore-origin parents.

treatment, where survival declined to 79% (Fig. S8). No changes in symbiont density (Fig. S9) or protein content (Fig. S10) were detected in response to either treatment or origin; however, temperature treatment had a significant impact on chlorophyll *a* content. On average, larvae exhibited a 19% reduction after 4 days at 32 °C in comparison to paired controls ($F = 12.544$, $df = 1$, $p = .001$; Fig. 2). A marginally significant interaction term was also detected, due to offshore larvae tending to lose more chlorophyll *a* post heat stress than inshore larvae ($p = .075$). Similar to the daily release data, the majority of the variance in chlorophyll *a* content was explained when a random effect of family was included in the model ($R^2 = 0.809$ vs $R^2 = 0.169$ for the fixed effects only model).

Symbiont density explained 43% of the variation in chlorophyll *a* at the end of the experiment (Fig. S6). No relationships were detected between cumulative fecundity (the total number of larvae released prior to beginning the experiment) and the change in trait values in response to temperature treatment.

3.3. Survival under acute thermal stress

Temperature treatment significantly impacted mortality, with > 99.7% of larvae surviving in controls, whereas only 25% of larvae survived 46 h of exposure to 36 °C in the experiment encompassing 12 larval families. Reef origin (inshore or offshore) did not significantly impact mortality risk. However, a substantial familial effect was evident, with outcomes ranging from less than half (hazard ratio [HR] = 0.45, Family 31) to over 3.4 times (HR = 3.45, Family 27) the average familial risk for the experiment (Fig. 3A).

While absolute mortality rates differed between the two replicate acute stress experiments (Fig. 3, e.g. fraction surviving at 22 vs 24 h), the same general patterns were detected in the 6 family experiment. Again, 99.4% survival was observed in control treatments, whereas survival was reduced to 34% under elevated temperature. Similarly, no effect of reef origin was evident, but familial effects were large, with a range of less than a third (HR = 0.27, Family 26) to nearly 10 times (HR = 9.87, Family 27) the average mortality risk (Fig. 3B). For families represented in both experiments, the rank order of hazard ratios was highly consistent ($R^2 = 0.85$, $P < .05$; Thermal tolerance ranking: 26 ~ 28 > 3 > 4 > 27, Fig. 3). Families 26 and 28 were generally tolerant of elevated temperature, whereas family 27 always exhibited 100% mortality in the 36 °C treatment.

No significant relationships were detected in regressions of familial hazard ratios on daily release phenotypes averaged by family (volume, host protein content, symbiont density or chlorophyll *a*) or bleaching in the moderate thermal stress experiment, calculated as the difference in chlorophyll *a* content between control and heat-treated larvae by family. Nor was there a relationship between familial hazard ratios and cumulative fecundity, calculated as the total number of larvae released prior to beginning the experiment.

4. Discussion

Variation in larval quality can facilitate survival and reproductive success in spatially and temporally variable environments, contributing to adult population dynamics in a changing climate (Beaumont et al., 2009; Burgess and Marshall, 2011). We found that several larval traits, including volume, symbiont density, chlorophyll *a*, and protein content varied by day of release, reef zone of origin, and individual *P. astreoides* family. In particular, family was the dominant factor influencing differences in larval physiology. Of the four traits that were quantified, > 60% of the variation in chlorophyll *a*nd protein content was explained by family. In comparison, day of release and origin explained < 20% of the total variation. This result has substantial implications for the interpretation of prior studies which focused on variation in the physiology of brooded coral larvae. To our knowledge, the majority of work which examined larval physiology by day of release, reef origin, or other experimental treatment (e.g. Edmunds et al., 2001; Edmunds et al., 2011; Goodbody-Gringley et al., 2018; Putnam et al., 2010; Putnam and Gates, 2015; de Putron et al., 2017) relied on pooling larvae from different maternal colonies and performing analyses on the composite. Only a handful of studies clarified that an equal number of larvae were sampled from different families to control for this source of variation (Cumbo et al., 2012; Hartmann et al., 2017; Rivest et al., 2017). Given the variation in planulation among individual parent colonies over a single reproductive cycle and the large contribution of individual family in explaining the physiological variances examined here, it is possible that a large proportion of the variation previously attributed to day of release or origin may in fact have been the result of familial effects, if care was not taken to normalize the representation of different families in the larval pools.

Family also played a significant role in determining the probability of larval survival under 36 °C acute temperature stress. The rank order of thermal resilience among families was preserved in the two technical replicates of this experiment, despite incorporating larvae from

different days of release (Fig. 3). Larvae released from families 26 and 28 were generally more tolerant than those from families 27 and 4. A prior study which used this same acute stress assay to quantify thermal tolerance of *A. millepora* larvae also found a significant family effect, but a significant portion of the variation in larval performance was also attributable to the parent's reef of origin: parents from a warmer location conferred significantly higher thermo-tolerance to their offspring in comparison to parents from the cooler location (Dixon et al., 2015). Adult *P. astreoides* in the lower Florida Keys exhibit differences in thermal tolerance depending on their reef of origin, with inshore-origin corals exhibiting greater bleaching resistance than offshore-origin corals Kenkel et al., 2015b; Kenkel et al., 2013; Kenkel and Matz, 2016). Although survival was clearly linked to family, we did not find any association between survival and parental reef origin. The most (26 and 28) and least tolerant (27) families all originated from the offshore reef site.

One explanation for this finding is that larval thermal tolerance differs fundamentally from adult thermal tolerance, possibly as a consequence of significant maternal effects (Lockwood et al., 2017). Another possibility is that our assay examining survival under acute thermal stress is quantifying a different aspect of thermal tolerance than the traditional bleaching response metrics, such as the reduction in symbiont or chlorophyll concentrations. The moderate thermal stress experiment which included these most and least tolerant offshore families (26, 27 and 28) in addition to a set of inshore families, showed that while family 27 again exhibited the greatest mortality under heat stress, larval bleaching responses (quantified as the change in chlorophyll *a* content) were similar to previously reported adult bleaching phenotypes (Kenkel et al., 2015b; Kenkel et al., 2013); offshore larvae tended to lose more chlorophyll than inshore larvae (Fig. 2). Moreover, survivorship under acute stress was unrelated to changes in chlorophyll *a* content under this milder temperature stress, nor was it explained by mean daily release phenotypes. The ecological significance of larval survival under acute temperature stress remains unclear. However, it may be an important phenotype to study with ongoing global warming when extreme temperature regimes such as heat waves occur more frequently (Jentsch et al., 2007).

In *Pocillopora damicornis*, elevated temperature reduced maximum quantum yield of photosystem II in larvae but not adult colonies, suggesting that early life stages might be more vulnerable to thermal stress (Putnam et al., 2010). While we did observe a loss of chlorophyll *a* following a 4-day exposure to 32 °C, the density of Symbiodiniaceae cells and soluble host protein content were unaffected. The weaker correlation between chlorophyll *a* and symbiont cell density following the moderate thermal stress exposure ($R^2 = 0.43$) in comparison to the daily release measures ($R^2 = 0.61$), suggests a decoupling of bleaching mechanisms. Prior work has shown that loss of symbiont cells can occur without pigment reduction (Jones, 1997) and decreases in chlorophyll *a* content can occur without losing symbionts (Venn et al., 2006). Our results suggest the occurrence of the latter, where chlorophyll *a* is eliminated through photo-oxidative reactions that yield colorless compounds during pigment loss, or photobleaching, which may not necessarily lead to symbiont expulsion (Venn et al., 2006). The propensity for photobleaching in Symbiodiniaceae can also be altered by thermal acclimation (Takahashi et al., 2013), suggesting that variable thermal tolerance may influence the extent of bleaching. Similarly, although larval families exhibited significant variation in mortality during our acute, 36 °C exposure, significant differences in mortality were not observed until larvae had experienced these conditions for > 24 h (Fig. 3). Similar patterns were observed in aposymbiotic *A. millepora* larvae, where 36 °C exposure times in excess of 24 h were necessary to elicit significant mortality (Dixon et al., 2015). Combined, this suggests that larvae of certain coral species can withstand these excessive temperatures, several degrees above typical summer maxima (Kenkel et al., 2015a; Manzello et al., 2019), for prolonged periods and calls for additional investigation into the thermal tolerance limits of larval life stages and how this impacts subsequent life stages. For example, negative latent effects were reported in brooded *A. humilis* larvae, where minimal mortality was observed in larvae exposed to high temperature but subsequent reductions in settlement rate and post-settlement survival were evident (Hartmann et al., 2013).

Not all families released larvae every day during the 5-day observation period and the number of larvae released varied greatly (Fig. S3), suggesting variation in the reproductive capacity of individual parent colonies. However, we did not detect any predictive relationships among the number of larvae released per parent colony and variation in daily release phenotypes, the change in physiological trait values in response to moderate thermal stress, or familial hazard ratios under acute thermal stress. Although our observations occurred during the peak planulation window for *P. astreoides*, this species is capable of releasing larvae through September (McGuire, 1998). In a multi-site survey along the Great Barrier Reef, fecundity varied significantly among colonies of *A. millepora* within a site, yet colony was a good predictor of fecundity (Tan et al., 2016). Conversely, *Agaracia humilis* was reported to exhibit interannual variation in fecundity in Curaçao, although individual colonies were not tracked (Hartmann et al., 2017). If individual *P. astreoides* colonies vary in their reproductive investment over time it may be that our fecundity measures do not accurately reflect individual reproductive output, and future studies should investigate this possibility.

Quantification of additional energetic traits may yield more informative relationships among daily release phenotypes or thermal tolerance responses. For example, lipids are important energy reserves and contribute to buoyancy of planktonic coral larvae (Harii et al., 2007; Richmond, 1987). Correlations between larval size and lipid content have been previously reported (Hartmann et al., 2013; de Putron et al., 2017). A positive relationship between triacylglycerol content and temperature treatment was observed in *P. damicornis* larvae, in addition to increased metabolism of both protein and lipid (Rivest and Hofmann, 2015). Population-level variation in lipid catabolism has also been observed (Rivest et al., 2017). Lipid reserves are predicted to influence mortality and recovery in adult corals in response to bleaching (Anthony et al., 2009). Consequently, evaluating the relationship between lipid content and family level variation in larval thermal tolerance represents an important avenue of future research.

To our knowledge, this study provides the first evidence of size differences in *P. astreoides* larvae from different reef zones in the Florida Keys. Earlier work showed that offshore-origin recruits were significantly smaller than inshore-origin recruits (Kenkel et al., 2015b), which aligns with the pattern we observed in larvae. In contrast to a study on *P. astreoides* in Bermuda that documented smaller individuals released earlier in the lunar cycle (de Putron et al., 2017), most families released larger larvae early in the April spawning event except for one offshore family (Fig. 1), although more exceptions might have been captured if more families were included. Size variation may determine settlement success in later developmental stages (Hartmann et al., 2013). Larger larvae are also more likely to contain higher endogenous energetic reserves and thus disperse farther in the field (Marshall and Keough, 2007). Larvae of three Pocilloporid coral species exhibited longer lifetimes, defined as time from free-swimming to death, in larger individuals than smaller ones (Isomura and Nishihira, 2001). Interestingly, the trend in symbiont density we observed in the current study (Fig. S3) agrees with a previous finding that symbiont abundance was higher in the last 3 days than the first 3 days of collection (Edmunds et al., 2001; Putnam et al., 2010; de Putron et al., 2017). If symbiont density and chlorophyll a content are reliable predictors of energy reserves, our results showed small but energy-rich offspring are likely to be released later in the spawning window. Compared to the observation of larger and more energy-rich larvae that are late releases in *P. damicornis* (Putnam et al., 2010), such deviation may indicate the unique physiology of *P. astreoides* larvae from the lower Florida Keys.

In conclusion, we find that the majority of variation in the

physiology of brooded *P. astreoides* larvae is explained by larval family. These effects may be the result of either fixed genetic differences, or maternal effects, and future work should aim to conduct additional genetic analyses to help distinguish among these mechanisms. Prior work established that symbiont community composition in *P. astreoides* is largely uniform in the Florida Keys, regardless of parent population origin (Thornhill et al., 2006; Kenkel et al., 2013), but finer-scale genotyping of *Symbiodiniaceae* is also needed to evaluate the contribution of maternally inherited symbiont types. Furthermore, additional data on other energetic and life-history traits may help shed light on variation among families. Regardless of the ultimate mechanism, it will be important for future studies to control for this variation by either tracking the familial origin of larvae or taking care to pool larvae originating from different families in equal proportions. Additional studies examining interactive effects between family and other previously identified sources of variation (e.g. day of release) will provide additional insights. Future work will also benefit from including juveniles and recruits in the phenotyping assay to allow for the dissipation of maternal effects and gain a better understanding of how parental effects influence later developmental stages.

Data accessibility

All data and R code can be downloaded at https://github.com/yingqizhang/PoritesSpawn.

Declaration of Competing Interest

The authors have no competing interests to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbpa.2019.110562.

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