Self-fertilization suppresses thermal tolerance in embryos of reef-building coral

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Abstract. Self-fertilization is unusual in most animals but common in some scleractinian corals, in particular, the family Faviidae. High levels of self-fertilization in many faviids may contribute to their large latitudinal range size and their dominance of some isolated, high latitude coral assemblages in eastern Australia. In this study, conducted at One Tree Island in the southern Great Barrier Reef, the thermal tolerance of self-fertilized *Goniastrea favulus* embryos was compared to outcrossed embryos across five temperature treatments (20ºC, 22ºC, 24ºC, 26ºC, and 28ºC). Response variables were fertilization success, development rate, and larval survivorship. Fertilization was high (85-100%) across all treatments in cross-fertilized embryos. In self-fertilized *G. favulus* fertilization was low in treatments below ambient (27 and 60 % at -4 and -2 ºC, respectively), though the effects on fertilization were not significant among the treatments. Development rates (i.e. mean times to the planula stage) were similar in selfed and outcrossed embryos. Development occurred more rapidly at raised temperatures for both groups. High mortality occurred at raised temperatures, at +4ºC especially, for both self and crossed-fertilized *G. favulus*. Survivorship curves, median lifespans, and overlaps in 95% confidence intervals suggest reduced survivorship in selfed embryos. These results suggest self-fertilization has a negative effect on dispersal potential in reef-building corals.

Key words: Coral, Self-fertilization, Thermal tolerance, Larvae, Dispersal.

Introduction

Self-fertilization, involving male and female gametes from a single individual, yields ecological benefits as well as costs. Selfing is generally rare, but common in some animals (Jarne and Auld 2006). The reef-building coral genus Faviidae, for instance, exhibits high selfing success (Miller and Babcock 1997; Knowlton et al. 1997). This reproduction method ensures genetic transmission, especially in animals with limited mobility, and is thought to be an evolutionary strategy enabling species persistence in conditions of low densities (Tomlinson 1966; Crow 1994; Jarne and Auld 2006). Like asexual reproduction, selfing maintains a genotype known to be successful in the maternal environment. However, the main associated cost is reduced fitness from lower genetic variation within a population (Jarne 1995; Trouve et al 2003; Jarne and Auld 2006).

This study tests the hypothesis that self-fertilized coral embryos are less thermally tolerant than crossed-fertilized embryos. The test species, *Goniastrea favulus*, is a faviid that has a wide geographical distribution from Japan to southern Australia and exhibits high rates of self-fertilization (Miller and Mundy 2005). It displays an unusual spawning method where eggs are discharged in mucous and sperm is subsequently released in multiple pulses (Kojis and Quinn 1981). Eggs of *G. favulus* are negatively buoyant and retained close to the parent colony during fertilization (Heyward and Babcock 1986; Miller and Mundy 2005).

In the marine environment, many species rely on a planktonic life stage for recruitment and dispersal (Strathmann 1985; Cowen and Sponaugle 2009; Connolly and Baird 2010). In scleractinian corals, planula larvae are transported in the water column before settling, metamorphosing, and growing into adult corals. Spawning and dispersal are therefore crucial for reef recruitment and replenishment. Larval ecology may help explain biogeography of marine organisms. For example, *Parvulastra exigua*, a cross and self-fertilizing sea-star lacks dispersive larvae and yet is widely distributed in Australia. The combination of fertilization methods may account for this paradox (Barbosa 2012).

Thermal stress is known to affect many aspects of larval ecology (Hughes et al. 2003; O'Connor et al. 2007). However, there are often significant differences in response among species. In *Acropora millepora* (Family Acroporidae) fertilization success decreases with temperatures 2-4°C above ambient and ceases at +6°C (Negri et al. 2007). In faviids and pectiniids, high fertilization rates as well as normal development occur at temperatures 5°C above ambient (Bassim et al. 2002, Negri et al. 2007). Indeed, faviids are particularly tolerant to warm and

cold temperature stressors, as survivorship and fertilization success in *G. favulus* embryos were found to be significantly greater than in *Acropora spathulata* across multiple temperature treatments (Woolsey et al. *in prep*). Poleward migration with increasing temperatures has been observed in corals and other marine animals (Greenstein and Pandolfi 2008; Figueira and Booth 2010; Yamano 2011) and thermal tolerance in dispersed propagules could promote range expansion in corals (Woolsey 2007).

It is necessary to understand phenotypic responses to temperature because larvae dispersed over long distances are likely to encounter temperature fluctuations. In addition, these responses may provide insight into long-term adaptive capacity to projected temperature changes in eastern Australia (Poloczanska et al. 2007; Lough 2008) that are expected to alter patterns of larval dispersal in many coral species (Ayre and Hughes 2004, Heyward and Negri 2010).

This study compares the thermal response of self-fertilized and crossed progeny of *G. favulus* to determine if early developmental success differs with fertilization type. It addresses the hypothesis that self-fertilization lowers environmental tolerance in coral embryos.

Material and Methods

Study location and collection

Six adult colonies of *Goniastrea favulus* (Family Faviidae) were collected from the southern coral flats of One Tree lagoon (23°30'S, 152°05'E), Australia. Colonies were collected at least 10 m apart to reduce the chance of genetic similarity. Corals were maintained in flow-through filtered seawater (FSW) in shaded outdoor aquaria at ambient light and temperature. Before spawning, three colonies were separated into three tubs for self-fertilization experiments while the remaining three colonies were kept in a separate tub for cross-fertilization experiments. Cross-fertilization was assumed in embryos collected from the tub with multiple colonies.

Sperm and eggs were released asynchronously over a half hour, and time of fertilization was taken as 1.5 hours after all spawning was complete. At this time (0 hours), embryos were transferred to 20 ml glass vials and distributed among temperature treatments (approximately 20 embryos in each vial x 3 replicates per treatment). Embryos in these vials were used to confirm fertilization at 2 hours post fertilization (hpf), by observing cleavage under the microscope. Once fertilization was confirmed, embryos remaining in the culture were distributed among temperature treatments.

Temperature treatments

Ambient temperature was defined as 24 °C, the average SST reading from on-reef sensors for the month prior to spawning (GBROOS, http://data.aims.gov.au/gbroos/). Water baths were set up in a temperature-controlled room at One Tree Island Research Station at 5 temperatures (20°C, 22°C, 24ºC, 26°C, 28°C i.e. -4°C, -2°C, ambient, +2°C, +4°C). Aquarium heaters, coolers, and pumps kept treatment baths stable and within 0.5°C of the target temperatures (monitored with HOBO data loggers). Larvae were maintained in UV-C treated, 0.2 µm FSW to prevent bacterial contamination. Water was changed daily after being heated or cooled to the appropriate temperature.

Response variables

Glass vials (50ml) were filled with 30-50 embryos and transferred among treatments. There were three replicates for each of the five temperatures. These vials were used to monitor development stage throughout the experiment. Fertilization data was collected at 6 hpf, using cleavage as an estimate of fertilization success.

The stage of development of 20 embryos in each 50 ml vial was recorded at 9 time points: 18, 24, 30, 36, 48, 72, 96, 120 and 144 hpf. The stages used were: 2-cell, 4-cell, multicell/morula, blastula, gastrula, pre-planula and planula.

Survivorship was measured by placing 50 embryos into 50ml glass vials at 2 hpf with three replicates in each of the five treatments. Counts of live embryos remaining from the original 50 in each survivorship vial were conducted at 8 time points: 18, 24, 30, 36, 48, 72, 96, 120 and 144 hpf.

Data Analysis

A fully factorial 2-way ANOVA was used to test for differences in mean fertilization success at 6 hpf and development rates among temperature treatments (fixed, 5 levels: -4, -2, ambient, +2, and +4 °C) and fertilization mode (fixed, 2 levels: crossed and selfed). After graphical analysis of the residuals, development data was log transformed and fertilization data was arcsine transformed to remove bias. Levene's Tests were used to confirm homogeneity. Tukey HSD post-hoc analysis determined differences among treatment levels. Analyses were completed using R and SPSS v19.

Survivorship

Non-parametric Kaplan-Meier product limit analyses were used to test for differences in median survivorship among temperatures and fertilization mode. Median survivorship (in hours) was considered significantly different when the 95%

confidence intervals did not overlap. Analyses were completed using SPSS v19.

Results

Fertilization success did not differ significantly among temperatures or among the larval groups (Table 1). In crossed embryos, fertilization was high in all treatments ranging from 85 ± 7.6% at 22ºC to 100% at 28ºC (Fig. 1). In self-fertilized embryos, mean fertilization rates were ≥ 80% treatment at and above ambient (Fig. 1). There was more variation in self-fertilized embryos, with low fertilization success at temperatures below ambient (27 ± 24.2% at 20ºC and 60 ± 27.8 % at 22ºC).

Factor	*df*	F	*p*
Temperature	4	2.49	0.076
Method	1	3.45	0.059
Temperature * method	4	2.74	0.060

Table 1: Results of 2-way analysis of variance (ANOVA), testing for significant differences between mean fertilization rates. Factors of the model were temperature (fixed, 5 levels) and fertilization method (fixed, 2 levels). Data arcsine transformed. df error= 20.

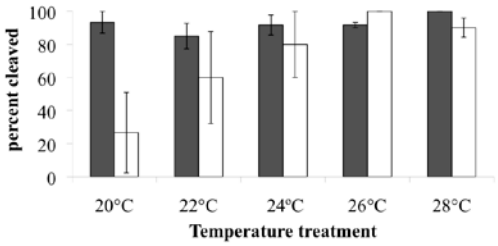

Figure 1: Fertilization success (mean proportion of eggs cleaved ± one SE) of crossed (dark bars) and self-fertilized (light bars) *G. favulus* embryos at temperatures above and below ambient. Fertilization success was measured by percent cleavage at 6 hpf.

Time to planula was shorter at higher temperatures but not affected by fertilization method (Table 2). Mean time to the free-swimming planula stage among crossed and selfed embryos was 124 hrs at 20°C, 91 hrs at 22°C, 82 hrs at 24°C, 73 hrs at 26°C and 72 hrs at 28°C (Fig. 2).

Factor	*df*	F	*p*	Treatments (°C)
Temperature	4	32.3	<0.001	20>22=24>26=28
Method	1	0.792	0.384	
Temperature* Method	4	2.44	0.081	

Table 2. Results of 2-way analysis of variance (ANOVA), testing for significant differences between mean time to planula among treatments. Factors of the model were temperature (fixed, 5 levels) and fertilization method (fixed, 2 levels). Data log transformed. df error= 20. Treatment order from Tukey HSD post-hoc test.

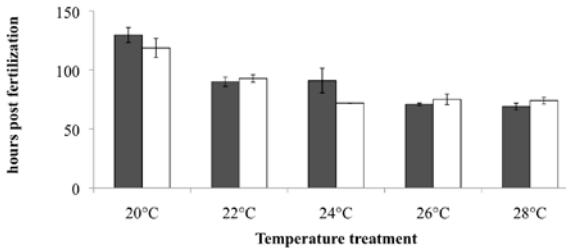

Figure 2: Time to the free-swimming planula stage (mean hours post-fertilization ± one SE) in crossed (dark bars) and self-fertilized (light bars) *G. favulus*.

For both fertilization types, survivorship was low at +4ºC (28ºC). The 28ºC median lifespan was 48 hrs in both crossed and self-fertilized embryos (Fig. 3). In cross-fertilized *G. favulus*, all temperature treatments besides 28ºC show overlap in confidence intervals, meaning there are no significant differences in median survival time among treatments. In self-fertilized *G. favulus* however, median survivorship and confidence intervals indicate significant differences among treatments (Fig. 3, Fig. 5).

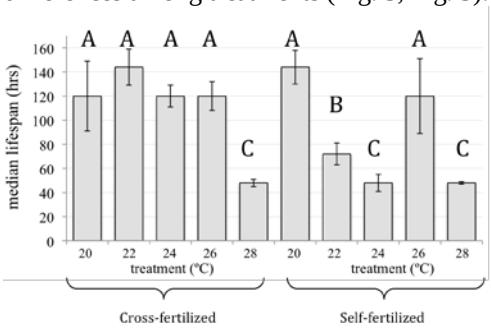

Figure 3: Kaplan-Meier median survivorship estimates for cross and self-fertilized *G. favulus*, n=150. Error bars show 95% Confidence Intervals. Treatments with overlap in Confidence Intervals are labeled as groups A, B, or C.

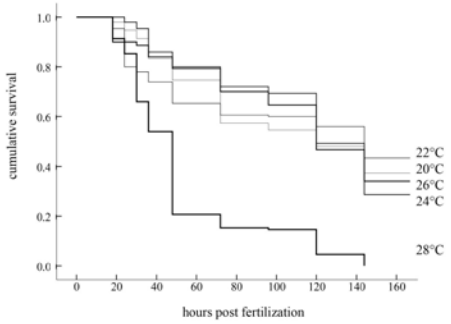

Figure 4: Kaplan-Meier median survivorship curves for crossed-fertilized *G. favulus* at temperatures above and below ambient (24°C), n=150.

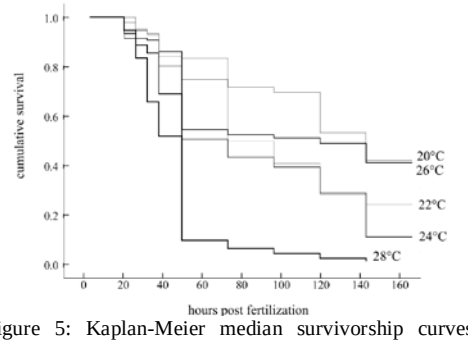

Figure 5: Kaplan-Meier median survivorship curves for self-fertilized *G. favulus* at temperatures above and below ambient (24°C), n=150.

Discussion

Neither development rate nor fertilization success was affected by fertilization type. Development rate was faster at higher temperatures, which is consistent with metabolic theory, and mean time to the free-swimming planula stage did not vary among larval groups. Although there was greater variation in self-fertilized embryos, fertilization success was also not significantly affected by fertilization method.

Survivorship data, however, indicate suppressed thermal tolerance in self-fertilized *G. favulus*. This suggests that self-fertilization, while potentially promoting growth on isolated or disturbed reefs, produces less healthy larvae and therefore reduces dispersal capabilities.

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