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Effects of temperature on reproductive timing and hatching success in a tropical fiddler crab

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The timing of reproduction is critical to reproductive success in many animal species. Parents that can perceive and respond to environmental cues and time the hatching/birth of their offspring to optimal environmental conditions show higher reproductive success. Intertidal ectotherms are under particularly strong selection because larval development rates are temperature-dependent, and larvae must hatch during the highest spring tides to avoid high levels of inshore predation. Here we investigate whether female fiddler crabs, *Austruca mjoebergi*, can mitigate the effects of high temperatures by adjusting the timing of reproductive events and/or by behavioural compensation. We experimentally manipulated incubation temperatures between 30 and 36 °C, based on natural and predicted temperature conditions, and found that hatching success decreased linearly with increasing temperatures. However, temperature had no effect on the timing of fertilization or hatching, suggesting that larval development rate was not temperature-dependent. Across the tested temperatures, females did not adjust egg size, the amount of yolk in each egg, larvae size or clutch size. In conclusion, high temperatures prevented clutches from reaching the hatching stage, but within the range of temperatures that facilitated hatching, there was no evidence of behavioural compensation and no discernible effect of temperature on reproductive timing.

ADDITIONAL KEYWORDS: *Austruca mjoebergi* – behavioural flexibility – global warming – incubation temperature – thermal stress.

INTRODUCTION

Understanding the factors leading to variation in reproductive success among individuals can provide key information about the direction of adaptation and evolution (Stearns, 1992). Organisms that are adapted to local environmental conditions and that begin reproductive activities (e.g. arrival at the breeding site, egg laying) at an appropriate time are more likely to have higher reproductive success (Verhulst & Tinburgen, 1991; Dickerson *et al*., 2005). For example, when the timing of breeding is synchronized with peak prey abundance, both adults and offspring benefit from higher food supply that can compensate for the energy demands of reproduction (Van Noordwijk *et al*., 1995). Offspring that develop at unfavourable times are likely to be selected against due to unfavourable climatic conditions (Love *et al*., 2010) and predation (Chivers *et al*., 2001; Touchon *et al*., 2006, 2013).

The timing of reproductive activities is determined by a variety of environmental cues (including temperature, precipitation, food abundance and predation risk) that contribute to breeding decisions (Cayuela *et al*., 2014; Touchon *et al*., 2006; Van Noordwijk *et al*., 1995). For example, the timing of breeding in most amphibians is sensitive to rainfall intensity (Marsh, 2000; Jensen *et al*., 2003; Cayuela *et al*., 2014). Some precipitation is needed to create suitable breeding habitats and prevent desiccation, but heavy rainfall can destroy suitable calling sites (Fukuyama & Kusano, 1992). Additionally, changes in spring temperature, which is highly correlated with prey abundance of avian species, has been found to cause spatial and temporal variation in egg laying date within and among populations (Nager & van Noordwijk, 1995; Schaper *et al*., 2012). Parents use these cues to time reproduction so that offspring are born into conditions that are suitable for survival.

The ability of parents to time reproduction so that offspring are born during optimal environmental conditions depends on their ability to perceive

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and interpret environmental cues that predict developmental duration, as well as their ability to respond to these cues (Perrins, 1970; Beever *et al.*, 2017). For example, female Arctic common eiders that were better at perceiving the high temperature variation during warmer springs, and therefore had offspring that hatched before the fully ice-free conditions, had the highest offspring survival (Love *et al.*, 2010). Many other species also exhibit flexible reproductive behaviours in response to environmental variation (Beever *et al.*, 2017). In ectotherms, developmental duration is often dependent on temperature (Gillooly *et al.*, 2002). This is of particular concern for marine ectotherms because seawater temperatures are rising rapidly and the rate of change is accelerating (IPCC, 2007). The incubation duration of many marine ectotherms is strongly temperature-dependent (Hoegh-Guldberg & Pearse, 1995). In fiddler crabs, for example, it is well documented that larvae develop faster at higher temperatures (Christy, 1982 , 2003; Sanford *et al.*, 2006; Reaney & Backwell, 2007; Kerr *et al.*, 2012). This could potentially impact reproductive timing and clutch survival.

STUDY SYSTEM

The mating cycle of the high-intertidal species of fiddler crab, *Austruca mjoebergi*, is strongly tide-dependent. The habitat is inundated only for a few days during the largest amplitude tides and larval release must occur during this limited time (Christy, 1982, 2011; Morgan & Christy, 1995). Larval development takes ~15 days, so mating generally occurs during the 3–4 days immediately before the dry, late-neap-tide period. The strong temporal constraints on mating and larval release times, along with the faster development of the larvae at higher temperatures, make this species particularly vulnerable to global warming. Unless these crabs can alter their reproductive behaviour or the timing of larval release, increased temperatures will push the appropriate time for mating into the dry, late-neap-tide period when surface activity is impossible due to desiccation and heat stress. We currently have no understanding of whether *A. mjoebergi* can adapt to these faster larval development rates under higher temperatures.

There are two non-mutually exclusive ways that females could compensate for a shorter incubation. First, they could shorten the time between mating and fertilization. At present, females select a mate on one of the four potential mating days and copulate shortly after entering the male's burrow (Goshima *et al.*, 1996). The male then guards the female until she extrudes her eggs onto her pleopods (Goshima *et al.*, 1996). In principle, females could still engage in the long and expensive process of mate choice during the current mating period (i.e. before the dry period) but delay fertilization for a few days, until a time that is consistent with the prevailing temperature. Second, females could slow the rate of egg development by adjusting the size of eggs they produce. There is a strong negative relationship between egg size and temperature in many crustaceans (Sheader, 1996). Egg size varies depending on the quantity of yolk laid down. Fiddler crabs are unusual in that they can rapidly produce yolk using multiple sites (hepatopancrease, ovaries and haemolymph; Quackenbush & Keeley, 1988). This allows females to alter yolk content shortly before fertilization. *Austruca mjoebergi* females could potentially extend the incubation period by producing larger eggs. However, this could reduce their fecundity due to a trade-off between egg size and number. Although a previous study has found no evidence that this occurs, it examined incubation temperatures within the normal species range (Clark & Backwell, 2016). We do not currently know how the crabs will respond under the more severe changes in climate that are predicted by the IPCC.

AIMS

In this study, we examine the effect of incubation temperature on the timing of reproduction in *A. mjoebergi*. This species occurs on the northern coast of Australia. Near-coastal sea surface temperature rise around Australia is typically 0.4–1 °C per decade and the largest increase is predicted to occur along the north-west coast of Australia (CSIRO/BOM, 2015). This study aims to determine whether females are able to adjust the time of fertilization to compensate for the shorter incubation duration expected at higher temperatures. We also investigate whether females adjust the development rates of their larvae by adjusting the size of their eggs and/or the amount of yolk in each egg; and if so, whether these adjustments result in smaller hatchlings and/or smaller clutches. Specifically, we address the following seven questions: (1) Does increased temperature affect fertilization success or hatching success? (2) Do females adjust the timing of fertilization depending on incubation temperature? (3) Does temperature affect incubation duration? (4) Do females release their larvae during the highest amplitude spring tides irrespective of incubation temperature? (5) Do females adjust egg size or the relative size of the yolk in their eggs in response to increased temperatures? (6) Do hatchlings differ in size when incubated at different temperatures? (7) Does clutch size differ at different incubation temperatures?

MATERIALS AND METHODS

We conducted fieldwork at East Point Reserve, Darwin, Australia (12°24′31.89″S, 130°49′49.12″E) and laboratory work at the North Australia Research Unit over a 5-month period during peak reproductive activity in 2016 (August to December).

Collection of females

We collected 113 females that had mated but had not yet fertilized their eggs. When female fiddler crabs are ready to mate, they wander around the mudflat and successively sample male burrows until they select a male, enter his burrow and stay underground. Once the female enters the burrow the male follows her in, and seals the burrow entrance. Mating usually occurs 1 or 2 h after the male seals the burrow. To ensure we collected females that had recently mated, we identified females that were ready to mate and tracked them until they entered a male's burrow and the males followed them in. Once the males sealed the entrance of the burrow we placed a plastic collar around the entrance to prevent other crabs from disturbing the mating pair, and to detect whether either of the mating pair left the burrow early (in which case the female was discarded). After 3 h, we dug the crabs up and measured the carapace width of both male and female. Three hours is long enough for mating to occur and for the female to store the sperm in her spermatheca. The eggs are only fertilized once they are extruded. The male was released after being measured and the female was placed in a cup with a small amount of seawater and transported to the laboratory within a few hours of capture.

Experimental manipulation of incubation temperature

After being brought back to the laboratory, females were randomly assigned to one of three temperature treatments. We manipulated the incubation temperature by using three six-well Digital Laboratory Water Baths (HH-6) set at 30, 34 and 36 °C. The water bath temperatures were monitored and found to fluctuate less than 1 °C from the assigned temperature.

The 30 °C incubation temperature is similar to conditions under which females normally incubate in the wild (Reaney & Backwell, 2007; Milner *et al*., 2010), while the two higher incubation temperatures (34 and 36 °C) encompass the range of temperatures predicted by the end of the 21st century under a scenario of high greenhouse gas and aerial emissions (i.e. rise of 4–6 °C; IPCC, 2007).

All females were kept in small, individual containers with 1 cm of seawater and a stone so that they could emerge from the water. The water inside the container was replaced every 2 days (the old water was suctioned out and replaced with freshly collected seawater). The water baths were covered with a lid to simulate the darkness of natural incubation burrows.

Females were monitored at the same time every day, and as soon as a female extruded her clutch onto her pleopods, successfully fertilizing the eggs, we collected 10–20 eggs (2000–5000 eggs per clutch) for measurements before placing her back in her incubation chamber with her remaining eggs. We continued to monitor the females until they released their larvae from their pleopods into the water. Once the larvae were released (i.e. the clutch hatched), we collected the water that contained them. The water was stirred evenly before we collected a 1-mL subsample for measurements. After releasing their clutches, the females were returned to burrows in the mudflats and larvae were released into the sea during the peak spring tide to give them the best possible chance of survival.

The time taken from mating to extrusion of the clutch (i.e. the point at which the eggs are fertilized) was defined as the pre-fertilization period. The time between clutch extrusion and larval release, or hatching, was defined as the incubation period. The number of larvae was estimated by multiplying the number of larvae/mL by the total volume of water (Reading & Backwell, 2007) and this measure was used as a proxy for clutch size.

We preserved the collected samples of eggs and larvae in 10% formalin (4% formaldehyde) to prevent egg shrinkage or expansion prior to measurement (see below) (Clark & Backwell, 2016). We photographed all the egg and larval samples using a Leica microscope (MC120HD) with digital camera. We measured total egg width and height as well as yolk width and height, and larval eye width, body width and height, spike length, and tail length (Fig. 1). All measurements were taken using ImageJ (Clark & Backwell, 2016).

Data analysis

All statistics were carried out using R 3.4.1 and SPSS 24. The effects of temperature on the pre-fertilization period and incubation period were investigated using general linear models (GLMs) and significance was determined using F-tests. The pre-fertilization and incubation periods were specified as dependent variables, incubation temperature as a categeorical explanatory variable and female size as a covariate. In the 36 °C treatment, only one female was able to successfully hatch her clutch. We therefore combined data from the 34 and 36 °C treatments into a high-temperature treatment for the incubation analysis only. Model residuals met the assumptions

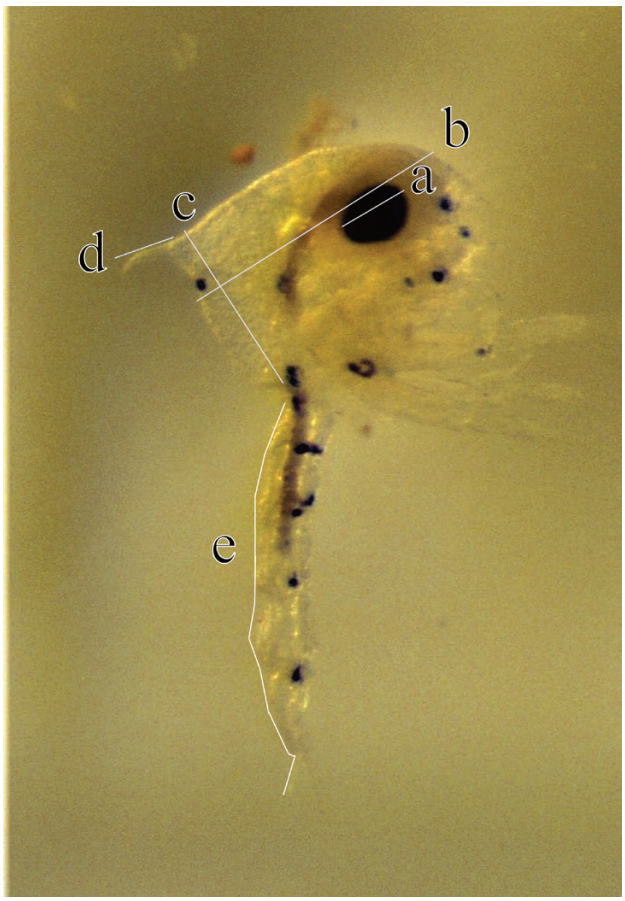

Figure 1. Illustration of larval measurements: (a) eye width, (b) body width, (c) body height, (d) spike length and (e) tail length.

of normality and homoscedasticity (Supporting Information, Appendix S1; Figs S1 and S2).]

The effect of temperature on the larval release day (i.e. how many days before or after the spring tide larvae were released) was also analysed using a GLM followed by F-tests to determine significance. In this model release day was specified as a dependent variable, temperature as a categorical explanatory variable and female size as a covariate. Larval release day provides a measure of how well the timing of larval release matched the expected release date. If larval release occurred before the spring tide (i.e. the expected release date), then the release day was negative, and if it occurred afterwards it was positive. Model residuals met the assumptions of normality and homoscedasticity (Appendix S1; Fig. S3).

We used a GLM with a binomial error structure (and quasibinomial error structure in the combined dataset for overdispersion) to examine the effects of temperature on both fertilization and hatching success. Chi-square tests were used to determine significance. We specified fertilization and hatching success as dependent variables and incubation temperature as an explanatory variable.

To examine the difference in egg and larval size under different temperature treatments, we used principal component analysis (PCA) to combine the information on egg size into a single variable. All measures of egg size (egg width and height, yolk width and height) loaded strongly and positively on PC1, explaining 86.72% of variation in the egg size data (Table 1). This suggested that the four measurements of egg size varied together, and therefore for simplicity we have called PC1 egg size. We also conducted a PCA to reduce the data on larval size into a single variable. For the larval size measurements (eye width, body width, body height, spike length, tail length), tail length alone loaded strongly and positively on PC1, which explained 96.85% of the variation in our larval size measurements data (Table 1) and thus PC1 represents the tail length of larvae. PC2 explained 1.61% of the variation in larval size, and was mainly attributed to body height. After extracting these two factors, we conducted a GLM using the factor score derived

from PCA described above. Model residuals met the assumptions of normality and homoscedasticity.

We present means ± 1 SE of our raw data unless otherwise stated. Alpha was set to 0.05.

RESULTS

We analysed data from 105 of the 113 collected mating pairs. Data from eight mating pairs were excluded, either because the female died during the experiment or because the incubation temperature fluctuated beyond the permitted ±1 °C range. Neither the size of the incubating female nor the size of the male that fertilized her eggs differed among the three temperature treatments (Table 2).

Fertilization and hatching success

Fertilization success (i.e. the number of females that extruded their eggs divided by the number of females tested) was 53.3% at 30 °C (16 of 30), 26.8% at 34 °C (11 of 41) and 37.1% at 36 °C (13 of 35). Temperature had marginal effects on fertilization success (GLM d.f. = 2,102, $P = 0.055$, Fig. 2A): there was a significant difference in fertilization success between 30 and 34 °C (Tukey test $P = 0.048$) but there was no difference between 30 and 36 °C (Tukey test $P = 0.323$) or between 34 and 36 °C (Tukey test $P = 0.602$).

Hatching success (i.e. the number of hatched clutches divided by the number of fertilized clutches) was 87.5% at 30 °C (14 of 16), 45.5% at 34 °C (5 of 11) and 7.7% at 36 °C (1 of 13). Temperature had effects on hatching success (GLM d.f. = 2,37, $P < 0.001$, Fig. 2B): there was a marginal difference in hatching success between 30 and 34 °C (Tukey test $P = 0.072$), a significant difference between 30 and 36 °C (Tukey test $P = 0.002$), but no difference between 34 and 36 °C (Tukey test $P = 0.135$).

Additional data

We were able to combine the data for hatching success with data collected from a different study (Clark & Backwell, 2016) because the methods were identical. This combined dataset gave us greater sample sizes and allowed us to increase the range of temperatures in our analyses (28–36 °C; see Supporting Information, Appendix S2). Using the same analyses outlined above, we obtained similar results. Hatching success showed a quadratic functional form across the experimental range of incubation temperatures; it was maximal at 30 °C and decreased significantly with increasing incubation

Table 1. The proportion of variance explained by each component of egg and larval size in the principal component analyses

	Egg factor	Larval factors	
	1	1	2
Variance explained by PC	86.72%	96.85%	1.61%
Egg width	0.356		
Egg height	0.303		
Yolk width	0.685		
Yolk height	0.559		
Body height		0.013	–0.993
Body width		–0.014	–0.078
Eye width		0.031	–0.072
Tail length		0.999	0.015
Spike length		0.009	–0.052

Table 2. Mean body size of sampled males and females in three temperature treatments.

	Temperature	*N*	Body size (mm, mean ± SE)	d.f.	*F*	*P*
Female	30	29	8.67 ± 0.20	2	2.292	0.106
	34	41	9.08 ± 0.15			
	36	35	9.14 ± 0.13			
Male	30	28	11.03 ± 0.37	2	0.603	0.544
	34	40	11.08 ± 0.20			
	36	35	10.73 ± 0.19			

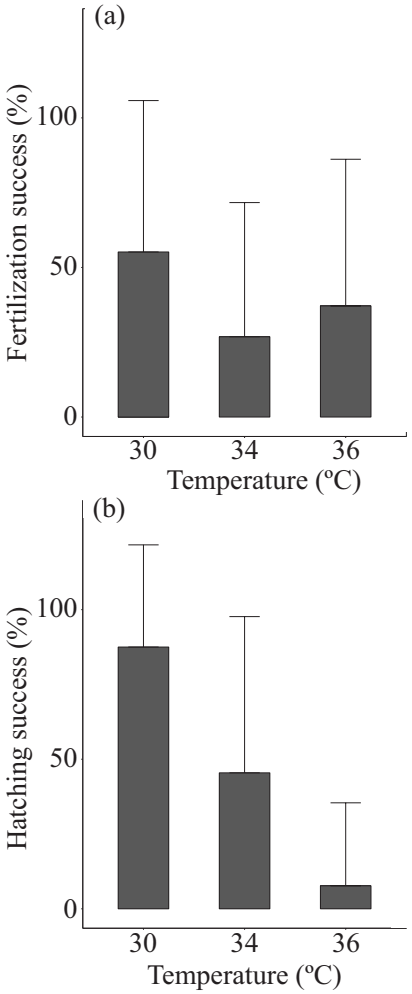

Figure 2. The effect of temperature on fertilization (A) and hatching success (B).

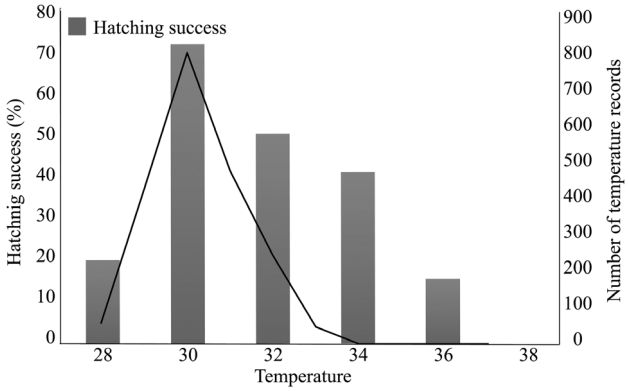

Figure 3. Hatching success from the combined dataset (i.e. our data and data from Clark & Backwell (2016)). Plot with data from Clark & Backwell (2016) showing the distribution of in total 2070 temperature readings in natural burrows during October–December 2012.

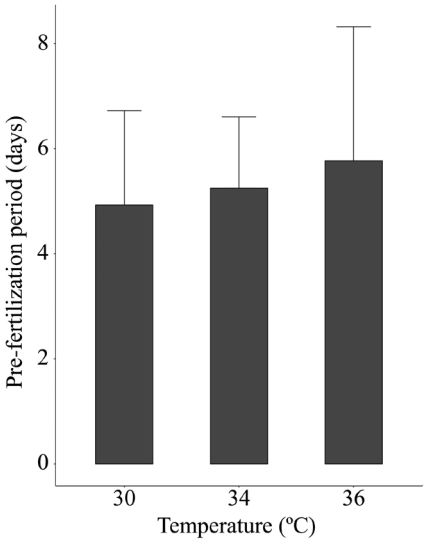

Figure 4. The effect of temperature on the pre-fertilization period.

temperatures (GLM d.f. = 4,77, $P = 0.001$, Fig. 3). We also relate the results to the natural incubation temperatures experienced by females in the field (Fig. 3; data taken from Clark & Backwell, 2016).

Pre-fertilization period and incubation period

Across all three temperature treatments, the average time between mating and clutch extrusion was 5.30 ± 0.31 days. Neither temperature nor female size affected the duration of the pre-fertilization period (temperature: GLM $F = 0.616$, d.f. = 2,36, $P = 0.546$, $N = 40$; female size: GLM $F = 0.032$, d.f. = 1,36, $P = 0.857$, $N = 40$; Fig. 4).

Across all three treatments, the average time between clutch extrusion and larval release was 15.8 ± 0.24 days. Neither temperature nor female size had an effect on the incubation period (temperature: GLM $F = 1.3517$, d.f. = 1,17, $P = 0.261$, $N = 20$; female size: GLM $F = 0.936$, d.f. = 1,17, $P = 0.347$, $N = 20$; Fig. 5).

Mismatch between spring tide and larval release day

Across all three temperature treatments, the average time between spring tide and larval release day was 0.20 ± 0.28 days. Neither temperature nor female size had an effect on the mismatch between spring tide and larval release day (temperature: GLM $F = 0.021$, d.f. = 1,17, $P = 0.886$, $N = 20$; female size: GLM $F = 0.683$, d.f. = 1,17, $P = 0.420$, $N = 20$; Fig. 6).

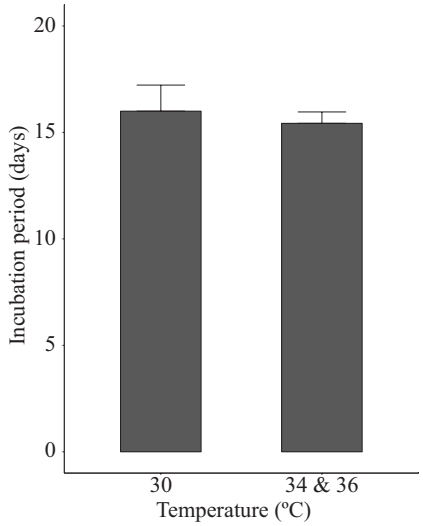

Figure 5. The effect of temperature on the incubation period.

Temperature effects on egg and larval traits

Neither incubation temperature nor female size had effects on egg size (Egg PC1, GLM $F = 0.111$, d.f. = 2,33, $P = 0.896$; female size: GLM $F = 0.069$, d.f. = 1,33, $P = 0.794$) or tail length (Larvae PC1, GLM $F = 0.936$, d.f. = 1,12, $P = 0.353$; female size: GLM $F = 1.151$, d.f. = 1,12, $P = 0.305$).

Clutch size was also not affected by incubation temperature. Females produced an average of 805.26 eggs per clutch (SD = 560.74; $N = 15$; GLM $F = 0.044$, d.f. = 1,15, $P = 0.84$; Fig. 7).

DISCUSSION

Environmental change, including increased temperatures, can result in powerful selection that alters ecological dynamics (Cameron *et al.*, 2013). Many correlational studies have demonstrated that temperature affects reproductive timing, clutch success and offspring phenotype (Charmantier & Gienapp, 2014). However, experimental tests are needed to determine causation. In this study, we experimentally manipulated fertilization and incubation temperatures for female fiddler crabs and observed their behavioural responses. Our data suggest that fiddler crabs may be vulnerable to future warming, as females could not successfully hatch a clutch of eggs under the temperatures that are predicted to occur within the next 80 years.

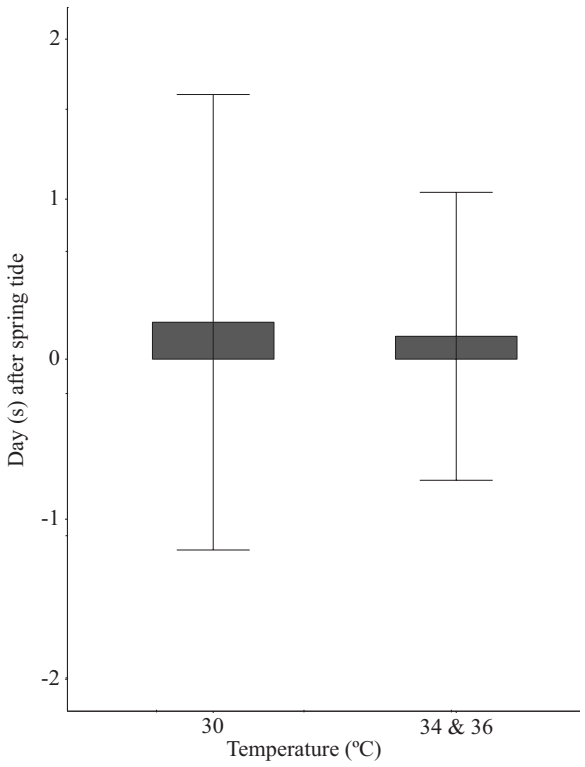

Figure 6. The effect of temperature on the mismatch of larval release day.

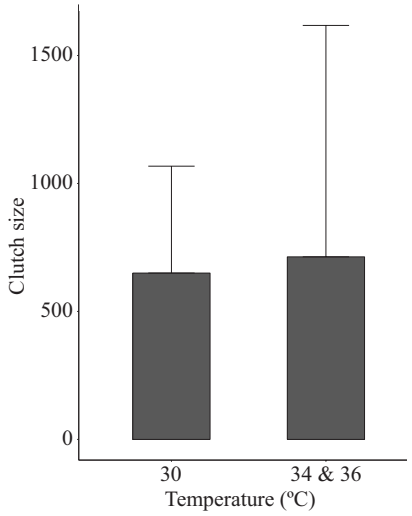

Figure 7. The effect of temperature on clutch size.

Clutch survival

The natural incubation temperatures experienced by female fiddler crabs and their clutches are relatively narrow (30.5 ± 1.1 °C). We therefore consider our experimental incubation temperature of 30 °C to represent 'natural' temperatures. In our experiment, 53% of females kept at 30 °C fertilized a clutch of eggs (the remaining 47% may have chosen not to fertilize their clutches due to disturbance, or may not have mated by the time we collected them). At higher temperatures, there was a drop in fertilization success: 27 and 37% of females fertilized their eggs at 34 and 36 °C, respectively. The chances that a fertilized clutch of eggs would survive to the hatching stage was highest at 30 °C, with 88% of clutches hatching. This dropped sharply at higher temperatures: 50% hatched at 34 °C and 8% hatched at 36 °C. A similar pattern was found when combining our data with previous data (Fig. 3): hatching success was highest at 30 °C and progressively decreased as temperatures moved away from this (i.e. 29, 31 and 32 °C) until almost no clutches hatched (i.e. 34 and 36 °C).

The dramatic decrease in hatching success at elevated incubation temperatures suggests that fiddler crabs are unlikely to cope with predicted future temperature increases. Intertidal organisms are known to have a wide tolerance of daily temperature fluctuations (Wilkens & Fingerman, 1965). Tropical fiddler crabs can experience air temperature ranges of 19 °C (Sibiger & Munguia, 2008). In our study species, air temperatures range from 30 to 49 °C (K. Kerr, unpubl. data). However, incubation temperatures are much more stable (29–32 °C) and here we show that incubating outside this natural range is problematic. This species, and probably many other intertidal ectotherms, has a limited ability to cope with extreme temperature changes (Deutsch *et al.*, 2008; Sunday *et al.*, 2014).

The inability to successfully hatch a clutch of eggs at the temperatures predicted to occur within the next 80 years could be devastating to the survival of this species. However, there may be other factors that could mitigate the effect of temperature. Burrow structure is known to influence burrow temperature (Reaney & Backwell, 2007), as is shade cover (Munguia *et al.*, 2017). It is possible that females incubating in wide, deep burrows in shaded areas of the mudflat would experience low enough temperatures to facilitate successful incubation.

Reproductive timing

Females fertilized their eggs an average of 5 days after mating. This duration was not affected by temperature. Females hatched their eggs an average of 16 days after fertilization. This duration was also not affected by temperature. As a result, there were no mismatches between the day of hatching and the highest spring tide (an average of 0.2 days at all temperatures).

Although it has been shown that temperature affects incubation duration in other fiddler crab species (Christy, 1982, 2003; Sanford *et al.*, 2006; Reaney & Backwell, 2007; Kerr *et al.*, 2012), we found no effect in *A. mjoebergi*. One possible explanation is that there is an upper limit to how much the development rate can change with temperature. In other fiddler crab species, larvae develop significantly faster under higher temperatures, resulting in shorter incubation periods under naturally occurring temperature ranges: an increase of 5 °C (24–29 °C) resulted in a 6-day decrease in incubation duration (13–19 days) in *Uca terpsichores* (Christy, 2003). An 8 °C temperature increase (18–26 °C) resulted in a 20-day decrease in incubation duration (15–35 days) in *Uca pugnax* (Sanford *et al.*, 2006). Similar effects were found in other species (Christy, 1982; Reaney & Backwell, 2007; Kerr, 2012). However, our study species lives at higher temperatures than most other fiddler crabs (air temperatures of 30–49 °C; K. A. Kerr, unpubl. data; incubation temperatures of 30.5 ± 1.1 °C). Temperatures greater than this may push the crabs beyond their physiological ability to compensate.

Another potential explanation for the lack of a temperature effect on incubation duration is behavioural compensation. Rather than directly adjusting the incubation *duration*, female *A. mjoebergi* may be able to adjust the development *rate*. Producing larger eggs or eggs with more yolk may slow egg development rates and therefore extend their incubation period. A previous study has shown that female amphipods produce smaller eggs at higher temperatures (Sheader, 1996). It is possible that *A. mjoebergi* females could increase egg sizes, but this may result in smaller

clutches because there is a known trade-off between egg size and clutch size (Sinervo & Licht, 1991; Rowe, 1994; Williams, 2001). However, in this study there was no effect of incubation temperature on either egg size or the size of the yolk within the eggs, or the size of the larvae. Clutch size was also not affected by temperature, suggesting female *A. mjoebergi* do not modify egg size under increasing temperatures and, more generally, that they are not able to adjust egg and clutch sizes in response to extreme environmental conditions. A similar pattern was found in newts, where ovipositing females exposed to non-reproductive temperatures barely changed egg size or early cleavage rates (Toufarová & Gvoždík, 2016). Although egg size plasticity has been documented in many taxa that experience environmental variation (e.g. Fox *et al*., 1997; Fischer *et al*., 2003; Galeotti *et al*., 2006; Allen *et al*., 2008), such adjustments in life-history traits are highly dependent on factors such as maternal nutrient status and age (Mousseau & Fox, 1998). Despite the fact that temperature did not affect egg or larvae sizes, high temperatures experienced during egg development may still have carryover effects on offspring fitness components, including immune response (Durant *et al*., 2010, 2012), locomotor ability (Braña & Ji, 2000; Wang *et al*., 2014), growth, survival and reproduction of offspring (Andrew *et al*., 2000; Durant *et al*., 2010).

Maternal behaviour is known to affect incubation temperatures under natural conditions. In our study species, females preferentially mate in wider, and therefore cooler, burrows in summer than in winter (Reaney & Backwell, 2007; Milner *et al*., 2010). In *Uca deichmani*, females control the temperature of their clutch by moving up or down the burrow shaft and even onto the sediment surface (Kerr, 2012). These behavioural modifications were not available to females in our experiment, but they may be able to use them under natural conditions.

Conclusions

The timing of hatching is extremely important in fiddler crabs. Larvae must be released close to the highest amplitude spring tide in order to avoid high inshore predation levels (Christy, 1982; Morgan & Christy, 1995). Here we show that females release their larvae on time even when temperatures vary by 4 °C. This suggests either that, unlike other species, incubation duration is not affected by temperature, or that females behaviourally compensate for shorter incubation duration. However, it is also possible that our experimental design was unable to detect the influence of temperature on incubation duration. We tested several possible mechanisms for behavioural compensation, including adjusting the timing of fertilization or the incubation rate by producing larger eggs, and found no evidence for either tactic. There are two potential explanations for these results. First, temperature may not affect larval development rate in this species. This is highly unlikely because it has a strong effect in all other fiddler crab species that have been examined, and because females of this species have been shown to behaviourally adjust incubation temperatures by selecting particular burrow features (i.e. 28–34 °C, Reaney & Backwell, 2007; Milner *et al*., 2010). Second, and more probably, females compensate for the effect of temperature on larval development rate in a way that we have not uncovered.

Although we found no effect of incubation temperature on the timing of reproductive events with a 4 °C rise in incubation temperature, we found that females were unable to carry clutches to the hatching stage when temperatures increased by ≥4 °C. Reductions in survival and reproduction are major pathways that can lead to species extinction under climate change (Bradshaw & Holzapfel, 2006; Parmesan, 2006).

The fact that we found no evidence for behavioural compensation in the timing of reproductive events, and that clutch survival decreased rapidly with increasing incubation temperatures suggests that these crabs are close to their thermal limits. We predict that a ±4 °C rise in seawater temperatures will have dire consequences for fiddler crab populations. However, there were a small number of clutches that survived the thermal stress of a 4 °C rise in incubation temperature. This may indicate the potential for adaptation to warming. More attention should be paid to the reproductive performance of intertidal species under climate warming.

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AUTHOR CONTRIBUTIONS

C.C.C. and P.R.Y.B. conceived the ideas and designed the methodology; C.C.C. collected the data; both authors analysed the data; C.C.C. led the writing of the manuscript. All authors contributed critically to the draft manuscripts and gave final approval for publication.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website.

AppendixS1. Residual plots and histograms of the residuals of the general linear model analyses.
Appendix S2. The combined dataset of present study with Clark & Backwell (2016).
FigureS1. Residual plot (A) and histogram (B) of the residuals of the analysis of the general linear model (GLM) for the pre-fertilization period.
Figure S2. Residual plot (A) and histogram (B) of the residuals of the analysis of the GLM for the hatching period.
Figure S3. Residual plot (A) and histogram (B) of the residuals of the analysis of the GLM for mismatch days.