ORIGINAL ARTICLE

Staying cool: the importance of shade availability for tropical ectotherms

C.-C. Chou [1] · D. M. Perez [1] · S. Johns [1] · R. Gardner [1] · K. A. Kerr [1] · M. L. Head [1] · E. L. McCullough [1,2] · P. R. Y. Backwell [1]

Abstract

Understanding how organisms mitigate the impacts of climate warming is one of the biggest challenges facing modern-day biologists. For tropical ectotherms, staying cool is critical for avoiding thermal stress, so individuals that are able to maintain territories in cool microhabitats are likely to gain fitness advantages. This study evaluated the importance of shade availability in the tropical fiddler crab, *Austruca mjoebergi*, by investigating temperature variation and behavioral responses (distribution, activity level, and time budget) in sun-exposed and shaded microhabitats. We found that the daily temperatures in sun-exposed microhabitats were significantly higher than in shady microhabitats, and that more male crabs held territories in the shade. Shaded males were active on the sediment surface for longer periods each day, allowing more time for feeding and more opportunities to detect mate-searching females. The greater number of males resident in the shade, and shorter distances between male neighbors in the shade, suggested that there was less available space for individuals to move into the shaded microhabitats. The behavioral differences between sun- and shade-living residents are likely to have consequences for male fitness. We highlight the importance of shade in providing thermal refugia for tropical ectotherms.

Significance statement

The impact of climate warming on tropical organisms could be less dire if individuals are able to access shade. Shade can offer thermal refugia for tropical organisms that may have important consequences for both behavior and fitness. Relatively low and stable temperatures in shaded areas allow male fiddler crabs to remain on the surface for longer compared to sun-exposed males, allowing for longer feeding periods and more mating opportunities.

Introduction

Understanding how organisms mitigate the impacts of climate warming through thermoregulatory behavior, and identifying

✉ C.-C. Chou
   Chun-Chia.Chou@anu.edu.au

1 Research School of Biology, The Australian National University, Canberra, ACT 0200, Australia

2 Present address: Center for Reproductive Evolution, Syracuse University, Syracuse, NY 13244, USA

the potential limits of behavioral thermoregulation, is a critical challenge in contemporary ecology. Body temperature often determines the rate of biochemical reactions (Angilletta et al. 2004; Glanville and Seebacher 2006). Therefore, the ability to maintain an optimal body temperature under warmer environmental conditions may act as a selective force (Mitchell and Bergmann 2016), determining not only individual performance (Kingsolver and Huey 2008; Podrabsky et al. 2008) but also population growth (Seebacher 2005). Many taxa, especially ectotherms who have evolved under relatively constant thermal environments, tend to have narrow thermal tolerance ranges (Kearney et al. 2009; Dillon et al. 2010) and do not have the physiological thermal safety margins to cope with temperature fluctuations (Sunday et al. 2014). This makes ectotherms extremely vulnerable to climatic change (Huey and Tewksbury 2009; Kingsolver et al. 2013), and their survival is likely to rely on behavioral thermoregulation and the

accessibility of thermal refugia (Forsman et al. 2002; Edgerly et al. 2005).

For tropical ectotherms, the primary thermal challenge is to stay cool (Kearney et al. 2009) and a thermal refuge, such as a burrow or a shady surface, can prevent an individual from direct sun exposure and dehydration (Seabra et al. 2011; Edgerly et al. 2005; Munguia et al. 2017). Shade availability can have profound effects on population density and distribution (Kearney et al. 2009; Lima et al. 2016), because individuals in the shade can stay active for longer periods of time, which increases their access to feeding resources (Downes 2001; Amo et al. 2007) and their chances of encountering potential mates (Martín et al. 2003; Reaney 2007). If individuals need access to shaded areas in order to thermoregulate, then shade availability will determine the maximum number of animals that can survive in that habitat (Lima et al. 2016), and we would expect to see differences in activity levels and time budgets associated with temperature of the microhabitats.

The fiddler crab, *Austruca mjoebergi*, is an ideal study organism for investigating the importance of shade availability for thermoregulation for several reasons. This species lives in mangrove clearings in the hot, tropical north of Australia. Areas shaded by mangroves do not receive direct sun exposure throughout the day, which creates differences in the maximum daily surface temperatures between the two microhabitats (53 °C in the sun and 36 °C in the shade). Each individual holds a burrow in the center of its territory, and the burrows are used for behavioral thermoregulation (Munguia et al. 2017). Crabs periodically enter their burrows to cool down and access the water within. High territoriality makes the crabs unable to shuttle between the sun and shade for regulating their body temperatures because they risk predation, territory theft by conspecifics, and dehydration if they move away from their territories. Individuals that live in exposed, sunny areas of the mudflat retreat into their burrows far more regularly than crabs living in the shaded areas of the mudflat (approximately every 8 min vs every 22 min; Munguia et al. 2017). Most females retreat inside the burrow and cease surface activity during the middle of the day, and so do most males in sunny areas (Munguia et al. 2017).

Truncation of the activity period due to refuge use usually imposes fitness costs (Downes 2001; Amo et al. 2007; Reaney 2007). This could be especially true in *A. mjoebergi*, since most important activities occur on the surface (Zeil et al. 2006). For example, territorial battles for burrows frequently occur on the mudflat surface (Milner et al. 2010a; Clark and Backwell 2017); individuals mainly consume nutritional sediment on the mudflat surface (Robertson et al. 1980; Reinsel and Rittschof 1995), and males need to be active on the surface and engaged in courtship (claw waving) to attract mate-searching females (see Reaney et al. 2008). It is thus likely that there is a fitness benefit of holding a territory in the shade and being able to stay active on the surface for longer periods of time.

A. mjoebergi individuals clearly use their burrow as an escape from thermal stress, but they may also have the option of selecting a territory in the shaded parts of the habitat. Is there an advantage to living in the shade? Do females preferentially select mates in the shade? Are crabs able to freely select a territory in the sun or shade, or are "real estate" costs prohibitive? The main aims of this study were (i) to quantify the habitat composition of sun/shade and compare the daily temperature variation in two microhabitats, (ii) to explore whether the differences in temperature causes different activity levels and time budgets of individuals, (iii) to explore if different temperature-dependent activity levels and time budgets in the two microhabitats incur different fitness-related outcomes (e.g., mating success, female choice, wave characteristics), and (iv) to determine if there is available space for individuals to move into the microhabitat with higher fitness potential.

Methods, statistics, and results

We studied a population of *Austruca mjoebergi* in the mangroves of East Point Reserve, Darwin, Australia (12° 24′ 032″ S; 130° 49′ 050″ E) from September to December each year from 2015 to 2017. All data were collected during the diurnal neap low tides, except for the final part of the experiment evaluating the space availability (see below for details), since this is the mating period in this species. It is not applicable to apply blind methods because our study involved focal animals in the field. *A. mjoebergi* are small (< 2 cm carapace width) crabs that live in dense, mixed-sex populations on inter-tidal mudflats across Northern Australia. Like all fiddler crabs, males have a single enlarged claw that they use to fight other males and that they wave to attract mate-searching females. Each crab, both male and female, holds a territory that consists of a small area of mudflat surface (10–20 cm in diameter) on which they feed. The males also use this area to court females. In the center of the territory is a burrow that is used as an escape from predators, a retreat from the high tide and high temperatures, a source of water, a heat sink, and a mating and incubation site. When ready to mate, a receptive female leaves her territory and wanders through the population of waving males, visiting several males before selecting a mate. The pair mates underground, in the male's burrow. Once the female extrudes her eggs, the male leaves, sealing the female underground to incubate her eggs for about 2 weeks. The larvae are released on a high spring tide so that they are rapidly washed out to sea where they are planktonic.

All statistics were carried out using R 3.4.1 and IBM SPSS 24. For clarity, we have combined the methods, statistics, and results sections for each of the questions individually.

What is the proportion of fiddler crab habitat covered by mangrove?

We delimited the part of the population on which we regularly worked and divided the study site into 988 grid squares (1.35 m^2 each) (Fig. 1). We marked each square as covered if > 50% of it was covered by mangrove trees (hereafter shaded plot) and sun-exposed if ≤ 50% was covered by mangrove trees (hereafter sunny plot) (Fig. 2). We counted the number of "shaded" and "sunny" grid squares and calculated the percentage of grid squares that were in the sun or shade. Seven hundred thirty-one grid squares were in the sun (a total area of 987 m^2), and 257 grid squares were in the shade (a total of 347 m^2). This translated into 74% of the study area being in the sun and 26% in the shade (Fig. 1). All data presented below was collected within this area of the mudflat.

What are the average and maximum temperatures in the sun and shade?

Temperature was measured under clear, sunny conditions during the experimental period. We randomly selected 117 points in the sun and 108 points in the shade and measured the surface sediment temperature using a CE QM7216 digital probe thermometer (DigiTech). The time of each reading was noted. We analyzed the data using a general linear model (GLM) (temperature as the dependent variable; sun/shade as a fixed factor; time of day as a covariate and the interaction between microhabitat and time of day). Model residuals met the assumptions of normality and homoscedasticity (see the residual plot and the histogram of the residuals in Fig. 1 of

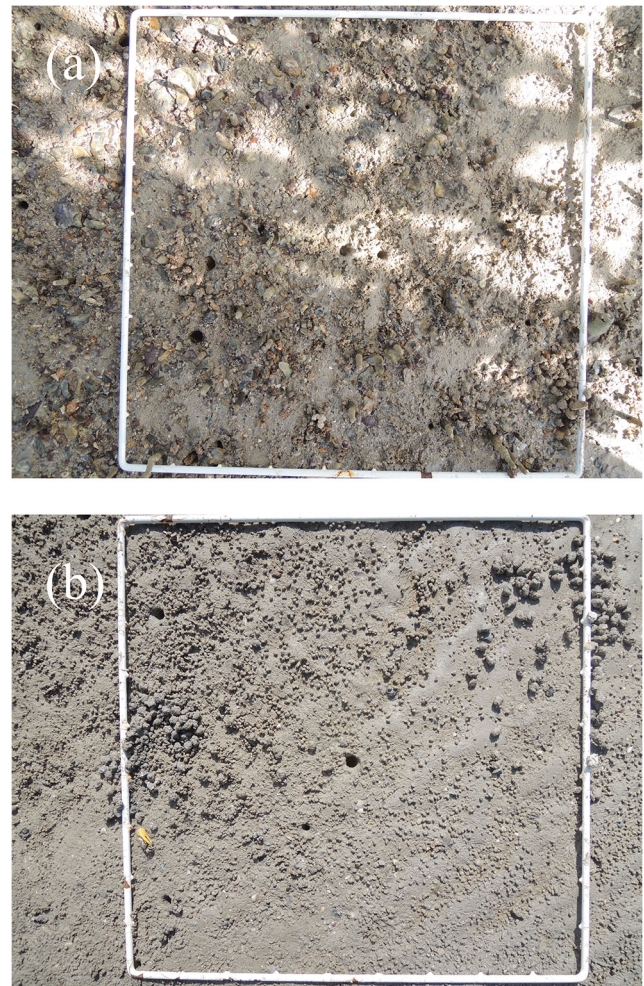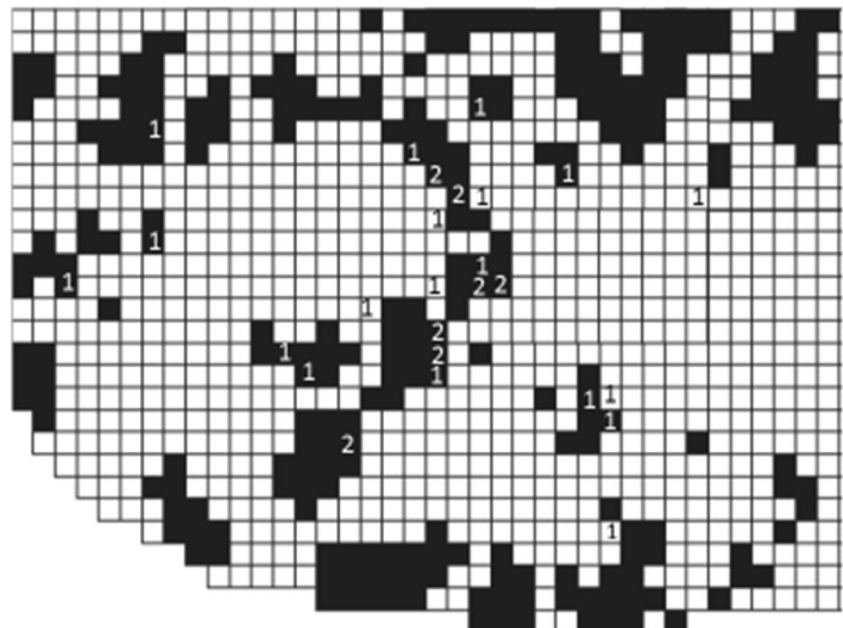**Fig. 2** Plots in the shade (**a**) and in the sun (**b**)

Fig. 1 The proportion of sunny (white) and shaded (black) microhabitats in the study site. Plots where mating occurred are indicated with a number, showing the number of observed matings

the supplementary document). The average sediment surface temperature in the sun was 37.26 °C (s.d. = 3.70, range = 32.70–52.60, $n = 117$), and in the shade it was 33.65 (s.d. = 1.23, range = 30.20–36.40, $n = 108$). Both microhabitat (sun/shade) and time of day had significant effects on sediment surface temperatures (time: $F = 49.70$, d.f. = 1,221, $P < 0.001$; sun/shade: $F = 122.08$, d.f. = 1,221, $P < 0.001$). The interaction between sun/shade and time of day was significant ($F = 22.91$, d.f. = 1, $P < 0.001$): the increase in temperature over the day was greater in the sun than in the shade (Fig. 3).

What is the number and density of crabs living in the sun and shade?

Data were collected every 15 min from 9 a.m. to 3 p.m. for 4 days. We demarcated 43 plots in the sun and 43 in the shade. Each plot was 35 × 35 cm. In each plot, we counted the number of waving males, the number of non-waving males, the number of resident females, and the number of wandering, mate-searching females. Plots were used once only; we alternated between sun and shade plots. A t test showed that there was no difference in the number of resident or mate-searching
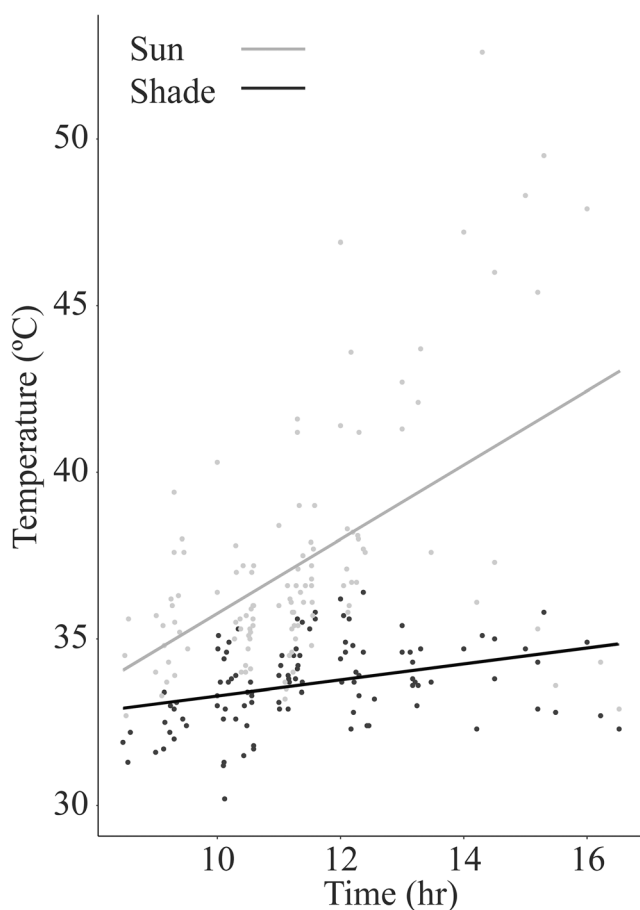

Fig. 3 Effect of time of day (x-axis) on sediment temperature in sunny (gray dots) and shaded (black dots) areas of the mudflat

females per plot in the sun and shade. There were, however, more males per plot (both waving and non-waving) in the shaded areas (Table 1), and hence, the total number of crabs per plot was higher in the shade than the sun (shade: $\overline{x} = 5.19$, s.d. = 3.28, $n = 43$; sun: $\overline{x} = 2.98$, s.d. = 2.17, $n = 43$; $t = 3.70$, d.f. = 84, $P < 0.001$). We also compared the proportion of waving males in relation to all males in each microhabitat using a two-proportion z test, and there were 1.5 times more waving males in the shade than in the sun (shade 45%; sun 29%, d.f. = 1, $P = 0.0243$). The ratio between males and females also differed in the sun and shade: in the sun, 59% of the crabs were males; in the shade, 77% of the crabs were males (Fisher's exact test $P = 0.025$).

Do males stay active later in the day when they live in the sun or shade?

We demarcated 35 plots (15 in the shade and 20 in the sun) at the start of the low tide period during the neap tides of four successive days (data from 5 shaded plots were excluded because they could not be classified as entirely shaded). The number of surface-active males present in the plot at 11 a.m., 1 p.m., and 3 p.m. was counted, and each plot (35 × 35 cm) was used on only 1 day (resulting in three readings for each of 35 plots). The daily variation in the number of surface-active males in sun and shade plots was then compared using linear mixed models (LMM). The number of males was the dependent factor while the time, the microhabitat, and the interaction between them were specified as fixed factors. In addition, plot number (plot ID) was included as a random factor into the model. Model residuals met the assumptions of normality and homoscedasticity (see Fig. 2 of the supplementary document). Both sun/shade and time of day had strong effects on the number of males staying on the surface (sun/shade: d.f. = 1,33, $F = 10.75$, $P = 0.002$; time: d.f. = 2,66, $F = 7.51$, $P = 0.001$). The interaction between microhabitat and time of day was significant (d.f. = 2,66, $F = 3.702$, $P = 0.030$): the decline in males on the surface as the day progressed was greater in the sun than in the shade (Fig. 4). There was also a significant variation in the number of males on the surface associated with plot ID (chi-square test, $P < 0.001$).

Do male time budgets differ between the sun and shade?

We scan sampled males from 20 plots (5 or 6 males per plot) over 3 days, 10 plots in the sun and 10 plots in the shade. Two researchers collected the data simultaneously, one in the sun and one in the shade (alternating between trials) so that the sun and shade areas were equally represented throughout each day. We recorded the behavior of six males sequentially, and repeated the scans 20 times for each set of males. The
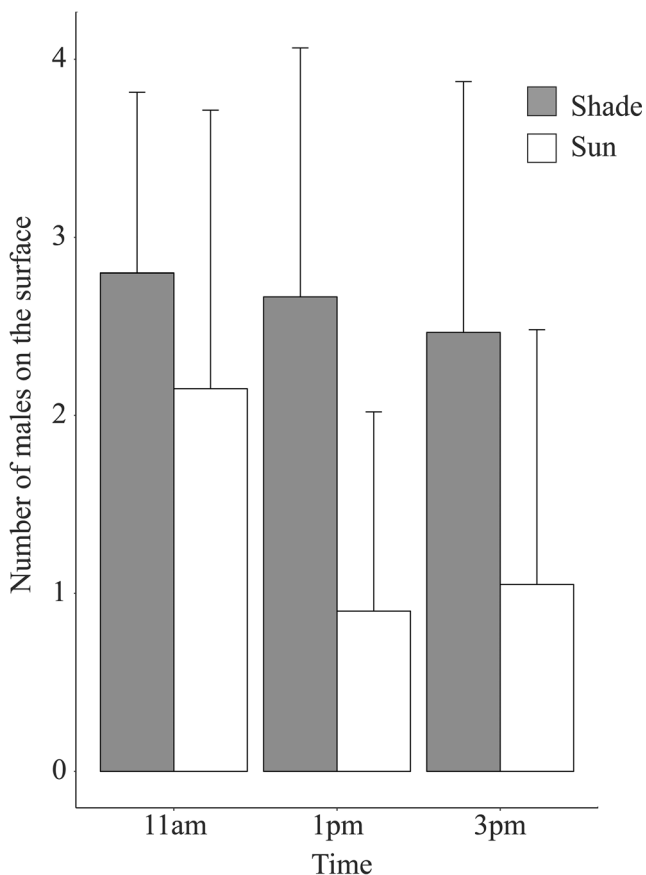
Table 1 Means, standard deviations, and total number of resident and mate-searching females and waving and non-waving males observed in the 43 plots sampled in sun and in shade areas in the mudflat. Student t test results indicate degrees of freedom and significance in the comparison between sun and shade for each group

		Resident females	Mate-searching females	Waving males	Non-waving males
Sun	$\bar{x}$	0.91	0.33	0.52	1.23
	sd	1.27	0.64	1.13	1.44
	(n)	43	43	43	43
	Total	39	14	22	53
Shade	$\bar{x}$	1.16	0.19	1.81	2.16
	sd	1.57	0.59	2.45	1.947
	(n)	43	43	43	43
	Total	50	8	78	93
	t	0.83	1.05	3.10	2.52
	d.f.	84	84	84	84
	P	0.41	0.30	0.003	0.014

following behavioral categories were noted: feeding, waving, underground (inside the burrow), standing at the burrow entrance, interacting with heterospecific fiddler crabs (*Tubuca elegans*), and "other" (a category that encompassed any behaviors not already covered, such as burrow maintenance and grooming; this category is not included in the analysis). We

compared males in the sun and shade using Student t tests. When surface-active, males in the sun and shade spent equivalent amounts of time waving, but males in the shade spent more time feeding, less time underground, less time at their burrow entrance, and less time interacting with *T. elegans*. Data are presented in Table 2.

Does mating naturally occur more often in the sun or shade?

We tracked 33 mate-searching females on the mudflat until they selected a mate. A researcher followed slowly behind a mate-searching female as she visited several males in the population. It is possible to prevent disturbing the female or the courting males by remaining 1.5 m away from them (see Reaney and Backwell 2007). A mating was confirmed when the female entered a male's burrow and remained there until the male either displayed mate-guarding behavior at the burrow entrance or sealed the burrow entry with the pair inside. This behavior indicates successful mating (see Peso et al. 2016). We plotted the position of the mating on an aerial photograph of the study site (see "Methods, statistics, and results" 1 above) and classified the mating sites as being in the sun or shade (Fig. 2). We used a Fisher exact test to compare the number of matings that occurred in the sun and shade with the amount of space that was available in each of these areas. Seven matings in the sun and 26 in the shade (21% in sun, 79% in shade) were documented. Compared to the proportion of the study site in the sun (74%) and the shade (26%), significantly more matings occurred in the shade than would be expected by chance (Fisher exact test, $P < 0.001$). However, when compared to the number of waving males that were present in the sun (0.52 males/35 cm^2 plot; 22%) and shade (1.81 males/35 cm^2 plot; 78%), the proportions were almost identical (Fisher exact test $P = 0.54$). Females do not

Fig. 4 The number of males on the surface of the mudflat at different times of day in the sun and shade microhabitats

Table 2 Means, standard deviations of five behavioral activities, feeding, waving, underground, at burrow entrance, and heterospecific interactions observed in males in sun and in shade areas in the mudflat. Student *t* test indicates degrees of freedom and significance for the comparison between sun and shade activities

		Feeding	Waving	Underground	At burrow entrance	Interacting with *Tubuca elegans*
Sun	$\bar{x}$ (s.d.)	7.67 (5.34)	2.83 (4.02)	6.12 (5.29)	1.33 (2.58)	0.45 (0.86)
	n	58	58	58	58	58
Shade	$\bar{x}$ (s.d.)	11.17 (6.15)	2.77 (3.55)	2.97 (4.80)	0.53 (0.10)	0.03 (0.18)
	n	60	60	60	60	60
	t (d.f.)	−3.29 (114.68)	0.09 (113.19)	3.39 (114.06)	2.19 (73.19)	3.59 (61.86)
	P	0.001	0.93	0.009	0.031	0.0006

specifically select to mate with males in the shade, but they are more likely to mate in the shade because there are more courting males there.

Do males living in the sun and shade differ in size?

We randomly selected the location of plots and marked the burrows of all surface-active males within 17 plots in the sun and 19 plots in the shade (35×35 cm). The minimum diameter of the burrow entrance of each burrow ($n = 80$ in the sun; 94 in the shade), which is closely correlated with male size (Reaney and Backwell 2007; see supplementary data), was measured. Burrow diameters (and hence male sizes) were larger in the shaded areas of the mudflat although this trend was not significant (shade: $\bar{x}$ burrow diameter = 8.9 mm, s.d. = 1.4, $n = 94$; sun: $\bar{x}$ burrow diameter = 8.5 mm, s.d. = 1.5, $n = 80$; *t* test: $t = 2.02$, d.f. = 192, $P = 0.08$).

Do females prefer males courting in the sun or shade?

We tested female mate preferences using custom-built robotic crabs. Each robot consisted of a plaster claw replica (molded from a real male claw) attached to a metal arm that protruded above the test arena and was controlled by a motor directly below the arena. The metal arm was programmed to imitate the claw waving pattern of this species. This system has been used successfully in several past studies (e.g., Kahn et al. 2013; Reaney et al. 2008, also see Fig. 3a of the supplementary document).

Choice trials were conducted in the field for 16 days, using mate-searching females that were captured on the mudflat just prior to experiments and kept in separate plastic cups with 1 cm of water. Handling was minimal to avoid escape responses. The number of trials conducted each day varied from 5 to 54 depending on the availability of females we obtained in the field and weather. The two robotic crabs were placed 15 cm apart in a 62 cm by 62 cm test arena covered with a smooth layer of sediment from the surrounding mudflat. Each robotic crab was 25 cm away from (and directly facing) the female release point (Fig. 3b of the supplementary document).

The female was placed under a small inverted transparent plastic cup attached to a remote release mechanism. The robotic units were activated, and, once the female had seen 3 waves from the robots, she was released and allowed to move freely towards her chosen male. A female was considered to have selected a male if she moved in a direct and steady path to one of the units and reached the edge of the robotic crab unit (Fig. 3c of the supplementary document). Trials were discarded if the female dashed on release, ran to the perimeter of the arena, or sat still for > 2 min after release. This increases the likelihood that all choices were made in the mate-searching context. Each female was used only once in each trial, and after their trials, they were released to continue mate searching in the population.

Both robotic crabs were identical: They waved at a rate of 16.8 waves/min and had 24-mm-long claws. The only difference between them was their shading. In the first set of trials, one of the robotic crabs was shaded using a 30×30 cm piece of suspended 70% shade-cloth while the other was in the full sun. In the second set of trials, both the female and one of the males were shaded. We switched the side that was shaded $\pm$ every fifth trial and alternated the robotic crab units used on each side between days. Before each trial, the arena was left for ± 30 min until the temperatures in the sun and shade had stabilized. We measured the sediment surface temperature in front of each of the robotic crab units and in front of the female release point using a CE QM7216 digital probe (DigiTech) (trial 1, shaded 35 ± 1.59 °C, $n = 70$; sun 40.4 ± 2.32 °C, $n = 70$; trial 2, shade 34.8 ± 1.79 °C, $n = 70$; sun 41.29 ± 2.36 °C, $n = 70$).

We compared female choices between sun and shade males using binomial tests (Table 3). Females had no preference for males displaying in the sun or in the shade. This was true whether the female was released in the sun or in the shade (Table 3). It is possible that female behavior was affected by the presence of the shade cloth per se, so the results could potentially be due to an interaction between female preferences for shaded males coupled with an aversion to shade cloth.

Table 3 Female size and the outcome of female choice. Binomial tests indicates the significance for the comparison of female choice between sun and shade

	Female size	Female choice	Binomial test P value
Trial 1			
$\bar{x}$	8.6	Shade	0.91
(s.d.)	(1.09)	36	
n	70	Sun	
		40	
Trial 2			
$\bar{x}$	8.6	Shade	0.28
(s.d.)	0.97	40	
n	70	Sun	
		30	

Female size did not differ between trials where the female chose the male in the sun or the male in the shade (GLM Exp.1: d.f. = 1,68, $F = 0.95$, $P = 0.33$; Exp.2: d.f. = 1,68, $F = 0.18$, $P = 0.67$).

Do male wave rate and wave structure differ between the sun and shade?

We video recorded the waving behavior of 56 males (28 males in the sun and 28 males in the shade) using video cameras (JVC GZ-EX 355 BAA, frame rate: 30 frames per second). A pair of sun and shade males was recorded simultaneously by two different observers to control for differences in behavioral activity over the course of the day. Male crabs with similar sizes were paired to avoid potential size-related behavioral biases (male size did not differ; paired t test $t_{28} = -0.958$, $P = 0.343$). Males were stimulated to wave by placing a tethered female 10 cm in front of their burrow (the female was attached to a nail by a 3-cm piece of cotton thread glued to her carapace). A scale bar was set in a vertical position next to the male's burrow, and we video recorded his waving for 1–3 min after he emerged from his burrow, noticed the female, and began waving at her. Following this, we captured the male and measured his carapace and claw length. Each female crab was used for fewer than 5 videos and was allowed to rest in a cup with seawater for at least 10 min before filming the next video. The recordings followed the methodology describe by Perez et al. (2016). The camera was positioned on a tripod 50 cm from the ground, ensuring less than 2° difference in the camera-to-crab angle between videos. In addition, the vertical scale placed near the crab allowed us to control for the angle between the camera and the crab. This enabled us to measure crab size and wave elements with accuracy.

We analyzed the videos using the software *digilite* created in MATLAB (The MathWorks, Inc., Natick, MA, USA) by Jan Hemmi and Robert Parker. Each video was analyzed frame by frame for 1 min after the male saw the female and started courting. We recorded the wave duration and wave rate for 902 waves from 56 males with the claw length as a scale to measure 173 wave heights relative to the upper carapace ridge from the 56 males (measured only when males were directly facing the camera). There are two types of waves in this species, a single wave and a double wave (Perez and Backwell 2017), and we measured and analyzed them separately. We compared the wave duration, wave height, and wave rate using LMM. Wave duration and height were considered as dependent factors, with microhabitat type as a fixed factor, and length of the upper claw as a covariate. Both pair number (i.e., the males that were simultaneously recorded in the sun and shade) and individual number (crab ID) were included as the random factors in the analyses of wave duration and height. Individual number was included because there were multiple measures of wave duration and wave height within each recording. The data met the assumptions of LMM (see Fig. 4–6 of the supplementary document). In the analyses of wave rate, only pair ID was included as a random factor to control for the variation arising due to the time at which the pair was recorded. Individual ID was not included in this analysis because each individual only had one wave rate.

Males in the sun produced double waves at a higher rate than males in the shade (Fig. 5b, d.f. = 1.51, $F = 6.193$, $P = 0.016$), but the microhabitat effect on the wave rate of the single wave was not significant (Fig. 5a, d.f. = 1,51, $F = 2.540$, $P = 0.117$). Males with longer claws had faster single wave rates (d.f. = 1, 51, $F = 4.338$, $P = 0.042$), but claw length had no effect on the double wave rate (double: d.f. = 1, 51, $F = 1.967$, $P = 0.167$). Both single and double waves produced by males in the shade were of longer duration than those of males in the sun (Fig. 5c and d, single: d.f. = 1, 43.04, $F = 16.966$, $P < 0.001$; double: d.f. = 1, 49.158, $F = 12.598$, $P < 0.001$), and the claw length had no effect on the wave duration (single: d.f. = 1, 39.74, $F = 0.329$, $P = 0.569$; double: d.f. = 1, 49.86, $F = 0.497$, $P = 0.484$). There was no difference in wave height of either single or double waves between sun and shade males (Fig. 5e and d, single: d.f. = 1, 31.95, $F = 0.423$, $P = 0.520$; double: d.f. = 1, 25.85, $F = 2.316$, $P = 0.140$). There was no effect of claw length on the wave height of both single waves and double waves (single: d.f. = 1, 32.45, $F = 1.345$, $P = 0.255$; double: d.f. = 1, 49.08, $F = 3.947$, $P = 0.052$). Pair number had no effect on wave rate, wave duration, or wave height of either wave types (chi-square test, $P = 0.607$ for the height of double wave and $P = 1$ for the rest). However, there was significant variation in wave duration and wave height of both wave types associated with crab ID (chi-square test, $P < 0.001$).
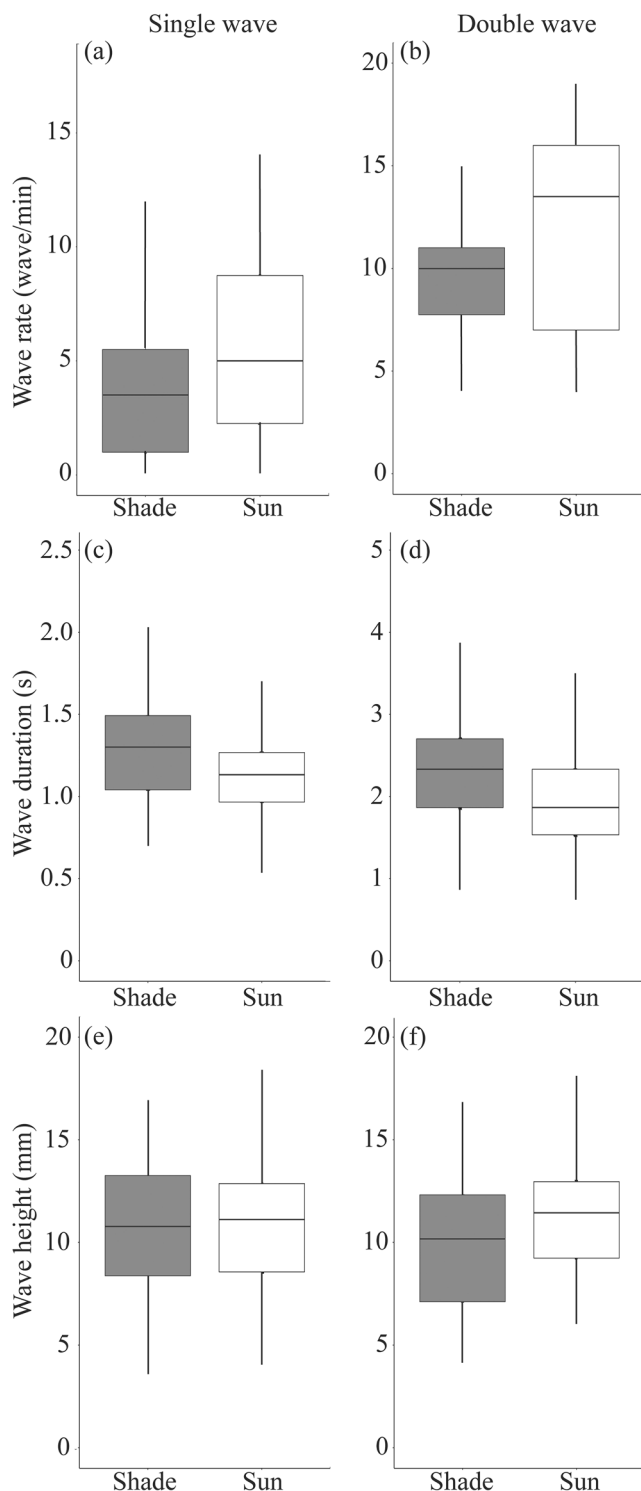
Fig. 5 The wave rate, duration, and height of single and double wave types in the sun and shade microhabitats

Is there empty space available for new crabs to move into the sun or shade?

We demarcated 20 plots (10 in the sun and 10 in the shade, each plot was 35 × 35 cm) at the start of a low tide period

during spring tide. During the spring tide, however, the area was covered by the high tide shortly before the plots were marked and were free of feeding pellets. At the end of the low tide period, we took photographs of the plots. For each plot, we counted the number of burrows and measured the nearest neighbor distances (NND) between males. In order to determine if there is still space for new crabs to establish territories, we calculated the percentage of area covered by feeding pellets in each plot by identifying the location of each pellet in the plot on digital photographs and marking the entire area of the quadrat that was covered by feeding pellets. Thereafter, each plot was divided into 81 grids (9 × 9) and was defined as used if the pellet coverage was over 50%. The percentage of used area can be derived as the ratio of the number of used grids and total grids (i.e., 81 grids). The 10 sun and 10 shade plots were compared using t tests. We also ran a Pearson's correlation between the number of burrows, the nearest neighbor distances, and the proportion of pellet cover in each plot (combining sun and shade plots). The arc-sin-transformed values of the proportion of the plot covered by feeding pellets were used as the dependent variable in the analyses.

There were more burrows and smaller NND in shaded plots than in the sun plots, but there was no difference in the proportion of the plot that was covered by feeding pellets (Table 4). There was a strong correlation between the number of burrows and the NND in each plot ($r = -0.77$, $P < 0.001$), but no correlation between the area covered by pellets and number of burrows ($r = 0.30$, $P = 0.19$) or the NND ($r = -0.10$. $P = 0.67$). Feeding pellets covered almost the entire surface in both sun and shade plots. Where the coverage was < 100%, it was usually due to the surface being covered by shells or dead leaves that prevented crabs from feeding. The feeding pellets were more spread out in the plots with fewer crabs (i.e., the sun plots), and there was, therefore, no correlation between the number of crabs and the area covered by feeding pellets. We suggest that the coverage of feeding pellets is not a suitable estimate of how much space is "available" for new crabs to inhabit.

Discussion

Hot tropical conditions may act as a strong selective force for ectotherms in terms of warming-induced activity restriction, given that these organisms often have very narrow thermal niches. Our findings demonstrate not only the significant role that shade habitat may have on the distribution and behavior of individuals, but more importantly, how the possession of a shaded territory can influence fitness. The assessment of how climate impacts a species relies on a comprehensive database that includes information about behavioral responses, environmental heterogeneity, and habitat utilization. In this study,

Table 4 The number of burrow, nearest neighbor distance (NND), and percentage of pellet cover in the sun-exposed and shaded plots. Student *t* test indicates degrees of freedom and significance for the comparison between the above three characters in the sun and shade

	Shade plots	Sun plots	*t* (d.f.) *P*
N	10	10	
$\bar{x}$ # burrows	10.5 (3.5)	5.4 (2.1)	3.91 (18) 0.001
$\bar{x}$ NND	8.15 (1.66)	11.56 (3.85)	2.57 (18) 0.019
$\bar{x}$ pellet cover	0.876	0.891	− 0.20 (18) 0.8413

we systematically address the impacts of thermal stress at each of these levels. We demonstrate the temperature difference between two microhabitats (i.e., sun-exposed and shaded) and compare the behavior of fiddler crabs in the two microhabitat types. We discuss how our results provide insights into how access to thermal refugia (i.e., shaded areas) may help this species, and potentially other ectotherms, cope with climate warming.

We present evidence that variation in tree coverage can create microhabitats that differ strikingly in surface temperature, which further affects the distribution and behavior of fiddler crabs. Vegetation cover is known to stabilize the temperatures of sediment surfaces (Powers and Cole 1976; Darnell et al. 2015). Tree density and canopy cover have been shown to influence the distribution and abundance of fiddler crabs (Peer et al. 2018). Our study site is partially shaded by mangrove trees: about a quarter of the area available to the crabs is in the shade, for at least part of the day, and three quarters is in the sun. The shaded areas are more thermally stable, with a mean diurnal temperature of 34 °C and a high of 36 °C. The sun-exposed areas are far hotter and more variable, with a mean of 37 °C and a high of 53 °C. Our study species occurred at densities that were nearly twice as high in the shade compared to the sun. There were significantly more males per plot in the shade than in the sun (both waving and non-waving), although the number of females per plot did not differ between two microhabitats. These differences in density imply that there are benefits associated with living in shaded areas, which may arise due to differences in temperature between the two microhabitats (see below).

The decrease in the number of surface-active males later in the day was far greater in the sun than in the shade, and males in the shade spent more time feeding on the sediment surface. Male *Uca pugilator* are more likely to maintain surface activity and wave more in shaded areas (Allen and Levinton 2014). These results support our expectation that individuals may obtain more opportunities to engage in fitness-promoting activities (i.e., longer time available for feeding) when they have access to a thermal refuge (see "Introduction"). It is less likely that males in the sun had higher efficiency in terms of nutritional intake and were thus able to meet the nutritional needs with the decreased feeding time. There is potentially a higher density of food in the sun-exposed areas due to higher light

conditions (greater microalgal growth) and the lower consumption pressures (lower crab density). These areas, however, dry out rapidly after they are exposed by the receding tide, and the sediment hardens. Fiddler crabs use chemoreceptors in the feeding claw and legs to detect food levels (Weissburg and Derby 1995). Sediments with lower water content prevent dactyl penetration and feeding behavior of fiddler crabs. As lower water content is more likely to happen in patches exposed to sun, males in the sun may not be able to consume the food even if it was available. Other studies have found that the water content of the sediment, rather than of the food content, is the major determinant of the foraging sites selected by fiddler crabs (Reinsel and Rittschof 1995).

Fiddler crabs have indeterminate growth, and feeding allows males to reach larger sizes more quickly. Larger males are preferred by females (Callander et al. 2012; Reaney and Backwell 2007) and more likely to defeat a challenger in a fight (Morrell et al. 2005). Males in the sun, on the other hand, spent more time at their burrow entrances, underground, and interacting with heterospecific fiddler crabs. The amount of time that males in the sun allocated to different behaviors suggests that they experience greater costs of living. For example, males holding territories in the sunny area may be more vulnerable to avian predators because they are more exposed; standing at their burrow entrance allows them to rapidly enter their burrow in the event of a predator attack (PRYB, unpublished data). Likewise, allocating time toward interactions with heterospecifics has previously been shown to be costly (e.g., wasting energy on directing courtship to a heterospecific female (Sanches et al. 2018). Despite differences in the time allocated to the above behaviors, there was no difference in the amount of time that males spent waving in the two microhabitats. This could simply be because the number of mate-searching females in the sun and shade did not differ, given that male fiddler crabs usually start waving when they spot a mate-searching female (Milner et al. 2010b).

Mating occurred almost four times as often in the shade than in the sun. Given that only a quarter of the study site is covered by shade, mating occurrences were very highly skewed towards shaded areas. This could result from three mechanisms: (1) Males in the sun are less likely to observe a mate-searching female and display the courtship to females because they need to spend more time underground

thermoregulating. (2) Females choose to mate with larger males who hold territories in the shade as they have mating preferences for larger males (Callander et al. 2012; Reaney and Backwell 2007). (3) Females mate randomly with respect to male microhabitat, but there are more males in the shade. As there are more males per unit area in the shade, and when this difference in density is taken into account, the proportion of mating occurring in the shade is almost identical to the proportion of males that lived in the shade (79% of mating occurred in the shade; 78% of males lived in the shade), it seems unlikely that females specifically select to mate with males in the shade, but rather they are more likely to mate in the shade because there are simply more males in the shade.

We expected that females would prefer males in the shade since they assess the burrow temperature when choosing a mate (Reaney and Backwell 2007); in addition, they are susceptible to predation, dehydration, and heat stress when they wander through the population searching for a mate (Koga et al. 1998; Perez et al. 2016), and all three of these risks are more likely to be greater in the open (sunny) areas. However, contrary to this expectation, females had no preference for identical robotic male crabs that courted in the sun or shade, when the searching female herself was either cool (shaded) or hot (in the sun). These results suggest that the thermal condition (nor any other factor associated with being shaded) of the male territory was not considered in the first stage of mate choice (i.e., when assessing wave performance). These results could also arise if the studied population faces relatively low predation (e.g., lower than species studied in the Americas: Koga et al. 1998; Perez et al. 2016) and if temperature-related risks that mate-searching females face (e.g., dehydration and heat stress) can be reduced by more frequent burrow visits (Bourdiol et al. 2018).

Males that held territories in the sun and shade differed in their waving behavior. Males in the sun produced waves with shorter durations (both single and double waves), faster rates (double wave), and higher height (double wave). In this species, double waves serve as a broadcast signal that stimulates receivers at long distances while single waves aim to signal receivers at short distance (DMP and PRYB, unpublished data). When a female is at a short distance, a male gives proportionally more single than double waves. However, double waves are still signaled to guarantee subsequent female approaches. Females do not have a preference for either waveform (Perez and Backwell 2017) or wave duration (DMP and PRYB, unpublished data). However, females have a very strong preference for males that wave higher (i.e., the height that the claw reaches during the peak of each wave) (DMP and PRYB, unpublished data) and at faster rates (Callander et al. 2012). One reason why males in the sun produced faster and higher double waves could be due to the effect of higher temperatures on their performance ability. The other reason could be that they are compensating for limited mating opportunities. The opportunity for sun-exposed males to notice mate-searching females is relatively low since males in the sun spend less time being active on the surface (Fig. 4) and more time underground (Table 2). Thus, sun-exposed males may allocate more effort to courtship (producing faster and higher double waves) once he saw a mate-searching female, but at the same time increasing the signal to attract distant females.

Given the advantages of living in shaded areas, we investigated the potential for competition between males to live in this microhabitat and found that there were more males in the shade than in the sun and that males occupying territories in the shade were also larger in size. Because there is a strong fighting advantage for larger males in this species (Morrell et al. 2005), the distribution patterns suggest that shaded areas are the preferred microhabitat, and that large males are more successful in competing with rival males over access to burrow ownership in these areas. It is possible to experimentally test if males prefer a shaded territory by releasing them in an arena with shaded and unshaded burrows and observing the burrow selection. In this study, we were unable to find direct evidence of whether there was space available for more crabs to move into the shaded areas; however, we expect that it was less likely that new residents could establish territories in the shade. The distance between two burrows that neighbor each other increases with the size of the residents (Clark and Backwell 2017). We found that the average distance between neighbors in the shaded areas was 8 cm. This was close to the typical size-neighbor distances previously found in this species (Clark and Backwell 2017; see also supplementary data). The high density of large male residents in the shade would make it difficult for a "new" male to fit between two existing territories without being evicted by one of his neighbors.

Climate warming can cause shifts in species ranges, or even local extinction if a species fails to access habitat suitable for its thermal niche. In this study, we provide specific mechanisms that directly explain temperature-induced changes in animal behavior, and the potential fitness costs underlying different habitat usage. Animals can buffer thermal stress through behavioral flexibility (Huey and Tewksbury 2009; Chapperon and Seuront 2011); however, thermoregulation behaviors can also incur costs (e.g., retreating into a burrow decreases food intake). This highlights the importance of shade availability, as a thermal refuge, in the context of climate warming. Although many studies have predicted detrimental effects of global warming on the loss of ectotherms (e.g., Sinervo et al. 2010; Kingsolver et al. 2013; Paaijmans et al. 2013), the impacts may be less dire if land managers protect and increase shade areas. We also advocate for more studies that examine the impact of shade loss given the critical importance of shade for ectotherm thermoregulation (Kearney et al. 2009; Kearney 2013). Approximately 7400 ha of mangroves in the Northern Territory of Australia suffered a

dieback in late 2015 potentially as a consequence of low sea levels, elevated salinity, low rainfall, and above average temperatures (Harris et al. 2017). Since changes in climate influence interactions between species and their habitats, it is necessary to incorporate information about these interactions if we plan to develop effective conservation management policies.

Data accessibility Data are available on Dryad Digital Repository.

Compliance with ethical standards

Ethical approval This research was approved by the Australian National University Animal Ethics Committee (permit A2015/54). We limited the handling and the amount of time each crab was used as much as possible. No crab was injured during the research, and they all continued their regular activities after release. The work was conducted under a research permit from the Darwin City Council (permit no. 3648724).

Conflict of interest The authors declare that they have no conflicts of interest.

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