Vol. 610: 99–108, 2019
https://doi.org/10.3354/meps12848

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published February 1

Too hot for sex: mating behaviour and fitness in the intertidal barnacle *Fistulobalanus albicostatus* under extreme heat stress

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ABSTRACT: Extreme weather events are predicted to increase under climate change, with serious consequences for ecosystems and populations. The effect of these events on mating and fitness are largely unknown. We examined the effect of heat stress on mating activity and reproductive success of the barnacle *Fistulobalanus albicostatus*. In aquarium tidal tanks, barnacles were subjected to low tide body temperatures (heat stress treatment, 39°C and extreme heat stress treatment, 45°C) based on field data from temperature biomimetic loggers, including on days of extreme heat. Barnacles were filmed post heat stress during high tide and their larvae collected. Barnacles exposed to the heat stress treatment rarely attempted to mate (<1% of individuals displayed mating activity) compared to controls (nearly 30% of barnacles mated). Barnacles exposed to heat stress still had lowered mating activity after 1 wk of non-stressful conditions. Heat-stressed barnacles almost never released larvae. In the extreme heat stress treatment, barnacles had additional negative impacts, decreasing mating activity by nearly 1 order of magnitude over a period of 3 wk. In conclusion, increases in heat stress under climate change have the potential to restrict mating with potential consequences for fitness. Our results provide evidence that extreme heat events can disproportionately affect mating behaviour.

KEY WORDS: Mating · Reproduction · Heat stress · Extreme weather

1. INTRODUCTION

Climate change is currently having, and will continue to have, a profound impact on patterns of animal distribution, behaviour and species interactions. Under climate change, shifts in air and water temperatures are confidently predicted to occur, along with increases in the frequency of extreme weather events (Coumou & Rahmstorf 2012, IPCC 2014). Many studies on ecological and biological responses to climate change have focussed on changes in distribution and adult mortality (Walther et al. 2002, Helmuth et al. 2006, IPCC 2014). Behavioural changes are, however, predicted to be the first place where the effects of human-induced environmental change, such as changes in temperature, will be seen (Tuomainen & Candolin 2011, Sih 2013). One significant way in which behaviour may change is an alteration in mating activity: its timing, frequency or location. Quantifying changes in reproductive or mating behaviour is especially important, as these changes are likely to have direct fitness consequences (Sinervo et al. 2010, Kingsolver & Buckley 2017).

Extreme temperature events have a disproportionate effect relative to their duration or frequency on animal populations and ecosystems (Jørgensen et al. 2006, Jentsch et al. 2007, Moreno & Møller 2011, Marrot et al. 2017). For example, the number of blue tit *Cyanistes caeruleus* fledglings in a brood is negatively correlated with the number of extremely hot days but not with the mean temperature (Marrot et al. 2017). Temperature determines when, where, how

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often and for how long animals mate, and the success of this mating (Rutowski et al. 1994, Hoffmann et al. 2003, Wilson 2005, Jiao et al. 2009, Zhang et al. 2016). Differences in mating behaviour may occur because animals are avoiding times or locations to limit heat stress (Sinervo et al. 2010, Munguia et al. 2017), are restricted by energetic constraints and reduced metabolic scope (Kordas et al. 2011, Sokolova et al. 2012), or are attempting to maximise conditions for subsequent offspring (Platt et al. 2003, Edwards & Richardson 2004). For some species, climate change may reduce mating opportunities by increasing periods where conditions are too stressful, whereas for other species, energetic restrictions may be removed and therefore mating could increase. The breeding season length and frequency of breeding in the three-spined stickleback *Gasterosteus aculeatus* has increased with higher winter temperatures, likely as a result of increased opportunities and individual energy reserves (Hovel et al. 2017). Additionally, as many habitats are already thermally heterogeneous at spatial scales relevant to organisms (Huey 1991, Raffaelli & Hawkins 1996, Helmuth & Hofmann 2001, Chapperon & Seuront 2011), certain habitats and microhabitats may become increasingly unsuitable for mating. Climate change has already started to alter reproduction across many taxa, including marine invertebrates, amphibians, mammals and birds, especially its timing (Walther et al. 2002, Parmesan & Yohe 2003, Tuomainen & Candolin 2011). For example, thick-billed murres (Brünnich's guillemots) *Uria lomvia* have been shown to be reproducing earlier in the year than previously recorded (Gaston et al. 2005). However, it is unknown how the relationship between temperature and mating is modulated under extreme temperature events (Parmesan 2006, Jentsch et al. 2007, Moreno & Møller 2011, Marrot et al. 2017).

Barnacles are an ideal study organism to investigate mating behaviour, as mating can easily be observed, realistic sample sizes can be achieved and because their sessile nature allows physiological and behavioural responses to heat stress to be disentangled from escape behaviour. Intertidal barnacles (Crustacea: Cirripedia) are mostly hermaphrodites with internal fertilisation taking place in the mantle cavity (Barnes et al. 1977, Barnes 1980, Charnov 1987, Murata et al. 2001). During mating, male-behaving individuals extend their penis out of their test and search for potential mates (Fig. 1A,B, and see the video in the Supplement at www.int-res.com/articles/suppl/m610p099_supp/). In tropical and subtropical shores, intertidal barnacles experience severe stress during summer low spring tides, and their body temperature can reach above 45°C during stressful periods (Chan et al. 2006, 2016). In barnacles, temperature affects gonad development (Page 1983, Berger 2009, Satheesh & Wesley 2009, Inatsuchi et al. 2010), brooding (Cimberg 1981) and reproductive output post-fertilisation (Kasten & Flores 2013, Freuchet et al. 2015). For example, variations in temperature have been linked to decreases in larval release rate (Kasten & Flores 2013, Freuchet et al. 2015) and frequency (Franco et al. 2015), and decreased quantity and quality of larvae (Freuchet et al. 2015).

We investigated the effect of both regularly experienced routine and extreme heat stress on mating activity in the outcrossing hermaphroditic mangrove barnacle, *Fistulobalanus albicostatus*, found in the mid- to high intertidal shore on mangrove trunks and branches, and rocky habitats in estuaries (Iwaki 1981, Anderson 1994, Chan & Leung 2007, C. Fraser pers. obs.). Specifically, we tested the hypothesis that when exposed to different levels of heat stress during high tide, the proportion of barnacles displaying mating activity will be lower compared to non-stressed barnacles, and fewer larvae will be produced (as a measure of fitness). In intertidal organisms, increases in air temperatures and solar radiation are often more important determinates of distribution and behaviour than water temperature (Helmuth & Hofmann 2001, Marshall et al. 2010, Huang et al. 2015), and therefore we focussed on changing environmental conditions at low tide, not high tide. In addition, we predicted that extreme heat stress will have a disproportionately negative effect on mating activity compared to routine heat stress that barnacles experience more regularly. If changes in mating activity are related to barnacles avoiding stressful conditions, rather than energetic constraints, then we predict that mating activity will recover after a period of less-stressful conditions.

2. MATERIALS AND METHODS

2.1. Study species

Fistulobalanus is commonly distributed across shores in the Indo-West Pacific, including Japan, Taiwan and southern China (Chan et al. 2009, Chang et al. 2017). Mating peaks from early summer to early autumn (May to October), with individuals producing around 20 broods yr^{-1} in Japan (Iwaki 1981); therefore, any changes in temperature at this time of year have the potential to alter reproductive output.

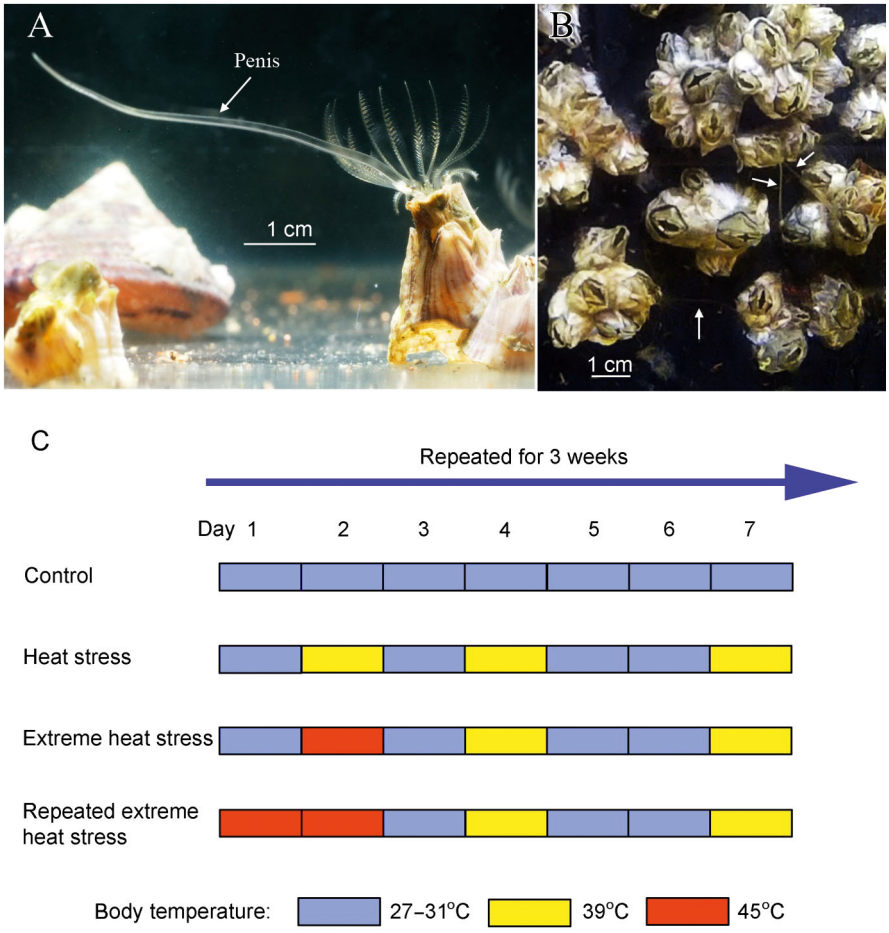

Fig. 1. (A) *Amphibalanus amphitrite* extending a long penis to search for 'female' barnacles during mating. (B) Screen shot from a video (see the Supplement) used to analyse reproductive behaviour. White arrows indicate extended penises. (C) Maximum body temperatures of barnacles (°C) among treatments during 'daytime' emersion each day over a period of 3 wk. Sampling to compare the effect of acute heat stress occurred after Day 2 emersion

2.2. Experimental setup

F. albicostatus were collected from the intertidal trunks of mangrove trees in Wa-Zhi-Wei in northern Taiwan (25° 9′ N, 121° 25′ E) and transported to the laboratory, where they were immersed in seawater tanks. In order to keep their bases intact, barnacles were collected with a thin layer of tree bark still attached to their base. Barnacles were lightly cleaned by flushing with seawater to remove mud and excessive bark, and separated into small clumps (1–15 mature barnacles clump^{-1}). Using black, neutral-pH silicone sealant (San Li Shih 2022), barnacle clumps were then attached to ceramic square tiles (147 mm^2), with clumps separated by 10–15 mm and each tile containing between 40 and 110 (Experiment [Expt] 1) or between 81 and 218 (Expt 2) individual barnacles. Each tile, with barnacles attached, was placed in an individual flow-through plastic tank (310 × 240 mm) on a semi-diurnal tidal cycle (Fig. 2). The flow-through tidal system was composed of a plastic experimental tank and sink tank placed below the experimental tank. Eight 5 mm diameter holes were drilled into the tank 45 mm above its base. These holes were connected to the sink tank via plastic tubing. A 15 mm diameter hole was cut at the base of the plastic tanks, in which a plastic tube was connected from this hole to an aquarium pump that pumped water from the sink to the experimental tank. Each tile represents an individual replicate for each treatment in the experiments described below. During simulated high tides, which lasted 6 h, the pump operated and barnacles were covered by 35–45 mm of natural seawater, and during low tide, also lasting 6 h, barnacle tiles were exposed to air. Water was changed twice a week, and barnacles were fed

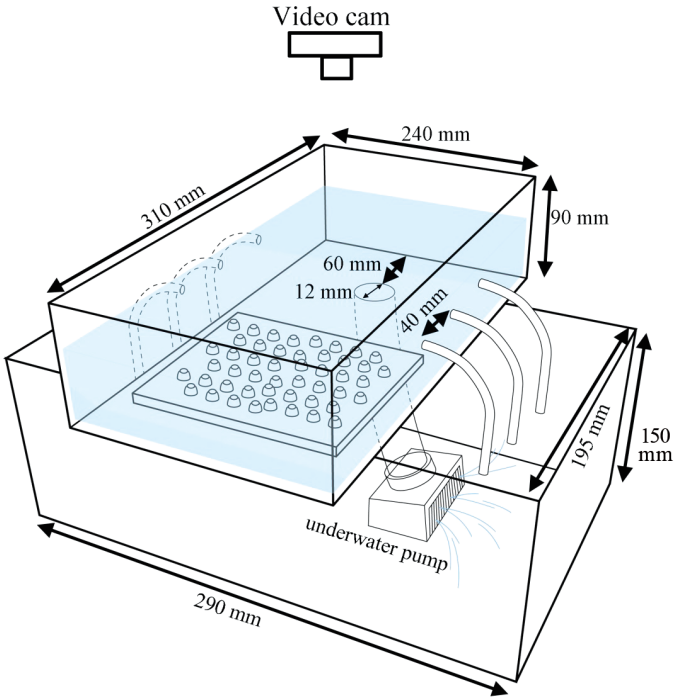

Fig. 2. Diagrammatic representation of the tidal tank system for mating experiments

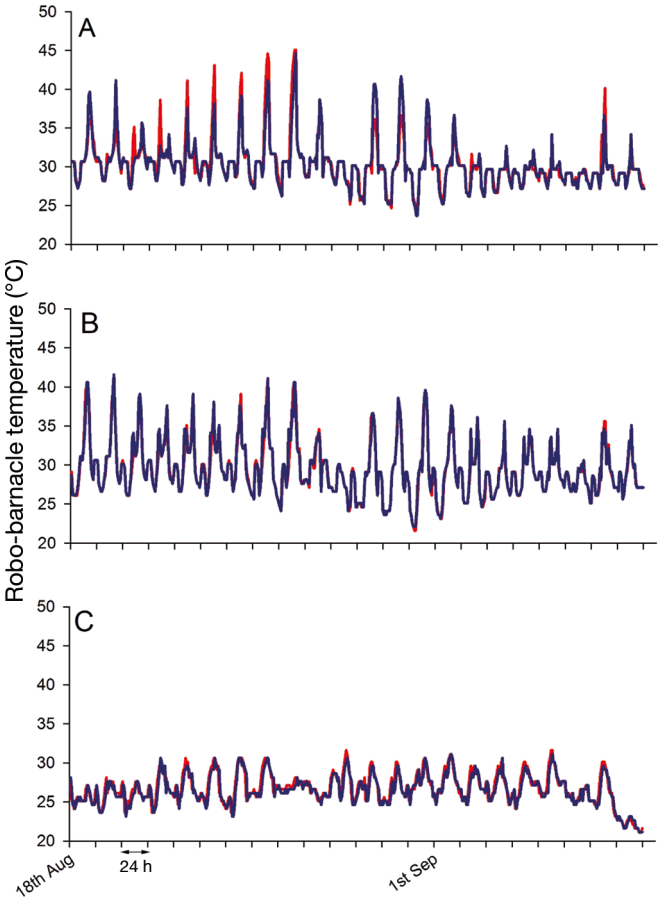

Fig. 3. Hourly robo-barnacle temperature data recorded over 3 wk. Two robo-barnacles (red and blue) were located in each microhabitat type: (A) exposed boulders, (B) sun-exposed mangrove tree trunks and (C) shaded mangrove tree trunks

daily with live *Artemia salina*. All applicable institutional and/or national guidelines for the care and use of animals were followed.

2.3. Baseline barnacle body temperatures during summer in the field

To assess the natural heat stress of *Fistulobalanus* in the field, temperature loggers encased in barnacle shells ('robo-barnacles'; following Chan et al. 2016) were attached to intertidal tree trunks (including east facing, sun-exposed; and west-facing, shaded habitat) and boulders (open habitat) in Wa-Zhi-Wei in northern Taiwan, to record the simulated hourly body temperature of barnacles over a period of 3 wk, from August to September 2016 (21 d). Although the robo-barnacles were made from the shell of a larger species (*Tetraclita* sp.), pilot studies showed that they recorded body temperatures similar to live, smaller-sized *Fistulobalanus* in the field and laboratory.

The maximum air temperature during August was in the top 6% of annual maximal temperatures over the past 70 yr (Taiwan Central Weather Bureau, www.cwb.gov.tw) and therefore represented routine and extreme heat events. During the deployment period, the temperature of the robo-barnacles increased from high tide to low tide, when the robo-barnacles were exposed (Fig. 3). Robo-barnacles on exposed boulders were recorded reaching a daily maximal temperature of 40°C on between 6 and 8 d, with the highest daily maximum temperature recorded being >47°C (Figs. 3 & 4C, Table 1). The average maximum gap between days of heat stress was 7 d (Table 1). On exposed boulders, on days where robo-barnacles reached above 40°C, temperatures were above 40°C for on average 180 min during the daytime low tide (Fig. 4C). Robo-barnacles attached to sun-exposed mangrove tree trunks reached 40°C on 1 to 4 d (Figs. 3 & 4B). Robo-barnacles located on shaded mangrove tree trunks generally recorded lower maximal daily temperatures of approximately 30°C when compared to robo-barnacles in other habitats (Figs. 3 & 4A).

Table 1. Temperatures recorded by biomimetic loggers ('robo-barnacles') in different micro-habitats over a period of 3 wk (21 d) in summer; na: not applicable

Habitat	Max. temperature (°C)	No. of days >40°C	No. of days >45°C	Mean maximum no. of days between days of heat stress (>40°C)
Mangrove trunk in shade	35.1	0	0	na
Mangrove trunk facing sun	42.1	1–4	0	7
Open boulders	47.1	6–8	1–4	7

2.4. Expt 1: Effect of heat stress on mating activity

Barnacles were exposed over 3 wk during simulated daytime low tide to 4 temperature treatments including no heat stress (control), routine heat stress, extreme heat stress and repeated extreme heat stress (Fig. 1C) (4 tiles per temperature treatment). Experimental temperatures in treatments were based upon temperatures recorded by temperature biomimetic loggers (robo-barnacles) in the field at different intertidal positions (see Section 2.3 and Table 1). In control treatments, barnacles were exposed to no heat stress (average maximum body temperature of barnacles = 27°C). In routine heat stress treatments, representing the stress level experienced by barnacles found on sun-exposed mangrove trunks, the average maximum body temperature of barnacles varied between 39°C on heat-stress low tides and 27°C on low-stress tides. Barnacle temperatures were held above 35°C on heat-stress low tides for a similar length of time as in the field (lab ~3 h, field ~3 h). In the extreme heat stress treatment, representing barnacles on boulders during a heat stress event, barnacles in experienced 1 tide of extreme heat (temperatures >40°C for over 2 h with a maximum body temperature of 45°C) and then followed the temperature pattern of the heat stress treatment. During the tide in which barnacles were exposed to extreme heat stress, barnacle body temperatures were held above 40°C for a similar length of time as in the field (lab ~2.5 h, field ~3 h). The repeated extreme heat stress treatment was identical to the extreme heat stress treatment, but with 2 consecutive days where during daytime low tide, barnacles were exposed to extreme temperatures. Temperatures slightly above 45°C (46–48°C) caused mass mortality in laboratory settings (78% dead after 1 d, C. Fraser unpubl. data). The maximum temperature was there-

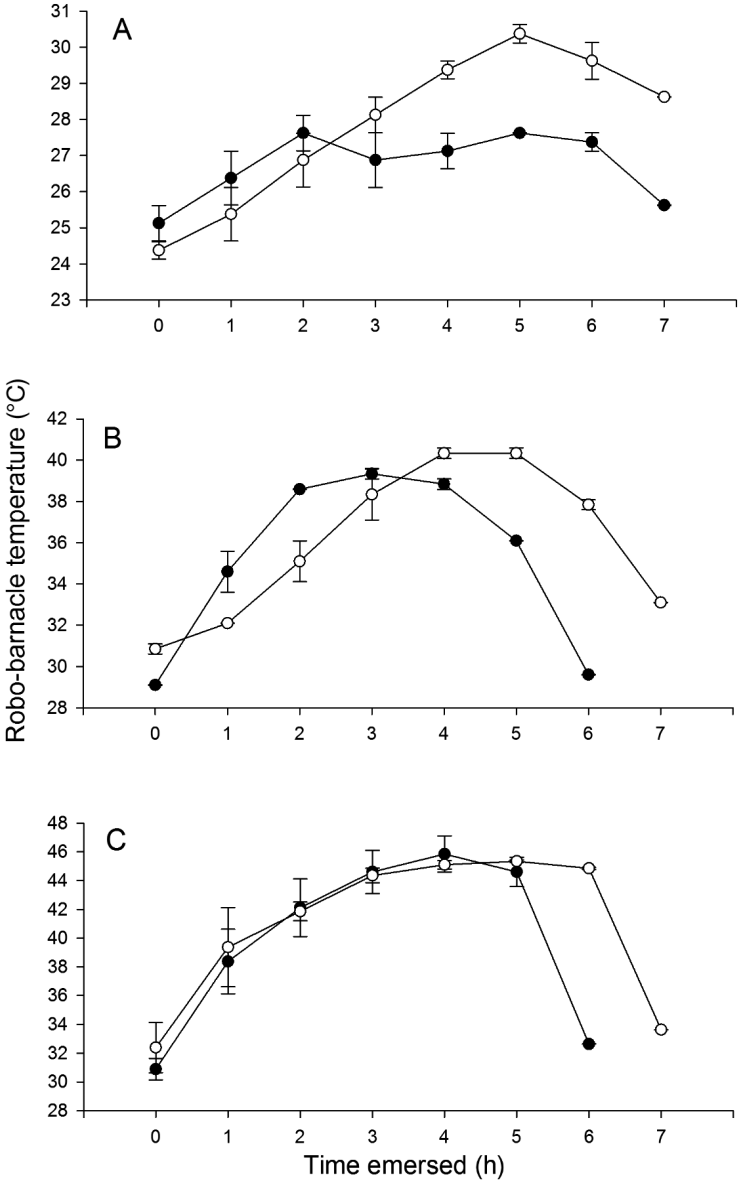

Fig. 4. Mean hourly temperature recorded by biomimetic loggers ('robo-barnacles'; n = 2) in the field during daytime low tide on 2 representative days located in each microhabitat type: (A) exposed boulders, (B) sun-exposed mangrove tree trunks and (C) shaded mangrove tree trunks. Black and white circles represent 2 individual robo-barnacles

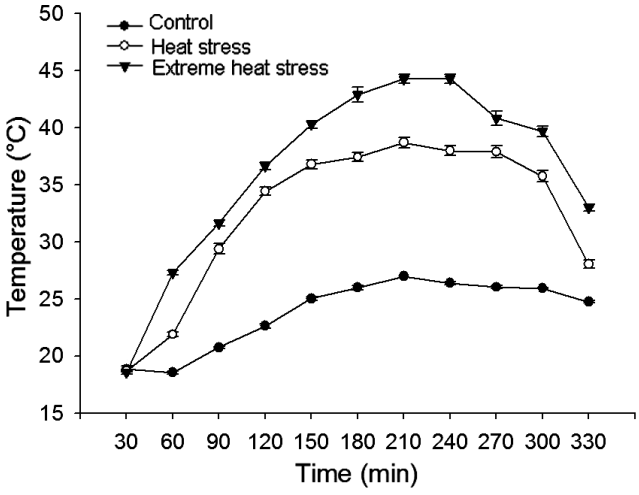

Fig. 5. Body temperature of barnacles (mean ± SE) among 3 treatments: control (black circles), heat stress (white circles) and extreme heat stress (black triangles)

fore kept at 45°C, despite higher temperatures being reached in the field (on 1–4 d). With a maximum temperature of 45°C, low levels of mortality were observed. Temperatures were varied daily on a weekly cycle.

Tiles and barnacles in all treatments were heated gradually using individual halogen lights (300 W) hung 30 cm above each tile (Williams et al. 2011, Han et al. 2013). The strength of the halogen lights was adjusted using a rheostat. In a pilot study to confirm that experimental temperatures reflected those of field based robo-barnacles, barnacle body temperature was measured using a thermocouple inserted through the operculum and into the barnacle (Fig. 5).

During high tide, tiles were videoed using a video camera (Patriot Sports Camera K600 1080p); the number of barnacles that displayed mating activity was counted for each tile and the total proportion calculated. During every high tide for 3 wk, barnacles were filmed for 3 h, and from within this footage, 2 blocks of 35 min were randomly selected for mating behavioural analysis using JWatcher (Blumstein et al. 2000). Mating activity occurred if an individual's penis was visible and actively searched for a mate or was inserted into another barnacle (Fig. 1B). To assess the acute effect of heat stress on mating activity, the proportion of barnacles that showed mating activity during immersion immediately after exposure to heat stress (27 vs. 39 vs. 45°C, see Fig. 1C) was compared among treatments. The data were analysed using a 2-way ANOVA (treatment: 4 levels, number of weeks exposed: 2 levels, $\alpha = 0.05$; n = 2). The assumption of homoscedasticity was tested using Cochran's *C*-test and the statistical analysis was done in WinGmav 5 (EICC, University of Sydney). Tukey's HSD test was used to compare means post hoc where necessary. Data from different tiles were used for the different weeks. The proportion of barnacles that engaged in mating activity at least once over the 3 wk sampling period was also compared amongst treatments, and data were analysed using a separate 1-way ANOVA (n = 4).

Barnacle nauplii larvae released from the barnacles on tiles were collected twice a week. Larvae collection started 12 d after the experiment began, to ensure that any larvae collected were the result of mating during the experimental period (Iwaki 1981), and continued for 14 d including after heat stress treatments concluded, as thermal stress can affect larval release (Kasten & Flores 2013)

2.5. Expt 2: Effect of recovery period on mating activity

Barnacle-covered tiles were exposed to different temperatures (no heat stress, routine heat stress or extreme heat stress treatment for 1 or 2 d, 3 tiles per treatment) to test the hypothesis that the proportion of heat-exposed barnacles displaying mating activity after 1 wk (average gap between heat stress in the field calculated from robo-barnacles, Table 1) of non-exposure will not differ from control treatments. Seven days after heat exposure, the total proportion of barnacles displaying mating activity was calculated based on video analysis. The hypothesis was tested using an asymmetrical 2-factor ANOVA of a single control (no stress treatment) with orthogonal comparisons (factors: temperature, number of days exposed to heat stress, Table 2, $\alpha = 0.05$; Underwood 1997) This analysis was built from ANOVA outputs using WinGmav 5, and final calculations were completed by hand.

Table 2. Asymmetrical ANOVA testing differences in the mean proportion of barnacles displaying mating activity among tiles exposed to differing levels of heat stress and for different lengths of time (1 low tide vs. 2 low tides). There were no significant levels of heteroscedasticity ($C = 0.41$)

Source	df	MS	*F*	p
All treatments	4	0.11	2.78	0.087
Control vs. heat stress	1	0.42	10.61	0.009
Heat stress level (HS)	1	0.11	2.84	0.123
Days exposed to heat stress (D)	1	0.02	0.49	0.498
HS × D	1	<0.01	<0.01	0.961
Residual	10	0.04		

3. RESULTS

3.1. Expt 1: Effect of heat stress on mating activity

Barnacles exposed to heat stress during low tide rarely attempted to mate during the subsequent high tide, with fewer than 1% of individuals seen with a visible and active penis extending out of their tests. The proportion of barnacles that displayed mating activity on control tiles was significantly greater than the proportion found in treatments exposed to heat stress ($F_{3,8}$ = 54.10, p < 0.001, Fig. 6A); however, extreme or repeated heat stress had no additional negative effect. A second week of exposure to heat stress also had no effect, as there was no significant increase or decrease in mating activity compared with the first week ($F_{1,8}$ = 4.69, p > 0.0.62). Measured over the the entire length of the experiment, the proportion of barnacles that exhibited mating decreased with increasing thermal stress ($F_{3,12}$ = 38.9, p < 0.001, Fig. 6B).

Heat-stressed barnacles almost never released larvae during the experimental period. On average, we found 0–1 larva over the entire period in treatments where barnacles were exposed to heat. In contrast, a large number of larvae (on average >100) were collected from the control tanks (Fig. 7).

3.2. Expt 2: Effect of recovery period on mating activity

After 1 wk of recovery time, barnacles exposed to any level of heat stress displayed reduced levels of mating activity compared to those that were not exposed ($F_{1,10}$ = 10.61, p < 0.05, Fig. 8, Table 2). Neither the number of days nor the level of heat stress to which barnacles were exposed had any significant effect (number of days: $F_{1,10}$ = 0.49, p = 0.498; heat stress level: $F_{1,10}$ = 2.84, p = 0.123).

4. DISCUSSION

High body temperatures during low tide led to a lower proportion of barnacles displaying mating activity and a reduction in larvae produced, supporting our predictions. In contrast to previous studies, we found no impact of extending the duration, or repeating, heat stress on barnacles that were exposed to heat stress (Schulte et al. 2011, Kingsolver & Woods 2016, Drake et al. 2017). Although extreme heat stress did not have an immediate additional effect, over a period of weeks this added stress led to

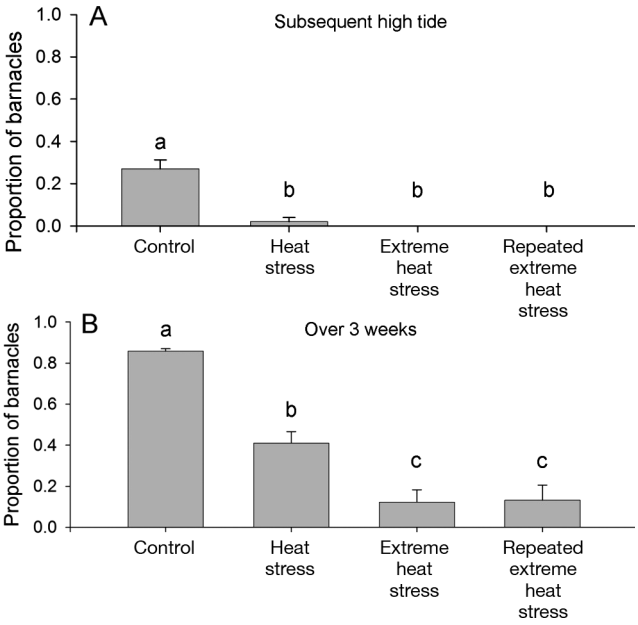

Fig. 6. Comparison of the mean (+SE) proportion of barnacles displaying mating activity (A) immediately after thermal stress and (B) at least once over the 3 wk period. Lowercase letters denote significant differences in mean proportions (ANOVA and Tukey's HSD post hoc tests, p < 0.05)

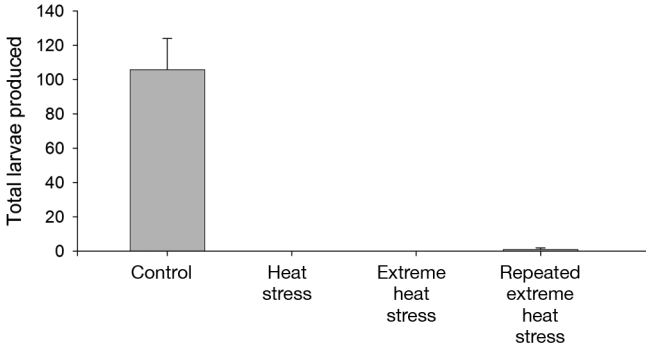

Fig. 7. Comparison of total larvae produced (+SE) among treatments

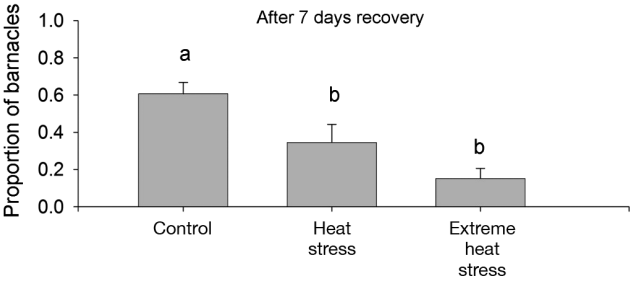

Fig. 8. Comparison of the mean (+SE) proportion of barnacles displaying mating activity after 7 d of recovery in no-stress conditions. Lowercase letters denote significant differences in mean proportions (ANOVA, p < 0.05)

a smaller proportion of barnacles engaging in reproductive behaviour. Whilst 9 d of heat stress across a 3 wk period halved mating activity, 3 d of extreme heat stress within that same period led to a decrease of nearly an order of magnitude. This effect is disproportionate to its duration, and highlights why increases in extreme weather events should be of concern when assessing potential climate change impacts. Determining whether increased extreme thermal events alter survival and/or reproduction has been identified as an important research area (Jørgensen et al. 2006, Kingsolver & Buckley 2017). Our findings address this need, and demonstrate that extreme events have the potential to significantly and disproportionately alter mating behaviour.

Shifts in behaviour under climate change have been linked to increased or decreased energetic constraints (Kordas et al. 2011, Sokolova et al. 2012), or to organisms shifting activities such as mating or foraging when conditions are less stressful (Kearney et al. 2009, Sinervo et al. 2010, Munguia et al. 2017). Organisms also employ physiological strategies to reduce the negative impacts of heat stress (Sokolova & Portner 2003, Sinclair et al. 2006, Sokolova et al. 2012). Many taxa, for example, produce heat shock proteins (HSPs) when under thermal stress (Sørensen et al. 2003, Tomanek 2010, Sokolova et al. 2012). HSP production has energetic costs (Somero 2002, Sørensen et al. 2003, Sokolova et al. 2012, Kingsolver & Woods 2016), increasing the ATP demands on cells, and can depress reproductive output (Silbermann & Tatar 2000). For instance, female *Drosophila* flies with greater HSP expression levels produced fewer offspring and had lower fecundity (Krebs & Loeschcke 1994, Silbermann & Tatar 2000). Increased temperature leads to increased metabolism, which can alter resource allocation (Gillooly et al. 2001, Brown et al. 2004). For example, at high temperatures barnacle growth is limited by higher metabolic costs (Nishizaki & Carrington 2015). Increased metabolism may also explain the observed shifts in mating behaviour (Page 1983, Brown et al. 2004, Berger 2009). That the effects of heat stress on mating activity persist long after the stress event has occurred indicates that the immediate decrease in mating activity is more likely a consequence of energetic constraints rather than barnacles avoiding the stressful conditions. Decreased mating activity during heat stress periods may also come from damage to reproductive cells from the high temperatures (Chakir et al. 2002, Alavi & Cosson 2005).

After a week of low-stress conditions, barnacle mating behaviour only partially returned to pre-stress levels. The ability, and speed, to recover from stress determines an organism's ability to survive and reproduce. Whilst some physiological parameters, such as heart rate, can return quickly to pre-stress levels (Santini et al. 2002, Morritt et al. 2007, Huang et al. 2015), the consequences for organisms in terms of growth and reproduction can persist (Baird & Marshall 2002, Somero 2002, Jørgensen et al. 2006, Rodriguez-Troncoso et al. 2016). For example, in flies, the proportion of males mating 7 d after heat stress decreased when exposed to higher temperatures, and time to recovery increased with test temperature (Jørgensen et al. 2006). A reduction in the time between heat stress events, especially extreme heat stress, may mean that organisms will have insufficient recovery time, and complete reproductive failure for that season/year may occur. Based on the frequency of heat stress events in the field (see Table 1 and Fig. 3) and the negative effect these events are predicted to have on mating, many barnacles would not have enough time to recover between events to successfully mate.

Animals in different microhabitats experience different levels of heat stress (Huey 1991, Raffaelli & Hawkins 1996, Angilletta et al. 2002). The potential for heat stress and extreme heat stress to seriously dampen mating and offspring production, as seen in our study, suggests that only individuals in certain microhabitats (for our species: mangroves; for rocky shore taxa: crevices; for estuarine crabs: shaded mangroves) where conditions are milder, can contribute offspring to future generations. This means that, even if climate change predictions indicate that a species can persist in a hotter microhabitat, those individuals may not necessarily help maintain a viable population as they are incapable of mating.

Acknowledgements. Thanks to assistants/students at the Biodiversity Research Center, Academia Sinica, and to Casey Lien for assistance with diagram creation. C.M.L.F. was funded by a post-doctoral fellowship from Academia Sinica. B.K.K.C. is supported by a grant from Ministry of Science and Technology (MOST), Taiwan (103-2621-B-001-005-MY3)

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Editorial responsibility: Steven Morgan, Bodega Bay, California, USA

Submitted: June 8, 2018; Accepted: December 8, 2018
Proofs received from author(s): January 29, 2019