



## OPEN Thermal acclimatisation to heatwave conditions is rapid but sex-specific in wild zebra finches

Anaïs Pessato<sup>1</sup>, Eve Udino<sup>1</sup>, Andrew E. McKechnie<sup>2,3</sup>, Andrew T. D. Bennett<sup>1,4</sup> & Mylene M. Mariette<sup>1,5</sup>

Under climate change, increasing air temperature average and variability pose substantial thermal challenges to animals. While plasticity in thermoregulatory traits could potentially attenuate this impact, whether thermal acclimatisation can occur quickly enough to track weather variability in hot climates is unknown in any endotherm, and sex differences have never been tested. We investigated acclimatisation responsiveness of male and female wild zebra finches to short-term (< 2 weeks) summer temperature fluctuations in the Australian desert. Hotter weather before respirometry trials triggered a typical acclimatisation response (especially at chamber temperature  $T_{\text{chamb}} \geq 40$ ). However, acclimatisation occurred remarkably rapidly: metabolic rate responded within just one day, while body temperature ( $T_b$ ) and evaporative cooling capacity (EHL/MHP) were best predicted by weather on the trial day; whereas evaporative water loss responded more slowly (1 week). Nonetheless, rapid acclimatisation only occurred in males, and females had higher  $T_b$  and lower EHL/MHP than males, potentially increasing hyperthermia risk. Furthermore, acclimatisation did not translate into greater acute heat tolerance (i.e. ability to tolerate  $T_{\text{chamb}} = 46$  °C). Our results therefore reveal surprisingly rapid acclimatisation and even anticipatory adjustments to heat. However, with no changes in acute heat tolerance, and in females, phenotypic flexibility may provide only limited buffering against the detrimental impact of heatwaves.

As a consequence of anthropogenic climate change, global surface temperature and the frequency of heatwaves are increasing dramatically<sup>1</sup>. In many regions, these changes have already resulted in loss of biodiversity<sup>2</sup>. Mass die-offs during heatwaves have been documented across diverse taxa<sup>3–5</sup>, presumably because conditions exceeded individuals' thermoregulatory limits. Indeed, based on species' physiological limits, widespread and severe population declines are predicted under future climates, through effects on survival and reproduction<sup>6–8</sup>.

Nonetheless, phenotypic plasticity or “phenotypic flexibility” (*sensu*<sup>9</sup>) in traits related to heat tolerance—whereby thermoregulatory performance is reversibly adjusted to prevailing conditions through acclimatisation—may have the potential to partly buffer the negative impacts of climate change<sup>10,11</sup>. If so, incorporating phenotypic flexibility in physiological traits into predictive models of species distributions may yield more accurate predictions of species vulnerability to climate change<sup>12,13</sup>. Crucially, however, such beneficial effects of physiological flexibility rely on thermal physiology tracking weather variability over time. While phenotypic flexibility can lessen the physiological cost of seasonal climate variation, such responses may become maladaptive when the weather varies faster than a species' acclimatisation capacities<sup>14</sup>. By contrast, if acclimatisation responses are rapid, organisms may be able to anticipate and prepare for upcoming weather by adjusting their physiology to recent or current conditions.

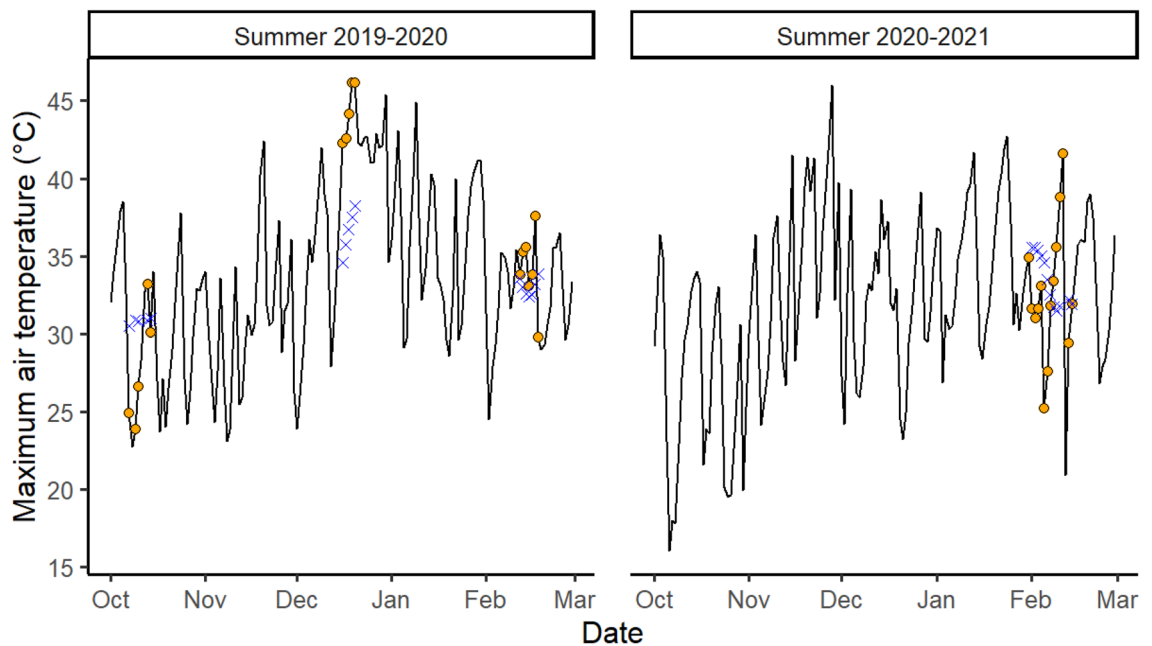
Acclimatisation to weather conditions, or acclimation to experimentally-manipulated thermal environments, are well-studied physiological responses occurring in many endotherms<sup>13,15,16</sup>. However, it is generally assumed

<sup>1</sup>Centre for Integrative Ecology, School of Life and Environmental Sciences, Deakin University, Geelong 3216, VIC, Australia. <sup>2</sup>South African Research Chair in Conservation Physiology, South African National Biodiversity Institute, Pretoria 0001, South Africa. <sup>3</sup>DSI-NRF Centre of Excellence at the FitzPatrick Institute, Department of Zoology and Entomology, University of Pretoria, Pretoria 0001, South Africa. <sup>4</sup>One Health Research Group, Melbourne Veterinary School, Faculty of Science, University of Melbourne, Werribee, VIC 3030, Australia. <sup>5</sup>Doñana Biological Station EBD-CSIC, 41092 Seville, Spain. email: m.mariette@deakin.edu.au; mylene.mariette@ebd.csic.es

that acclimation takes at least 2 weeks to arise, and experiments therefore typically use acclimation periods of 2–4 weeks, without investigating the effects of acclimation period duration<sup>15,17</sup>. Only a handful of studies have measured the time-course of acclimation or acclimatisation among endotherms. To the best of our knowledge, only two studies (5 species in total) have measured avian acclimation or acclimatisation over <7 days<sup>18,19</sup>. These authors found that, with the notable exception of the American tree sparrow (*Spizella arborea*; tested in both studies), acclimation to constant temperature in captivity does not occur within 8 days, and the weather in the past 14–30 days better explains variations in winter resting metabolic rate in free-living birds than weather on shorter timescales<sup>18,19</sup>. In rodents nonetheless, while acclimation took 2–5 weeks under constant temperature to reach a maximal level, all three species showed noticeable response within the first week of temperature change<sup>20–22</sup>. Overall, the evidence for acclimation timing is therefore very scarce and species-dependent. In addition, all avian or mammalian studies to date have exclusively focussed on responses to cold or mild conditions. With no data on the rates of thermoregulatory adjustments to hot conditions (maximum temperature tested = 30 °C), the functional significance of acclimatisation in alleviating or worsening the impact of increasing heatwaves on populations remains unclear. Furthermore, unlike for thermoregulation efficiency, acclimatisation of heat-tolerance limit in birds has received little attention<sup>23,24</sup>. Nonetheless, in white-browed sparrow-weavers (*Plocepasser mahali*), heat tolerance, measured as the air temperature at which severe hyperthermia was reached (i.e. body temperature  $\geq 44.5$  °C), was higher in summer than winter in one arid population (but not in two mesic populations)<sup>23</sup>, and in individuals acclimatised for 30 days to 42 °C compared to those kept at 30 °C or 36 °C<sup>24</sup>. Both studies together suggest that acclimatisation of heat tolerance may occur under very high temperatures, but this remains to be tested in other systems.

Hot deserts at subtropical latitudes are some of the most rapidly warming regions on the planet<sup>25</sup>, and are characterised by highly variable summer air temperatures and unpredictable precipitation<sup>26,27</sup>. Species inhabiting hot arid habitats are thus thought to be highly vulnerable to climate change, but they may also potentially be more physiologically plastic, if more variable climate (e.g. at higher latitudes) selects for greater flexibility. Whether this ‘climatic variability hypothesis’<sup>23,28</sup> (but see<sup>29</sup>) extends to more rapid acclimatisation to short-term weather variability has not been tested. Here, we investigated the timing of acclimatisation to summer weather conditions in free-living individuals of a desert specialist, the Australian zebra finch (*Taeniopygia castanotis*). We quantified rates of acclimatisation in both males and females, as thermoregulation capacities in the heat may differ between the sexes<sup>30</sup>, and females have been reported to have higher  $T_b$  than males in wild-derived captive zebra finches<sup>30</sup> and several other avian species<sup>31</sup>. In contrast to cold or thermoneutral conditions<sup>31,32</sup>, sexual dimorphism in thermoregulatory performance in the heat has rarely been tested, with studies finding mixed results<sup>24,30,33,34</sup>. Yet, evaluating sex differences in thermoregulation is essential, given the negative impact of extreme heat on reproduction<sup>35</sup> and the consequences this may have on population persistence under climate change<sup>36,37</sup>.

We used an open flow-through respirometry system to measure metabolic rate (MR), evaporative water loss (EWL) and body temperature ( $T_b$ ) of individuals exposed to air temperatures ( $T_{a\text{-chamb}}$ ) ramping gradually from 31 °C up to 46 °C. To characterize the timing of acclimatisation to natural weather fluctuations (Fig. 1), we considered the maximum air temperature ( $T_a$ ) at different time scales (i.e. on the day of the experiment ( $T_{0\text{day}}$ ),



**Figure 1.** Daily maximum air temperature (black line) during summers 2019–2020 and 2020–2021 on the days of respirometry measurements ( $T_{0\text{day}}$ ; orange points), and averaged over the 2 weeks prior ( $T_{-2\text{weeks}}$ ; blue crosses), recorded at Leigh Creek Airport. No field trip could occur during Oct 2020–Jan 2021 because of Covid-19-related border closures.

the day before ( $T_{-1\text{day}}$ ), or over the preceding 3 days ( $T_{-3\text{days}}$ ), 1 week ( $T_{-1\text{week}}$ ) and 2 weeks ( $T_{-2\text{weeks}}$ ), and then tested which time interval best explained the observed variation in thermoregulatory values. Similarly to changes observed in summer-acclimatised or heat-acclimated individuals across avian species<sup>15,23,38–40</sup>, we predicted that exposure to heatwave conditions before measurement would be associated with lower MR and higher evaporative cooling capacity (EHL/MHP), whereas EWL may be lower (to conserve water). We also expected these changes to be associated with lower  $T_b$  and greater acute heat tolerance after hot weather. In addition, we predicted that zebra finches, as desert specialists, may acclimatise rapidly in response to changing weather conditions, except, potentially, when temperature deviations from the day before are too large (measured as the difference in temperatures between  $T_{0\text{day}}$  and  $T_{-1\text{day}}$  ( $\Delta T_{0-1}$ ), or  $T_{-1\text{day}}$  and  $T_{-2\text{days}}$  ( $\Delta T_{1-2}$ )). Lastly, we predicted that free-ranging females may have higher  $T_b$  than males<sup>31</sup>, and that the sexes may differ in evaporative cooling capacity<sup>30</sup>, and, potentially, the timing of acclimatisation.

## Results

### Rapid acclimatisation in thermoregulatory traits

All thermoregulatory traits responded to weather conditions (measured as air temperature) prior to respirometry trials, at least at high chamber temperatures (i.e.  $T_{a\text{-chamb}} \geq 40$ ; above the upper critical limit of thermoneutrality  $T_{uc}$ ), as well as below the  $T_{uc}$  for body temperature  $T_b$  (i.e. null/base models [without weather predictors] not retained in best model sets, Table 1A). As expected, after hot weather, metabolic rate (MR) and  $T_b$  were significantly lower, and evaporative cooling capacity (EHL/MHP) significantly higher (CIs excluded zero, Table 1B, Fig. 2). These changes occurred surprisingly rapidly, with the air temperature the day preceding the trial ( $T_{-1\text{day}}$ ) explaining thermoregulatory values better (i.e.  $T_{-1\text{day}}$  in best model sets) than longer-term weather predictors for all traits except EWL (Table 1A). Notably,  $T_{-1\text{day}}$  was 2.3 to 9.8 AICc units lower than the typical acclimatisation period of 2 weeks. Remarkably, even the maximum air temperature on the day of measurement ( $T_{0\text{day}}$ ) influenced  $T_b$  and EHL/MHP (Table 1, Fig. 2), indicating that individuals were prepared for upcoming conditions, probably by responding to morning temperatures at time of capture (Pearson correlation between  $T_{0\text{day}}$  (i.e. maximum temperature) and the morning temperature at time of capture:  $r = 0.98$   $p < 0.001$ ). For all three traits, adding the amplitude of temperature deviation from the day before ( $\Delta T$ ) did not improve models, suggesting that large sudden weather changes did not impede rapid acclimatisation. These effects of  $T_{-1\text{day}}$  and  $T_{0\text{day}}$  were significant at  $T_{a\text{-chamb}}$  both below and above the  $T_{uc}$ , although weaker in the former (base/null model retained  $< T_{uc}$  for MR and EHL/MHP; Table 1). By contrast to other traits, evaporative water loss adjusted more slowly: EWL at moderate  $T_{a\text{-chamb}}$  ( $< T_{uc}$ ) did not vary with weather, and EWL at high  $T_{a\text{-chamb}}$  ( $\geq 40$  °C) only adjusted within a week, decreasing following a hot week (Table 1, Fig. 2). When removing seasonal variation by restricting analyses to February trips, results were qualitatively unchanged, with short- to medium-term weather ( $T_{-1\text{day}}$ ,  $T_{0\text{day}}$ ,  $T_{-1\text{week}}$ ) explaining significant variations in MR, EHL/MHP and EWL (respectively) at high  $T_{a\text{-chamb}}$ , whereas longer-term weather ( $T_{-2\text{week}}$ ) did not. Effects for  $T_b$  were however no longer detected, possibly because the sample size was reduced by half (Table S4).

### Sexual dimorphism in thermoregulatory capacities

The timing of acclimatisation differed between the sexes. At high  $T_{a\text{-chamb}}$  ( $> T_{uc}$ ), only males adjusted all thermoregulatory traits to short-term weather. Males' evaporative cooling capacity,  $T_b$  and MR showed the same patterns as in the pooled dataset, responding to  $T_{0\text{day}}$  and  $T_{-1\text{day}}$  (although, for EHL/MHP, the base model was included in the best model set:  $\Delta\text{AICc}$  with  $T_{0\text{day}} = 1.5$ , Table S7A). By contrast, females' evaporative cooling capacity (EHL/MHP) and  $T_b$  did not adjust to weather, and their MR response was more ambiguous and potentially slower than males' ( $T_{-1\text{day}}$ ,  $T_{-3\text{days}}$ , and  $T_{-1\text{week}}$  all significant, Table S7). Furthermore, EWL in males responded to weather 1–3 days before measurements at high  $T_{a\text{-chamb}}$ , whereas there were no significant weather predictors for EWL in females. At  $T_{a\text{-chamb}} < T_{uc}$ , sex differences were less consistent and weaker (null model included in best model sets, except for females'  $T_b$ ): only males adjusted EHL/MHP (as for  $> T_{uc}$ ), but only females adjusted  $T_b$  and MR to recent weather. EWL below the  $T_{uc}$  was not significantly affected by weather in either sex, similar to the pooled dataset (Table S7).

Consistent with these sex differences in acclimatisation timing, females had overall higher  $T_b$  than males, both below and above the  $T_{uc}$  (i.e. sex significant in the pooled dataset: Table 1B, Fig. S2), and lower EWL and EHL/MHP than males at  $T_{a\text{-chamb}} > T_{uc}$  (Table 1, Fig. 3). By contrast, MR did not differ between the sexes (Table 1, Fig. 3). Taken together, these results reveal that females had lower and less flexible heat dissipation capacity than males at high air temperature, associated with a higher  $T_b$  at high but also mild  $T_{a\text{-chamb}}$ .

### No acclimatisation or sex differences in acute heat tolerance

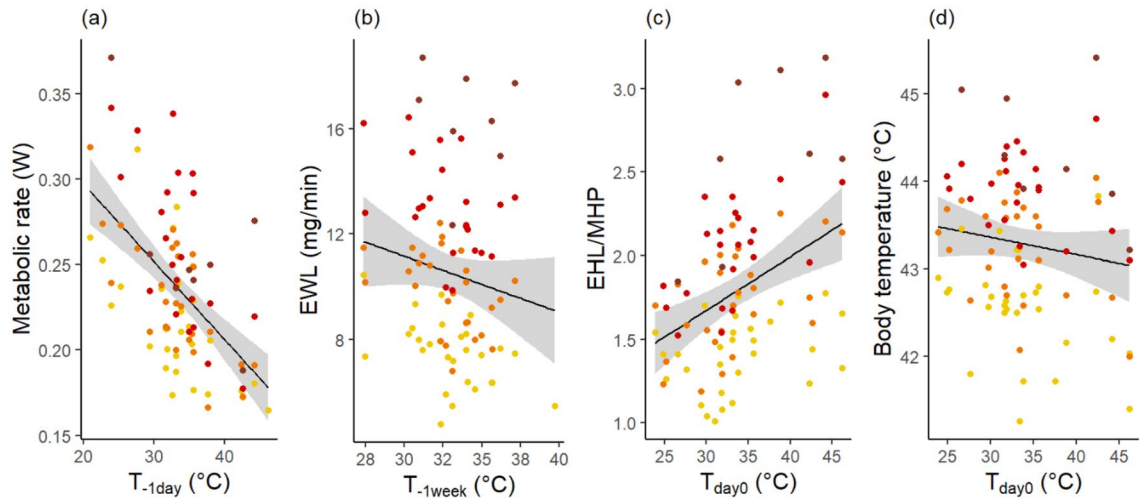
In spite of thermoregulatory traits rapidly adjusting to weather variations (i.e. air temperatures), no clear benefits for acute heat tolerance were found. Indeed, none of the weather predictors had a significant effect on either the probability of reaching  $T_{a\text{-chamb}} = 46$  °C or trial completion probability (15 min at  $T_{a\text{-chamb}} = 46$  °C), although for both proxies, some weather predictors explained some variation (i.e. retained in overall dataset model set: Table 2, and in February dataset: Table S5). The weakness of weather effects may be partly related to the fact that, in contrast to results for thermoregulatory traits, sudden changes from previous conditions ( $\Delta T$ ) appeared to have some influence on heat tolerance (i.e. retained in the top model set, Table 2A), albeit not significantly (Table 2B). In agreement, heat tolerance proxies did not improve after heatwaves in either sex, when the sexes were considered separately (Table S8). Finally, males and females achieved similar heat tolerance thresholds (i.e. sex not retained in top models for either heat tolerance proxy, Table 2A), even though their evaporative cooling capacity differed.

Metabolic rate	$T_{a-chamb} < T_{uc}$					$T_{a-chamb} > T_{uc}$				
(A) Model	df	LL	AICc	$\Delta AICc$	w	df	LL	AICc	$\Delta AICc$	w
Null	3	103.7	-201.0	1.1	0.268					
+ $T_{-1day}$	4	105.4	-202.1	0	0.470	5	179.3	-347.9	0	0.839
Metabolic rate	$T_{a-chamb} < T_{uc}$					$T_{a-chamb} > T_{uc}$				
(B) Predictors	Est	SE	CI			Est	SE	CI		
Intercept	0.218	0.007	<b>0.21; 0.23</b>			0.237	0.005	<b>0.23; 0.25</b>		
$T_{a-chamb}$						0.042	0.005	<b>0.03; 0.05</b>		
$T_{-1day}$	-0.043	0.012	<b>-0.07; -0.02</b>			-0.053	0.009	<b>-0.07; -0.03</b>		
Evaporative water loss	$T_{a-chamb} < T_{uc}$					$T_{a-chamb} > T_{uc}$				
(A) Model	df	LL	AICc	$\Delta AICc$	w	df	LL	AICc	$\Delta AICc$	w
Base	4	-79.2	167.3	0	0.280					
+ $T_{-1week} + sex$						7	-131.8	279.0	0	0.373
Evaporative water loss	$T_{a-chamb} < T_{uc}$					$T_{a-chamb} > T_{uc}$				
(B) Predictors	Est	SE	CI			Est	SE	CI		
Intercept	3.465	0.151	<b>3.17; 3.76</b>			10.567	0.197	<b>10.20; 10.94</b>		
Mass	0.788	0.305	<b>0.19; 1.38</b>			1.491	0.378	<b>0.78; 2.20</b>		
$T_{a-chamb}$						5.624	0.199	<b>5.22; 6.01</b>		
$T_{-1week}$						-1.049	0.389	<b>-1.78; -0.32</b>		
Sex						0.892	0.395	<b>0.15; 1.64</b>		
Evaporative cooling capacity	$T_{a-chamb} < T_{uc}$					$T_{a-chamb} > T_{uc}$				
(A) Model	df	LL	AICc	$\Delta AICc$	w	df	LL	AICc	$\Delta AICc$	w
Null	3	20.1	-33.8	0	0.409					
+ $T_{-1day}$	4	20.7	-32.6	1.2	0.223	5	2.0	6.8	1.0	0.153
+ $T_{0day}$	4	20.3	-31.9	2	0.152	5	2.4	5.9	0	0.247
+ $T_{0day} + sex$						6	3.4	6.3	0.5	0.195
+ $T_{-1day} + sex$						6	3.2	6.6	0.7	0.171
Evaporative cooling capacity	$T_{a-chamb} < T_{uc}$					$T_{a-chamb} > T_{uc}$				
(B) Predictors	Est	SE	CI			Est	SE	CI		
Intercept	0.642	0.021	<b>0.60; 0.68</b>			1.781	0.044	<b>1.69; 1.87</b>		
$T_{a-chamb}$						0.657	0.038	<b>0.58; 0.73</b>		
$T_{0day}$	0.094	0.042	<b>0.01; 0.18</b>			0.278	0.087	<b>0.11; 0.45</b>		
$T_{-1day}$	0.100	0.042	<b>0.02; 0.18</b>			0.263	0.085	<b>0.09; 0.43</b>		
sex						0.199	0.084	<b>0.03; 0.37</b>		
Body temperature	$T_{a-chamb} < T_{uc}$					$T_{a-chamb} > T_{uc}$				
(A) Model	df	LL	AICc	$\Delta AICc$	w	df	LL	AICc	$\Delta AICc$	w
+ $T_{0day} + sex$	6	-43.4	100.4	0	0.583	6	-36.2	85.4	0	0.225
+ Sex						5	-37.4	85.6	0.2	0.208
+ $T_{-1day} + sex$						6	-36.4	85.8	0.4	0.188
Body temperature	$T_{a-chamb} < T_{uc}$					$T_{a-chamb} > T_{uc}$				
(B) Predictors	Est	SE	CI			Est	SE	CI		
Intercept	41.380	0.103	<b>41.18; 41.58</b>			43.305	0.086	<b>43.13; 43.48</b>		
$T_{a-chamb}$	0.052	0.016	<b>0.02; 0.08</b>			1.329	0.055	<b>1.22; 1.44</b>		
$T_{0day}$	-0.623	0.205	<b>-1.01; -0.23</b>			-0.351	0.168	<b>-0.69; -0.02</b>		
$T_{-1day}$						-0.328	0.164	<b>-0.65; -0.01</b>		
Sex	-0.688	0.208	<b>-1.09; -0.29</b>			-0.521	0.173	<b>-0.86; -0.18</b>		

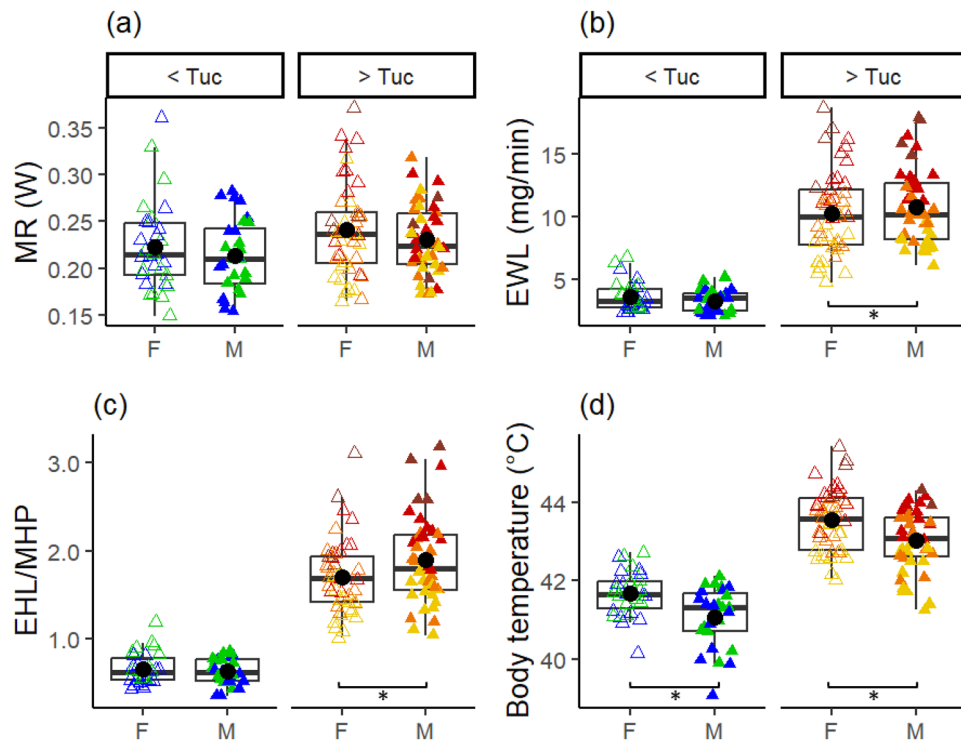
**Table 1.** (A) Top model set ( $\Delta AICc \leq 2$ ), and (B) model-averaged estimates of predictors included in the top models, for metabolic rate, evaporative water loss, evaporative cooling capacity and body temperature, below and above  $T_{uc}$  on the full dataset ( $n = 29$  birds). A predictor has a significant effect (bold) if CI excludes 0. Female is the reference group for sex. *df*: degree of freedom, *LL*: log-likelihood,  $\Delta AICc$ : difference in AICc scores between the best model and the model being compared, *w*: model weight, *Est*: parameter estimate, *SE*: standard error, *CI*: 95% confidence interval.

## Discussion

Our study demonstrates that zebra finches adjust thermoregulatory performance in response to prevailing weather, but sexes differ in such phenotypic flexibility. Remarkably, adjustments in metabolic rate, evaporative



**Figure 2.** Effects of weather predictors on thermoregulation at  $T_{a-chamb} \geq T_{uc}$  ( $38\text{ }^{\circ}\text{C}$ ). Effects of (a) the maximum air temperature the day before measurement ( $T_{-1day}$ ) on metabolic rate; (b) the average maximum air temperature 1 week prior to measurement ( $T_{-1week}$ ) on evaporative water loss (EWL); and (c,d) the maximum air temperature on the day of measurement ( $T_{day0}$ ) on (c) EHL/MHP and (d) body temperature. Colours corresponds to  $T_{a-chamb}$  (yellow =  $40\text{ }^{\circ}\text{C}$ , orange =  $42\text{ }^{\circ}\text{C}$ , red =  $44\text{ }^{\circ}\text{C}$  and brick =  $46\text{ }^{\circ}\text{C}$ ). Regression lines and confidence intervals (grey area) display significant effects from model-averaging. Excluding the point at  $T_{-1week} = 39.7\text{ }^{\circ}\text{C}$  for EWL did not affect significance.



**Figure 3.** Sex differences in (a) metabolic rate (MR), (b) evaporative water loss (EWL), (c) evaporative cooling capacity (EHL/MHP) and (d) body temperature, at mild (left) and high (right) chamber temperatures, (i.e.  $T_{a-chamb}$  below or above  $T_{uc} = 38\text{ }^{\circ}\text{C}$  respectively). Black dots show the mean in females (open triangles) and males (filled triangles). Colours corresponds to  $T_{a-chamb}$  (blue =  $30\text{ }^{\circ}\text{C}$ , green =  $35\text{ }^{\circ}\text{C}$ , yellow =  $40\text{ }^{\circ}\text{C}$ , orange =  $42\text{ }^{\circ}\text{C}$ , red =  $44\text{ }^{\circ}\text{C}$  and brick =  $46\text{ }^{\circ}\text{C}$ ). \* indicates a significant sex effect (i.e. CI excludes zero). Body temperature as a function of  $T_{a-chamb}$  is shown in Fig. S2.



Models	$T_{a-max} = 46\text{ }^{\circ}\text{C}$ (yes/no)					Trial completion				
	df	LL	AICc	$\Delta\text{AICc}$	w	df	LL	AICc	$\Delta\text{AICc}$	w
(A)										
Null	1	-20.2	42.5	0.5	0.152	1	-20.2	42.5	1.3	0.072
Base	2	-18.8	42.0	0	0.191	2	-18.6	41.7	0.5	0.105
+ $\Delta T_{0-1} + T_{0day}$	4	-16.7	43.0	1.0	0.115					
+ $T_{-2weeks}$	3	-18.4	43.7	1.7	0.081					
+ $T_{0day}$						3	-17.1	41.2	0	0.138
+ $T_{-1day}$						3	-17.2	41.4	0.2	0.125
+ $T_{-1week}$						3	-17.3	41.4	0.2	0.123
+ $\Delta T_{1-2} + T_{-1day}$						4	-16.4	42.4	1.2	0.075
+ $T_{-3days}$						3	-17.8	42.4	1.3	0.073
Predictors	$T_{a-max} = 46\text{ }^{\circ}\text{C}$ (yes/no)					Trial completion				
	Est	SE	CI			Est	SE	CI		
(B)										
Intercept	0.660	0.405	-0.17; 1.49			-0.758	0.446	-1.67; 0.15		
Capt. time	1.587	0.963	-0.38; 3.55			1.764	1.002	-0.28; 3.81		
$T_{0day}$	0.189	0.915	-1.69; 2.07			1.505	0.915	-0.28; 0.81		
$\Delta T_{0-1}$	-2.039	1.248	-4.60; 0.52							
$T_{-1day}$						1.457	1.046	-0.68; 3.60		
$\Delta T_{1-2}$						1.887	1.606	-1.41; 5.18		
$T_{-3days}$						1.195	0.945	-0.74; 3.13		
$T_{-1week}$						1.624	1.062	-0.55; 3.80		
$T_{-2weeks}$	-0.702	0.836	-2.41; 1.01							

**Table 2.** (A) Top model set ( $\Delta\text{AICc} \leq 2$  from best model), and (B) model-averaged estimates of predictors included in the top models for  $T_{a-max}$  ( $46\text{ }^{\circ}\text{C}$  or less) and trial completion (i.e. stayed 15 min at  $T_{a-chamb} = 46\text{ }^{\circ}\text{C}$ ). A predictor has a significant effect (bold) if CI excludes 0. Abbreviations as in Table 1.

cooling capacity and body temperature occurred very rapidly—within a day—to summer air temperature fluctuations, whereas evaporative water loss changed within 1 week (Fig. 2). Interestingly, only males rapidly adjusted all four thermoregulatory traits at high  $T_{a-chamb}$ , with MR, EWL and  $T_b$  decreasing and EHP/MHP increasing, following or during a hot day. Accordingly, males had lower  $T_b$  at all  $T_{a-chamb}$  and higher EHL/MHP at high  $T_{a-chamb}$  than females, although their acute heat tolerance remained similar to that of females. Overall, our study reveals very rapid sex-specific acclimatisation to heatwave conditions in a desert passerine, but without changes to acute heat tolerance. These findings highlight the need to thoroughly evaluate acclimatisation capacities across species, so the role of phenotypic plasticity in responding to climate change can be elucidated.

Metabolic rate, body temperature and evaporative cooling capacity adjusted surprisingly rapidly to weather conditions on the day before, or even on the day of measurements. Importantly, these changes were not driven by longer-term or seasonal effects, since weather in the preceding 2 weeks had no effect, and results were maintained when only February data were considered. Our results are contradictory to the notion of acclimatisation occurring over time scales of weeks in endotherms, and, at first sight, appear to contrast with previous findings on rodents demonstrating that under constant  $T_a$ , complete acclimation takes several weeks to establish<sup>20–22</sup>. However, in nature, environmental temperatures are very rarely constant, as they usually vary both within and between days. Whether the maximum acclimation levels measured in the lab are actually attainable under natural fluctuating conditions is therefore questionable. Remarkably however, at least in wild zebra finches, the magnitude of the changes we found is similar to those typically observed after 3–4 weeks of acclimation<sup>39,40</sup>. Indeed, in our study, MR decreased by 39%, and EHL/MHP increased by 57%, over  $T_{-1day}$  values increasing from 21 to  $46\text{ }^{\circ}\text{C}$  (Fig. 3). Such decrease in MR ( $1.5\% \text{ MR} \cdot ^{\circ}\text{C}^{-1}$ ) is similar to that measured after 2–4 weeks of acclimation in captive zebra finches<sup>39,41</sup>, or other wild-caught passerines<sup>17,40</sup>. Interestingly, the rapid thermoregulatory adjustments we document were not negatively impacted by sudden changes in thermal conditions ( $\Delta T$ ). Taken together, our findings support our hypothesis that the capacity for rapid physiological acclimatisation may have adaptive value in some habitats, such as arid unpredictable habitats (as in the Australian desert: Fig. 1)<sup>26,27</sup>. Further studies on a range of species and environments are nonetheless needed to determine whether acclimatisation is more rapid among species inhabiting desert environments, and to measure the fitness impact of such acclimatisation. Nevertheless, rapid acclimation may be more widespread than currently acknowledged, given that, in the temperate zone, American tree sparrows also acclimate very rapidly<sup>18,19</sup>, and that in great tits (*Parus major*) short-term (i.e. past week) weather explained variation in MR whereas that experienced previously (i.e. in the fortnight from week 2 to 4 before testing) did not<sup>42</sup>.

We also found that EWL responded more slowly than MR at high  $T_{a-chamb}$  and phenotypic flexibility in EWL was not evident at  $T_{a-chamb} < T_a$ . This is consistent with the literature, where the direction of change in EWL with acclimation temperature is less consistent across studies than for MR, and effects can differ below and above the  $T_{uc}$ <sup>23,38,40</sup>. This might be because of the underlying mechanisms allowing MR versus EWL flexibility (e.g. changes

in muscle versus skin ultrastructure<sup>43–45</sup>) and/or because cutaneous (principal component of  $E_{WL} < T_{uc}$ ) and respiratory  $E_{WL}$  (occurring mostly  $> T_{uc}$ ) may respond differently to acclimation<sup>39</sup>. Furthermore, other factors such as developmental plasticity, have been shown to contribute to variation in  $E_{WL}$ , including at adulthood in the zebra finch<sup>30</sup>. Lastly, beyond temperature,  $E_{WL}$  may acclimate to humidity, as for example, in house sparrow (*Passer domesticus indicus*) fledglings acclimated to dry conditions<sup>46</sup> or nestlings acclimated to desert environments<sup>45</sup>. More studies are clearly needed on the drivers and time course of  $E_{WL}$  acclimation, including in a range of habitats where selection strength by lethal dehydration may differ.

Despite efficiently adjusting their thermoregulation capacities to heatwave conditions, zebra finches' tolerance of acute heat exposure under experimental conditions did not improve. Instead, sudden weather changes might have disrupted acclimation of heat tolerance, although more data is needed to confirm this non-significant effect. These results are nonetheless consistent with the interpretation that temperatures at our study site may not have been high or stable enough for heat tolerance to improve, given white-browed sparrow-weavers increased heat-tolerance only at very high acclimation or acclimation temperatures<sup>23,24</sup>. Indeed, it is possible that flexibility of traits other than thermoregulation capacity that contributes to individual thermal limits (e.g. heat-shock protein regulation, mitochondrial efficiency<sup>47</sup>), may have restricted heat tolerance adjustments. In addition, we found that heat tolerance did not vary between the sexes, even though thermoregulation differed, as also found by<sup>33</sup>.

To our knowledge, our study provides the first evidence among endotherms for sex-specific thermal acclimation, with male zebra finches acclimating rapidly whereas females showed no response. This is particularly interesting considering that zebra finches have no sexual dimorphism in body-size or microsite use<sup>48</sup> and little behavioural differences<sup>49</sup>. By contrast, in ectotherms, a recent meta-analysis found that on average females show greater heat tolerance plasticity than males (but only among free-living animals<sup>50</sup>). In addition, we showed that female zebra finches have higher  $T_b$  and lower EHL/MHP than males, consistent with previous findings in captive wild-derived individuals<sup>30</sup> and, in the case of  $T_b$ , other avian species<sup>31,34</sup>. Most previous studies have found either no sex dimorphism in  $T_b$ <sup>24,33</sup> or higher  $T_b$  in females<sup>31,34</sup>, but there is to date no clear explanation for female higher  $T_b$ , or even for the presence or absence of sexual dimorphism in thermoregulatory traits across species. Nonetheless, implications of such sexes differences for acute heat tolerance are unclear, since we found no sexual dimorphism in that trait, and heat tolerance may not acclimate as readily as other thermoregulatory traits<sup>23,24</sup>. Overall, our study suggests that, in some species, females may be at higher risk of (chronic, if not lethal) hyperthermia during heatwaves than males. These results are particularly concerning for population growth rates under climate change if female heightened susceptibility reduces breeding opportunity and reproductive success<sup>36</sup>, given females are often not tested in physiological studies, or sex is not reported (e.g.<sup>19,39,41,51</sup>).

Finally, our thermoregulatory values for free-living zebra finches are comparable to those for domestic or wild-derived captive populations of this model species, measured with a similar methodology<sup>30,39</sup>. There were however some differences, although they may have been driven by differences in acclimation conditions from those studies (e.g. mild constant  $T_a$  in captivity vs high and fluctuating in the wild). Indeed, at high  $T_a$ , EHL/MHP was higher in wild individuals than individuals from captive populations (e.g. at  $T_{a-chamb} = 44$  °C: mean = 2.1 vs 1.3 respectively<sup>30</sup>), possibly because of lower  $E_{WL}$  in captive populations<sup>39,51</sup>. Accordingly, heat tolerance also appeared to be higher in wild zebra finches: 65% tolerated  $T_{a-chamb} = 44$  °C for the whole 20-min stage duration versus only 27% in captive birds acclimated to 25 °C<sup>30</sup>. However, consistent with published values of zebra finch thermal limits<sup>51</sup>, at  $T_{a-chamb} = 46$  °C, we had to terminate 45% of the trials on wild birds before 15 min (i.e. end of stage) to avoid the death of individuals showing severe signs of heat-stress. The heat tolerance limit of wild zebra finches thus may fall into the lower range of similarly-sized desert passerines<sup>52</sup>. However, EHL/MHP (mean = 2.6 at  $T_{a-chamb} = 46$  °C) is above values described so far for this order<sup>52,53</sup>. This greater evaporative cooling capacity is partly attributable to the zebra finch being a drinking species<sup>54</sup>, but also potentially to the use of vocal panting (increasing heat dissipation through evaporative water loss<sup>55</sup>) and programming by prenatal “heat-calls”<sup>56</sup>. Indeed, incubating zebra finch parents produce heat-calls at high temperatures through an extreme form of panting, or “vocal panting”, which adaptively programs offspring development and phenotype for heat (e.g. higher reproductive success and heat tolerance<sup>30,47,56</sup>).

## Conclusion

To the best of our knowledge, this is the first study investigating the time course of acclimation of traits related to thermoregulation in the heat and sex differences in thermal acclimation in endotherms. Against the generally accepted view, we found that acclimation to heat occurred remarkably quickly—allowing individuals to track daily weather fluctuations, at least in a species adapted to highly variable weather. Our findings therefore strengthen the argument for considering phenotypic plasticity in climate change models to achieve realistic predictions. Importantly however, such phenotypic flexibility may not lessen the impact of heatwaves on populations, since heat tolerance did not respond, and only one of the sexes acclimated. Our study highlights the need for further investigation of the acclimation capacities of species to heat, in order to understand the threat posed by climate change to biodiversity of endotherms.

## Materials and methods

### Study species and capture site

We examined heat tolerance and thermoregulation in free-living adult zebra finches ( $n = 31$ , 15 females and 16 males) during the austral summers of 2019–2020 and early 2021 (Fig. 1). To limit sources of inter-individual variation (and minimize impact on reproduction), we specifically targeted individuals moulting a few wing or tail feathers, since zebra finches tend to interrupt moulting during breeding<sup>57</sup>. The study took place at Wooltana station, South Australia (GPS: S 30.41324°, E 139.42035°) where daily maximum  $T_a$  ranged from 21 to 46 °C

during data collection (Fig. 1). Birds were caught between 7:00 and 11:30 am, which corresponded to 1–5 h after sunrise. Since sunrise time varied slightly between field trips, we standardised the capture time (hereafter, “capture time”) by expressing it as the number of hours since sunrise (i.e. time at capture [7:00–11:30 am] minus sunrise time [6:08–6:57 am]). Birds were trapped at two capture sites 4 km apart, using feeder walk-in traps (filled with seed mix for a few days every 1 to 6 months) or in proximity of the feeder using mist nets. Birds were transported to a field laboratory ( $\leq 12$  km away). We injected a temperature-sensitive passive integrated transponder (PIT) tag (Biomark, Boise, USA) subcutaneously into the bird’s flank. The PIT tags were covered by feathers and no noticeable moult in this area was observed. In small birds, including the zebra finch, subcutaneous PIT tags give similar values as those implanted intraperitoneally; subcutaneous PIT tags do not require surgery (superficial cut) and limit risk of fatal injuries<sup>58</sup>. The time since PIT tag injection also does not appear to affect measurement (Pessato and Mariette; unpublished data). We calibrated a subset of PIT tags in a water bath against a type-T thermocouple (BAT-12, Physitemp Instruments Inc., Clifton NJ, USA). Pit tags were accurate within  $0.31 \pm 0.06$  °C across water temperature ranging from 40 to 46 °C.

All procedures were approved by Deakin University Animal Ethics Committee (B18-2017) and performed in accordance with Australian guidelines and regulations for the use of animals in research. This study was conducted in compliance with the ARRIVE guidelines (<https://arriveguidelines.org>).

### Experimental heat challenge protocol

Before respirometry measurements, birds were held in a cage with no food but ad libitum water. Based on the predicted mean retention time for food in a digestive tract for a 12 g bird ( $\sim 50$  min<sup>59</sup>) and to reduce interindividual variation, we applied a fasting time of 2 h when birds had seeds in their crop at capture or 1 h if the crop was empty. Just before starting each trial, birds were offered water by depositing drops on their bill, and were then weighed (mass  $\pm 0.01$ , HT-120, A&D, Japan).

Heat exposure consisted of a stepped series of increasing air temperature ( $T_{a\text{-chamb}}$ ) in the metabolic chamber. The  $T_{a\text{-chamb}}$  was initially maintained at  $\sim 31$  °C for 45 min (within the zebra finches’ thermoneutral zone<sup>60</sup>); but below the average maximum  $T_a$  over the two summers  $\sim 32.8$  °C, Fig. 1), followed by 20-min stages at 35 °C, 40 °C, 42 °C, 44 °C and a 15-min stage at 46 °C. Trials were considered ‘complete’ when the individual remained in the chamber for 15 min at  $T_{a\text{-chamb}} = 46$  °C. The trial was stopped early if the bird showed loss of balance, an abrupt drop in the water and CO<sub>2</sub> traces, high body temperature ( $T_b > 45$  °C) or prolonged escape behaviour<sup>55,61</sup>. Following the heat-exposure, birds were allowed to recover at thermoneutrality ( $T_{a\text{-chamb}} = 35$  °C) for 10 min. Respirometry trials lasted on average 2h45 (range: 2h19–3h07). This stepped exposure protocol, involving brief periods at each  $T_{a\text{-chamb}}$  stage, yields similar results to exposure to each  $T_{a\text{-chamb}}$  for longer periods and is ethically (and practically) preferable<sup>62</sup>.

After measurements, birds were weighed, offered water and then transferred to a recovery cage for  $\sim 30$  min with ad libitum water and finch seed mix, before release at their capture site. None of the birds died during the trials.

### Respirometry measurements during experimental heat challenge

We used an open flow-through respirometry system described in details by Pessato et al.<sup>55</sup> to measure CO<sub>2</sub> production and EWL. Briefly, all birds were placed individually in a metabolic chamber made of transparent plastic (1.5 L, 8 × 18 × 11.5 cm), containing a thermocouple to measure  $T_{a\text{-chamb}}$ , a perch and a plastic mesh layer above a 5-mm layer of mineral oil. The chamber was placed into a dark temperature-controlled cabinet (Outermark, 99 × 51.5 × 48.5 cm) regulated by a temperature controller (ir33, Carel) connected to a thermocouple. We maintained very low humidity in the metabolic chamber (range: 0.1–0.9 kPa in excurrent air) by regulating incurrent airflow at flow rates of 2–3.5 L min<sup>-1</sup> (depending on  $T_{a\text{-chamb}}$ ) with a mass flow controller (Alicat scientific Inc., USA, calibrated and accurate within 0.008 L min<sup>-1</sup>). Baseline and excurrent chamber air were sequentially subsampled and pulled by a pump (SS4 subsampler, Sable Systems) at  $\sim 240$  mL min<sup>-1</sup> through the H<sub>2</sub>O analyser (RH-300, Sable Systems) and CO<sub>2</sub> analyser (CA-10, Sable Systems). Daily, the H<sub>2</sub>O analyser was zeroed using pure nitrogen (5.0, Coregas, Australia) and spanned with humidified air produced by a dew point generator (DG-4, Sable Systems). The CO<sub>2</sub> analyser was zeroed and calibrated every 3 days using pure nitrogen and certified gas with a known CO<sub>2</sub> concentration (1005 ppm, Coregas). Both analysers were connected to a computer interface (Expedata software and analog–digital converter UI2, Sable Systems).  $T_b$  was monitored every 10 s and recorded using a PIT tag reader (Biomark, Boise ID, USA); aberrant values ( $n = 3$  out of 27,498 readings) were discarded.

Throughout the experiment, bird activity (i.e. movement) was monitored every 30 s for 5-s scans using infrared video cameras (mini CCD camera with IR, Signet). We scored activity following<sup>30</sup> and restricted analyses to data to calm birds (i.e. sleeping, resting or stepping for small displacement) during measurement and in the 10 min prior.

### Respirometry data processing

In Expedata, for each  $T_{a\text{-chamb}}$  stage (31 °C, 35 °C, 40 °C, 42 °C, 44 °C, 46 °C), we selected the 1-min window with lowest and least variable CO<sub>2</sub> and H<sub>2</sub>O values, after at least 29 min at the initial  $T_{a\text{-chamb}}$  stage ( $T_{a\text{-chamb}} = 31$  °C), 9 min at  $T_{a\text{-chamb}} = 35$  °C, 40 °C and 42 °C, and 6 min at  $T_{a\text{-chamb}} = 44$  °C and 46 °C. We used the  $T_b$  in the 1-min window (accounting for 99% equilibrium time, ranging from 2 to 3.45 min depending on the flow rate<sup>63</sup>). We calculated (resting) metabolic rate (MR) and evaporative water loss (EWL) using equations 9.5 and 9.6 from<sup>64</sup>, and assuming a respiratory exchange ratio of 0.71 or 0.83, depending on crop content. After the trial, some birds ( $n = 5$ ) still had seeds in their crop; therefore we assumed a respiratory exchange ratio of 0.71 (oxyjoule equivalent = 27.8 J ml<sup>-1</sup> CO<sub>2</sub>) for birds with an empty crop at capture time ( $n = 8$ ), and of 0.83 (oxyjoule equivalent = 24.9 J ml<sup>-1</sup> CO<sub>2</sub>) for others (i.e. digesting seeds<sup>65,66</sup>). We also estimated evaporative cooling capacity, as



evaporative heat loss over metabolic heat production (EHL/MHP) as EHL/MR. EWL was converted to evaporative heat loss (EHL in W) assuming a latent heat of vaporisation of  $2.4 \text{ J mg}^{-1} \text{ H}_2\text{O}^{67}$ .

### Weather variables

We used weather data from Leigh Creek Airport meteorological station (station 017110, <http://www.bom.gov.au>) situated 95 km from Wooltana (the closest meteorological station to Wooltana (Arkaroola, 18 km away) had missing data; Pearson correlation between these stations:  $r = 0.91$ ,  $p < 0.001$ ). We used daily maximum  $T_a$  as we were interested in acclimatisation to heatwaves (correlation between maximum and minimum daily  $T_a$ :  $r = 0.84$ ,  $p < 0.001$ ). We extracted the daily maximum  $T_a$  on the day of experiment ( $T_{0\text{day}}$ ), and the day before the experiment ( $T_{-1\text{day}}$ ). We also computed the average daily maximum air temperature over the preceding 3 days ( $T_{-3\text{days}}$ ), 1 week ( $T_{-1\text{week}}$ ) and 2 weeks ( $T_{-2\text{weeks}}$ ). To evaluate whether large deviations from conditions on the day prior to measurements affected acclimatisation, we also computed the difference in temperatures between  $T_{0\text{day}}$  and  $T_{-1\text{day}}$  ( $\Delta T_{0-1}$ ) and  $T_{-1\text{day}}$  and  $T_{-2\text{days}}$  ( $\Delta T_{1-2}$ ).

### Statistical analyses

All analyses were performed using R (v3.6.1) in RStudio (v1.1.1335). Of 31 birds used for measurements, we obtained thermoregulatory data for 29 birds, because of issues with humidity control in two trials. The PIT tag was not detected for one bird at  $T_{a\text{-chamb}} = 31 \text{ }^\circ\text{C}$ , so the sample size for  $T_b$  was  $n = 28$  birds at  $T_{a\text{-chamb}} = 31 \text{ }^\circ\text{C}$  (and  $n = 29$  at other  $T_{a\text{-chamb}}$  stages).

To investigate responses at mild and high  $T_{a\text{-chamb}}$ , we considered separately  $T_{a\text{-chamb}}$  stages below or above the upper critical limit of thermoneutrality ( $T_{uc}$ , i.e., inflection  $T_{a\text{-chamb}}$  in MR and EWL), identified at  $T_{a\text{-chamb}} = 38 \text{ }^\circ\text{C}$  in this data set, using broken line analyses (see supplementary information). In each dataset (below and above the  $T_{uc}$ ), we first defined the base model for each thermoregulatory variable (MR, EWL, EHL/MHP and  $T_b$ ), using linear mixed models (LMMs) with mass,  $T_{a\text{-chamb}}$  stage and/or capture time as predictors, and bird ID as a random factor, and selecting the model with the lowest Aikake Information Criterion corrected for small sample size (AICc<sup>68</sup>). Then, we identified the best weather predictors explaining variation in thermoregulatory variables, by adding one weather predictor at a time (Table S1) to the selected base model (Table S2), using LMMs and AICc. This approach allows testing which predictor, among a suite of correlated parameters (which thus cannot be considered jointly in a model), best explains the observed variation<sup>69</sup>. To build the model set for each thermoregulatory variable, we first (i) tested for the effects of temperatures at different timescales by adding to the base model either  $T_{0\text{day}}$ ,  $T_{-1\text{day}}$ ,  $T_{-3\text{days}}$ ,  $T_{-1\text{week}}$  or  $T_{-2\text{weeks}}$  as a predictor (model 2–6, Table S1). We then (ii) tested for the effect of sudden changes in temperatures by adding to the model with  $T_{0\text{day}}$  or  $T_{-1\text{day}}$ , the deviation in temperature as either  $\Delta T_{0-1}$  (i.e.  $T_{0\text{day}} - T_{-1\text{day}}$ ) or  $\Delta T_{1-2}$  (i.e.  $T_{-1\text{day}} - T_{-2\text{days}}$ ) respectively (model 7–8, Table S1). Finally, (iii) to determine whether females and males differed in thermoregulatory performance, we fitted the same models as described above with sex as an additional predictor (model S1–S8, Table S1). We then used model averaging to test the significance of each parameter retained in the “top models”, within two AICc units of the best model, using the “model.avg” function from *MuMin* package and the conditional average method<sup>69,70</sup>. A predictor had a significant effect if its confidence interval excluded zero<sup>71</sup>. This method<sup>70</sup> does not correct for multiple comparisons, which was not required in our case<sup>72</sup>.

To investigate the best predictors of heat tolerance, we fitted generalized linear models (GLMs) on two proxies of heat tolerance: the maximum  $T_{a\text{-chamb}}$  stage reached by an individual ( $T_{a\text{-max}} = 46 \text{ }^\circ\text{C}$  or less) and whether or not the trial was completed (i.e. individual tolerated 15 min at  $T_{a\text{-chamb}} = 46 \text{ }^\circ\text{C}$  without showing sign of prolonged hyperthermia or activity). We used the model set and methods described above but without  $T_{a\text{-chamb}}$ .

To verify the observed acclimatisation patterns were not driven by hidden seasonal effects, we repeated the above analyses on the data collected in February only (2020 and 2021,  $n = 19$  birds with thermoregulatory data; Table S3).

Finally, to identify sex-specific predictor variables, we repeated the above procedure (model 0 to 8; Table S1) but considering males and females separately (and without sex as a predictor, Table S6). We used this split dataset approach rather than including interactions between sex and weather predictors because we were not aiming to test whether a particular time scale had opposite effects on the sexes, but instead, whether the same time scale was independently selected as best explaining variation in both of the sexes.

### Data availability

Data are available on Mendeley: <https://data.mendeley.com/datasets/kn6m7cg2p8/1>.

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## Author contributions

A.P. and M.M.M. conceptualised the study and designed the experiment with the help of A.E.M. and A.T.D.B.; A.P., E.U. and M.M.M. collected the data; A.P. analysed the data with advice from A.E.M. and M.M.M.; A.P. and M.M.M. wrote the first draft of the manuscript; E.U., A.E.M. and A.T.D.B. contributed to the revision of the manuscript. All authors gave final approval for publication.

## Competing interests

The authors declare no competing interests.

## Additional information

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**Correspondence** and requests for materials should be addressed to M.M.M.

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